Szkoła Główna Gospodarstwa Wiejskiego
w Warszawie
Instytut Nauk o Żywności

mgr inż. Anna Kot

Wpływ składu surowcowego, obróbki ultradźwiękami i nowych dodatków stabilizujących na właściwości fizyczne mieszanek lodowych oraz strukturę krystaliczną lodów spożywczych

The influence of raw materials, ultrasound and new stabilizers on the physical properties of ice cream mixes and the crystal structure of ice cream

Rozprawa doktorska
Doctoral thesis

Rozprawa doktorska wykonana pod kierunkiem
dr hab. Anny Kamińskiej-Dwórznickiej, prof. SGGW
Katedra Inżynierii Żywności i Organizacji Produkcji
Promotor pomocniczy
dr hab. Sabina Galus, prof. SGGW
Katedra Inżynierii Żywności i Organizacji Produkcji

Warszawa, rok 2023

WPŁYNĘŁO DNIA
2023 -10- 1
Instytut Nauk o Żywności
Sekretariat
INOŻ/30/2023

Oświadczenie promotora rozprawy doktorskiej

Oświadczam, że niniejsza rozprawa została przygotowana pod moim kierunkiem i stwierdzam, że spełnia warunki do przedstawienia jej w postępowaniu o nadanie stopnia naukowego doktora.

Data 09.10.2023 r. Podpis promotora Anna Kamińska-Dwórnicka

Oświadczenie autora rozprawy doktorskiej

Świadom/a odpowiedzialności prawnej, w tym odpowiedzialności karnej za złożenie fałszywego oświadczenia, oświadczam, że niniejsza rozprawa doktorska została napisana przez mnie samodzielnie i nie zawiera treści uzyskanych w sposób niezgodny z obowiązującymi przepisami prawa, w szczególności z ustawą z dnia 4 lutego 1994 r. o prawie autorskim i prawach pokrewnych (tj. z dnia 28 października 2022 r., Dz.U. z 2022 r. poz. 2509 ze zm.)

Oświadczam, że przedstawiona rozprawa nie była wcześniej podstawą żadnej procedury związanej z uzyskaniem stopnia naukowego doktora.

Oświadczam ponadto, że niniejsza wersja rozprawy jest identyczna z załączoną wersją elektroniczną.

Przyjmuję do wiadomości, że rozprawa doktorska poddana zostanie procedurze antyplagiatowej.

Data 09.10.2023 r. Podpis autora pracy Anna List

Streszczenie

Wpływ składu surowcowego, obróbki ultradźwiękami i nowych dodatków stabilizujących na właściwości fizyczne mieszanek lodowych oraz strukturę krystaliczną lodów spożywczych

W prezentowanej pracy zbadano wpływ iota karagenu, hydrolizatów iota karagenu oraz wpływ homogenizacji ultradźwiękowej na właściwości fizyczne mieszanek lodowych oraz lodów spożywczych, mlecznych oraz wegańskich. Badania były podzielone na etapy, które rozpoczęto od otrzymania hydrolizatów iota karagenu, wykorzystując metodę hydrolizy kwasowej oraz enzymatycznej z dwoma enzymami: β-galaktozydazą oraz laktazą przemysłową. Dla otrzymanych hydrolizatów przeprowadzono analizy SEC i FTIR, w celu określenia masy cząsteczkowej i układu grup funkcyjnych. Kolejne etapy dotyczyły zastosowania homogenizacji ultradźwiękowej oraz otrzymanych hydrolizatów jako stabilizatorów w mieszankach lodowych. W tym celu określono stabilność emulsji, wielkość cząstek, właściwości reologiczne oraz przeprowadzono analizę mikroskopową. Ostatnim etapem pracy było zbadanie właściwości fizycznych tj. puszystości, czasu topnienia i temperatury krioskopowej oraz struktury krystalicznej lodów po 24 godzinach, miesiącu i 3 miesiącach przechowywania w stałej temperaturze -18°C.

W wyniku przeprowadzonych badań wykazano, iż substancje stabilizujące pozyskane na drodze hydrolizy kwasowej i enzymatycznej iota karagenu, mogły korzystnie ograniczać proces rekrystalizacji w modelowych roztworach sacharozy. Najlepsze rezultaty osiągnięto dla roztworu modelowego z dodatkiem białka i hydrolizatu po laktazie przemysłowej – średnica kryształów nie przekraczała 17 µm. Przeprowadzone dla hydrolizatów analizy SEC i FTIR potwierdziły, że na inhibicję procesu rekrystalizacji ma wpływ struktura hydrolizatów i umiejscowienie grup funkcyjnych, a nie tylko redukcja ich masy cząsteczkowej.

Iota karagen oraz jego hydrolizaty przyczyniły się do poprawy stabilności oraz właściwości reologicznych mieszanek lodowych, zarówno mlecznych jak i wegańskich. Zastosowane dodatki stabilizujące w obu rodzajach lodów korzystnie wpłynęły na podwyższenie puszystości lodów (kształtowała się na poziomie 31-33%). Zastosowanie homogenizacji ultradźwiękowej wykazało lepszy wpływ na stabilność niż zastosowanie homogenizacji mechanicznej, wpłynęło również na zmniejszenie wielkości kuleczek tłuszczowych. W mieszankach wegańskich wartość mediany D_{50} po procesie dojrzewania mieściła się w zakresie 9,76-28,50 µm, przy czym najmniejsze wartości zauważono w próbkach z dodatkiem hydrolizatu enzymatycznego iota karagenu po β-galaktozydazie oraz po zastosowaniu homogenizacji ultradźwiękowej. Zarówno w lodach mlecznych, jak i wegańskich, ultradźwięki skutecznie inhibitowały proces rekrystalizacji (średnica kryształów nie przekraczała 19 µm) oraz wydłużały czas topnienia (czas topnienia przekraczał 30 minut). Przeprowadzone badania pozwoliły wykazać, że dodatek wybranych stabilizatorów w połączeniu z obróbką ultradźwiękową może korzystnie wpłynąć na optymalizację produkcji lodów spożywczych – pod względem ekonomicznym oraz środowiskowym.

Słowa kluczowe – mieszanka lodowa, emulsja, hydroliza, rekrystalizacja, lody wegańskie

Summary

The influence of raw materials, ultrasound and new stabilizers on the physical properties of ice cream mixes and the crystal structure of ice cream

The aim of this work was to examine the effect of iota carrageenan, iota carrageenan hydrolyzates and the effect of ultrasonic homogenization on the properties of ice cream mixes and ice cream, dairy and vegan ice cream. Studies were divided into stages, which started from the preparation of iota carrageenan hydrolyzates, resulting in acid and enzymatic hydrolysis with the enzymes: β-galactosidase and industrial lactase. SEC and FTIR analyses were performed for the obtained hydrolyzates to determine the molecular weight and arrangement of functional groups. The next stages include combining ultrasound homogenization and the obtained hydrolyzates as a stabilizer in the ice cream mixes. Therefore, such analyses as stability of the emulsion, particle size, rheological properties and microscopic analysis were investigated. The final stage of research was connected with the examination of the properties of ice cream, i.e. overrun, melting time and cryoscopic temperature, as well as the crystal structure of ice cream after 24 hours, one month and 3 months of storage at a constant temperature of -18°C.

As a result, it was noted that stabilizers obtained by acidic and enzymatic hydrolysis of iota carrageenan could beneficially inhibit the recrystallization process in model sucrose solutions. The most favourable results were achieved for the model solution with the addition of protein and after industrial lactase hydrolysis – the crystal diameter did not exceed 17 μm. Moreover, SEC and FTIR analyses performed on the hydrolyzates confirmed that the inhibition of the recrystallization process was influenced by the structure of the hydrolyzates and the location of the functional groups, and not only by the reduction of their molecular weight.

Iota carrageenan and its hydrolyzates contributed to improving the stability and rheological properties of ice cream mixes, both dairy and vegan. The stabilizers used in both types of ice cream had a beneficial effect on increasing the overrun of the ice cream (at the level of 31-33%). The use of ultrasound homogenization contributed to a better effect on stability than the use of mechanical homogenization and additionally reduced the sizes of fat globules. In vegan ice cream mixes, the median D_{50} value after the maturation process was in the range of 9,76-28,50 μm, and the lowest values were observed in samples with the addition of iota carrageenan enzymatic hydrolyzate after β-galactosidase and after the use of ultrasound homogenization. In both dairy and vegan ice cream, ultrasound homogenization effectively inhibited the recrystallization process (crystal diameter did not exceed 19 μm) and extended the melting time (melting time exceeded 30 minutes). Finally, the conducted research showed that the addition of selected stabilizers in combination with ultrasound treatment may have a positive impact on the optimization of food ice cream production – in economic and environmental terms.

Keywords – ice cream mix, emulsion, hydrolysis, recrystallization, vegan ice cream

Spis treści

WYKAZ PUBLIKACJI STANOWIĄCYCH PRACĘ DOKTORSKĄ

[P1] Kot, A., Kamińska-Dwórznicka, A., Antczak, A., Jakubczyk, E., Matwijczuk, A.: 2022. Effect of ι-carrageenan and its acidic and enzymatic hydrolysates on ice crystal structure changes in model sucrose solution. *Colloids and Surfaces A - Physicochemical and Engineering Aspects,* 643, 1-12. http://dx.doi.org/10.1016/j.colsurfa.2022.128744 IF:5,518, MEiN: 70.

Prze[P2] Kot, A., Kamińska-Dwórznicka, A.: 2022. Vegan ice cream: A review. *Przemysł Spożywczy,* 12(76), 30-34. http://dx.doi.org/10.15199/65.2022.12.5 IF:0, MEiN: 20.

[P3] Kot, A., Kamińska-Dwórznicka, A., Barańska, A.: 2020. Study on the properties of vegan ice cream based on almond drink. *Zeszyty Problemowe Postępów Nauk Rolniczych,* 600, 21-30. https://doi.org/10.22630/ZPPNR.2020.600.3 IF:0, MEiN: 20.

[P4] Kot, A., Kamińska-Dwórznicka, A., Galus, S., Jakubczyk, E.: 2021. Effect of different ingredients and stabilisers on properties of mixes based on almond drink for vegan ice cream production. *Sustainability,* 13(21), 1-17. https://doi.org/10.3390/su132112113 IF:3,889, MEiN: 100.

[P5] Kot, A., Kamińska-Dwórznicka, A., Jakubczyk, E. 2022. Study on the influence of ultrasound homogenisation on the physical properties of vegan ice cream mixes. *Applied Sciences,* 12(17), 8492. https://doi.org/10.3390/app12178492 IF:2,7, MEiN: 100.

[P6] Kot A., Kamińska-Dwórznicka A.: 2023. The influence of ultrasound homogenization on recrystallization during storage of vegan ice cream. *Journal of Food Process Engineering,* e14472. https://doi.org/10.1111/jfpe.14472 IF:2,889, MEiN: 100.

[P7] Kot, A., Jakubczyk, E., Buniowska-Olejnik, M., Kamińska-Dwórznicka, A.: 2023. The effect of iota carrageenan and its hydrolysates on the stability of milk ice cream mixes. *Polish Journal of Food and Nutrition Science*, 73(2), 196-204. https://doi.org/10.31883/pjfns/166382 IF:2,736, MEiN: 100.

[P8] Kot, A., Kamińska-Dwórznicka, A., Jakubczyk, E.: 2023. The effectiveness of ultrasound homogenisation on the recrystallisation process in milk ice cream. *Applied Sciences*, 13(13), 7561, 1-16. https://doi.org/10.3390/app13137561 IF:2,7, MEiN: 100.

Wartości współczynników Impact Factor poszczególnych publikacji podano w oparciu o dane udostępnione w InCites Journal Citation Report 2022, punkty ministerialne podano w oparciu o „Wykaz czasopism naukowych i recenzowanych materiałów z konferencji międzynarodowych wraz z przypisaną liczbą punktów", stanowiących załącznik do komunikatu Ministra Edukacji i Nauki z dnia 17 lipca 2023 roku. Sumaryczny IF publikacji wynosi 20,432. Liczba punktów ministerialnych wynosi 610 pkt.

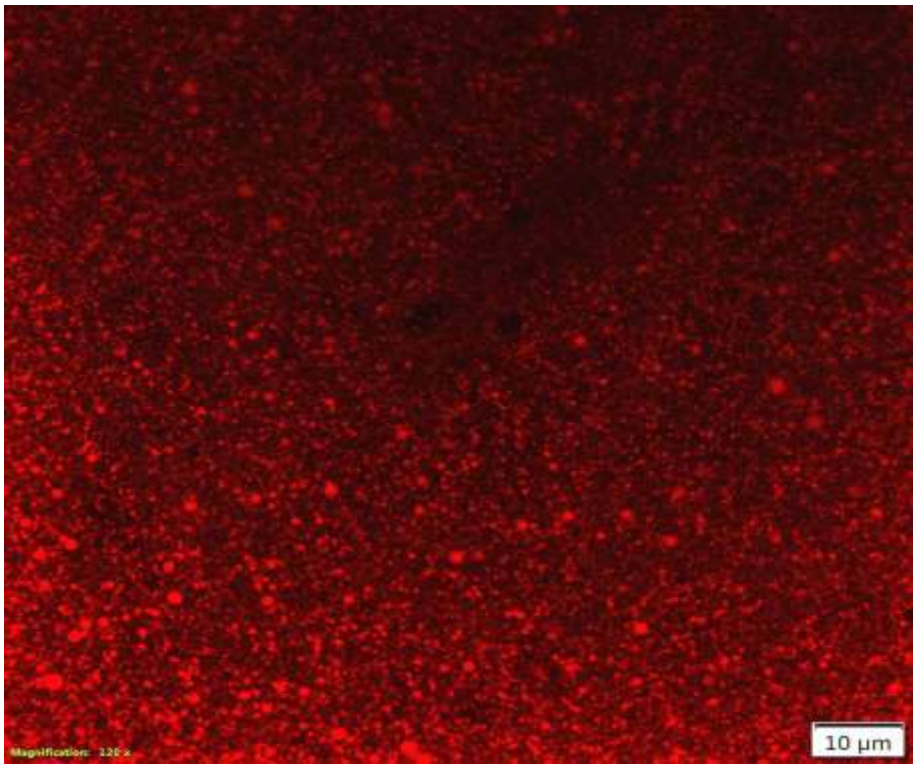
WSTĘP

Ze względu na swoje walory sensoryczne, lody spożywcze cieszą się ogromnym zainteresowaniem wśród szerokiej grupy konsumentów. Z tego też powodu produkcja lodów jest zaliczana do profitowych i dość szybko rozwijających się branż przemysłu spożywczego [Góral i wsp. 2018; Akdeniz i Akalin 2019]. Szacuje się, że produkcja lodów będzie się poszerzać w tempie rocznym, na poziomie 4,2% w latach 2022-2030. Wzrost ten napędzany jest przez zapotrzebowanie na innowacyjne smaki, rodzaje lodów czy nasze potrzeby zdrowotne [Internet 1]. Już w 2022 roku, w samej Unii Europejskiej wyprodukowano 3,2 biliony litrów lodów co stanowi wzrost o 5% do roku poprzedniego [Internet 2].

Pod względem fizykochemicznym lody można zdefiniować jako multidyspersyjny układ, który powstaje w wyniku rozproszenia poszczególnych składników w różnych fazach. Wyróżnia się aż pięć różnych faz, tj. piana, emulsja, zawiesina kryształów lodu, roztwór rzeczywisty substancji rozpuszczonych oraz koloid ze względu na dodatek hydrokoloidowych substancji stabilizujących. Struktura lodów w wyniku zdyspergowania powietrza w mrożonej cieczy, złożonej z 2/3 objętości wody. Lody stanowią układ pianowy, ponieważ ciecz jest fazą dyspergującą, a fazą zdyspergowaną powietrze. Natomiast w fazie wodnej lodów występuje również roztwór rzeczywisty cukrów oraz soli mineralnych [Goff i wsp. 1999; Kamińska-Dwórznicka i wsp. 2019]. Otrzymanie pożądanej tekstury lodów, która spełnia wymagania konsumentów, jest jednym z kluczowych cech tego produktu. Chociażby dlatego, iż tekstura lodów jest ściśle związana z odczuciem kremowości w trakcie spożycia. Podstawowym i jednocześnie najważniejszym czynnikiem wpływającym na teksturę lodów są obecne kryształy lodu, a w szczególności ich kształt, wielkość oraz rozmieszczenie. Małe rozmiary kryształu lodu (10-20 μm) nadają właściwej i pożądanej struktury produktowi finalnemu. Natomiast kryształy większe niż 40-55 μm powodują wyczuwalną piaszczystość czy też tworzenie się niepożądanych grudek w produkcie [Gaukel i wsp. 2014; Fiol i wsp. 2017].

Nie tylko struktura krystaliczna ma istotny wpływ na jakość lodów, ale również stabilność mieszanki lodowej. Odpowiednia stabilność, dotycząca braku zmian w rozmiarach czy wielkości cząstek, przyczynia się do pozyskania pożądanej tekstury lodów czy chociażby możliwości kontrolowania tzw. shelf-life produktu [Cheng i wsp. 2015b; Pal 2019]. Początkowa stabilność mieszanek lodowych kształtuje się już na etapie homogenizacji, w której dochodzi do jednoczesnej adsorpcji białek i emulgatorów. W trakcie dojrzewania mogą zachodzić zmiany nie tylko w przypadku tłuszczu, ale również uwadnianie białek czy

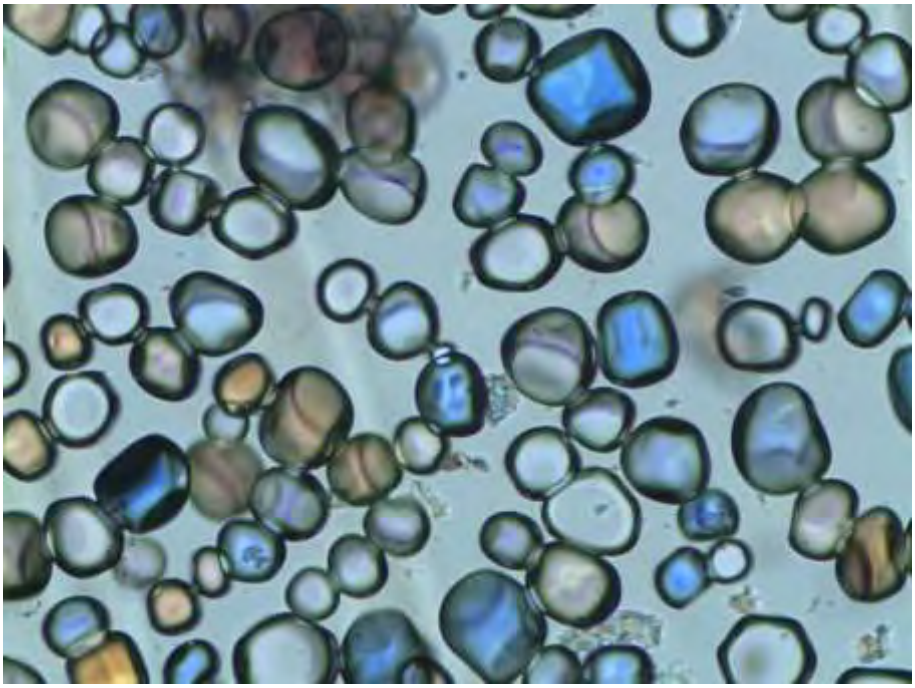
stabilizatorów. Wszystkie te zmiany mogą wpływać na bardziej kremową konsystencję lodów i w konsekwencji większą akceptowalność przez konsumentów [Gelin i wsp. 1994; Goff 1997; Mendez-Velasco i Goff 2012]. Warto również zaznaczyć, iż w przypadku mieszanek lodowych pewien rodzaj destabilizacji nie musi być uznawany jako pogorszenie właściwości fizycznych emulsji. Zostało udowodnione, iż niewielki poziom destabilizacji może być korzystny dla kształtowania się struktury krystalicznej lodów. Finalnie taki produkt może charakteryzować się dłuższym czasem topnienia oraz gładszą teksturą [Berger i White 1971; Goff i wsp. 1989; Koxhlot i wsp. 2001].



Rysunek 1. Przykład destabilizacji tłuszczu w mieszankach lodowych (Zbiór własny).

Problemem, który dotyczy struktury krystalicznej lodów, jest proces rekrystalizacji. To niepożądane zjawisko następuje zwykle na skutek fluktuacji temperatury czy długoterminowego przechowywania lodów poniżej temperatury zeszklenia. W wyniku rekrystalizacji może dojść do wzrostu rozmiarów kryształów lodu, zmiany ich kształtu, liczby, czy też rozmieszczenia w strukturze produktu. Może odbywać się to na drodze koalescencji lub migracji. Koalescencja polega na łączeniu się dwóch lub więcej kryształów w jeden. Natomiast migracja zwykle następuje w wyniku topnienia kryształów i migracji cząsteczek wody w stronę powierzchni większych kryształów, powodując przyrost

ich średnicy [Adapa i wsp. 2000; Ndoye i Alvarez 2015; Zhu i wsp. 2019; Kiran-Yildirim i wsp. 2021].



Rysunek 2. Przykład koalescencji w lodach (Zbiór własny).

Jednym ze sposobów zapobiegania procesowi rekrystalizacji jest dodatek stabilizatorów. Dodatek tego typu substancji do lodowej receptury pozwala zmniejszyć, a nawet całkowicie wyeliminować zjawisko rekrystalizacji. Ponadto celem stabilizatorów jest nie tylko ograniczenie tego niepożądanego zjawiska w lodach, ale również ograniczenie czasu topnienia, zmniejszanie migracji cząsteczek wody, wzrost lepkości oraz gęstości układu, utrwalenie emulsji, a nawet zmniejszenia uczucia zimna w trakcie konsumpcji [Regand i Goff 2003; Gaukel i wsp. 2014].

Obecnie najpowszechniej stosowanymi stabilizatorami są karageny. Są to biodegradowalne, liniowe polisacharydy z grupy substancji żelujących, zagęszczających, stabilizujących oraz emulgujących [Regand i Goff 2003; Soukoulis i wsp. 2008]. W zależności od ilości oraz położenia grup sulfonowych wyróżnia się trzy rodzaje karagenów: kappa, iota oraz lambda. W prezentowanej pracy poświęcono uwagę działaniu jednej z tych frakcji – iota karagenu. Iota karagen posiada zdolność do tworzenia mocnych i elastycznych żeli, które są bardzo stabilne podczas mrożenia i topnienia. Wykazuje również powinowactwo do jonów wapnia [Thrimawithana i wsp. 2010; Kiran-Yildirim i wsp. 2021].

Sam mechanizm działania karagenów cały czas podlega badaniom i generuje liczne kontrowersyjne dyskusje. Tłumaczy się chociażby, iż zdolność hydrokloidów do ograniczenia procesu rekrystalizacji może wynikać ze zmniejszania się ruchliwości cząsteczek wody ze względu na ich właściwości wiązania wody lub tworzenia szczelnej bariery dla wzrostu kryształów [Gaukel i wsp. 2014]. I tak na przykład, w oparciu o badania przeprowadzone przez Kamińską-Dwórznicką i wsp. [2015, 2016], wykazano, że hydrolizaty kappa karagenu znacznie lepiej ograniczały proces rekrystalizacji niż sam kappa karagen. Zależność ta była związana z redukcją masy cząsteczkowej hydrolizatów.

Zainteresowanie wykorzystaniem karagenów wynika z badań przeprowadzonych przez Gaukel i wsp. [2014], dotyczącej specjalnego białka AFP, czyli antifreeze protein, które skutecznie ogranicza proces rekrystalizacji lodu. Sposób interakcji kappa karagenu z powierzchnią lodu jest zbliżony do mechanizmu działania wspomnianego białka, a dodatkowo charakteryzuje się silnymi właściwościami inhibitującymi proces rekrystalizacji. Modyfikowanie łańcucha karagenów jest możliwe dzięki procesowi hydrolizy zarówno kwasowej, jak i enzymatycznej. Podejmuje się kroki w celu redukcji tego łańcucha polisacharydowego, ponieważ według obecnego stanu wiedzy zmniejszenie liczby grup sulfonowych może polepszyć elastyczność otrzymanego hydrolizatu. Oznacza to, iż otrzymany hydrolizat może skuteczniej zachowywać się w roztworach niż sam karagen [Karlsson i Singh 1999]. W badaniach Kiran-Yildrim i wsp. [2021] wykazano, iż kappa karagen, który posiada mniej grup sulfonowych, ma skuteczniejsze działanie inhibitujące proces rekrystalizacji niż iota karagen, który w swojej budowie ma dwie grupy sulfonowe. Nasuwa to wniosek, iż liczba oraz położenie grup funkcyjnych może oddziaływać na zachowanie się stabilizatorów w trakcie zamrażania.

Kolejną techniką mającą na celu ograniczenie rekrystalizacji w lodach oraz polepszeniem ich właściwości fizycznych, a jednocześnie sensorycznych, jest aplikacja ultradźwięków. Ta innowacyjna metoda pozwala na podwyższanie jakości, zwiększanie efektywności procesu oraz wydłużenie życia – „shelf life" – produktu. Dodatkowo ultradźwięki mogą być przyszłościową techniką w przemyśle spożywczym ze względu na: niskie koszty, prostotę użytkowania, szybkość działania, brak toksyczności, przyjazność środowisku oraz ograniczenie zużycia energii [BahramParvar i wsp. 2013; Akdeniz i Akalin 2019]. W przypadku produkcji lodów ultradźwięki mogą być kompleksowym rozwiązaniem z tego względu, iż mają zdolność do wspierania zarodkowania kryształu lodu, przyspieszania przepływu masy i energii, kontroli wzrostu oraz kryształów w trakcie zamrażania czy redukcji całkowitego czasu zamrażania. Na przykładzie badań Islam i wsp. [2015] czy Xu

i wsp. [2015] stosowanie kawitacji akustycznej w trakcie zamrażania grzybów i rzodkiewki przyczyniło się do zmniejszenia wielkości kryształów lodu, w porównaniu do próbek niepoddanych działaniu ultradźwięków. Ultradźwięki mogą zostać wykorzystane także na innych etapach produkcji lodów, jak pasteryzacja, gdzie stwierdzono, iż można je stosować zamiennie z tradycyjną pasteryzacją, nie powodując pogorszenia jakości produktu [Nazarewicz i wsp. 2022] lub w połączeniu z homogenizacją, pozytywnie oddziałując na stabilność emulsji [Tüker i Dogan 2021]. Wykazano również, iż zastosowanie ultradźwięków może poprawiać właściwości emulgujące białek na przykładzie białek roślinnych, chociażby ze względu na zmniejszenie rozmiaru cząstek i poprawę ich rozpuszczalności [Taha i wsp. 2018]. Ujednolicenie mieszanki może mieć z kolei wpływ na uzyskanie mniejszych kryształów lodu w trakcie procesu zamrażania. Udowodniono, że zmniejszenie wielkości kuleczek tłuszczu i zmniejszenie odległości między nimi ma potem wpływ na budowaną strukturę krystaliczną. W konsekwencji może to oddziaływać na poprawę efektywności procesu produkcyjnego, jednocześnie pozwalając na otrzymanie lodów o odpowiedniej jakości oraz akceptowalności przez konsumenta [Adapa i wsp. 2000].

W publikacji **P2**, wchodzącej w skład pracy doktorskiej, przedstawiono aktualne informacje dotyczące produkcji lodów wegańskich, a w szczególności stosowanych składników oraz najnowszych technik produkcyjnych. Szerokie zainteresowanie wegańskimi produktami wynika ze zmieniających się trendów żywieniowych oraz większej świadomości żywieniowej konsumentów. W związku z czym również w branży lodziarskiej tworzy się produkty na bazie surowców roślinnych. Na przestrzeni ostatnich lat nasza dieta zyskała ogromne znaczenie, z uwagi na dbałość o zdrowie i dobre życie. W przypadku lodów to mleko krowie jest jednym z głównych składników. Natomiast według aktualnego stanu wiedzy jest ono również jednym z podstawowych alergenów dla człowieka. Szacuje się, iż w krajach rozwiniętych nawet do 3% dzieci w wieku do 1 roku, może mieć alergię na mleko krowie. Ponadto w przypadku osób zmagających się z nietolerancją laktozy nie jest to również rekomendowany składnik. Dodatkowo poprzez wdrażanie rozwiązań związanych ze zrównoważonym rozwojem, na skutek zmian klimatycznych, konsumenci są zachęcani oraz edukowani do ograniczenia spożywania produktów pochodzenia zwierzęcego lub wprowadzenia diety wegańskiej. Nasz sektor żywności jest odpowiedzialny za 30% całkowitej emisji dwutlenku węgla. Ponadto, jak zauważono w badaniach Konstanas i wsp. [2019], produkcja lodów dostarcza 4 $kgCO_2$ eq./kg wyprodukowanych lodów spożywczych na bazie składników pochodzenia zwierzęcego. W związku z czym, aby przeciwdziałać zmianom klimatu, warto wdrożyć zmiany nawet w produkcji lodowych deserów.

W składzie lodów wegańskich, zgodnie z ich definicją, nie powinny znajdować się żadne produkty pochodzenia zwierzęcego, lecz ich roślinne alternatywy. Składniki w lodach spożywczych można podzielić na trzy kategorie: główne, w których skład wchodzą białka, węglowodany i tłuszcz oraz druga grupa związków, czyli emulgatory i stabilizatory. W zależności od receptury w składzie lodów mogą znaleźć się inne dodatki takie jak orzechy lub czekolada [Clarke 2004]. Dość popularnymi stabilizatorami w lodach są karageny, mączka chleba świętojańskiego, guma guar czy guma ksantanowa. Stabilizatory posiadają zdolność do wiązania wody, poprawiają teksturę oraz wpływają na smak lodów [Kamińska-Dwórznicka i wsp. 2015; Akbari i wsp. 2019].

W recepturze lodów wegańskich częstym zamiennikiem białka mleka krowiego są białka roślinne. Aktualnie ogromną popularnością cieszy się białko grochu, które cechuje się pożądanymi właściwościami emulgującymi, żelującymi oraz właściwościami odżywczymi a przede wszystkim niskim kosztem. Kolejnym białkiem roślinnym, które można spotkać w recepturze lodów wegańskich, jest białko sojowe. Natomiast z powodu rosnącej alergenności tego białka również poszukuje się jego zamienników. Stosuje się np. białko z ziemniaka, które w połączeniu ze stabilizatorami daje możliwości utrzymania wysokiej jakości lodów [O'Sullivan i wsp. 2016a; Lomolino i wsp. 2020].

Również w przypadku zamiany mleka krowiego w formie płynnej, dość często wykorzystuje się napój sojowy, kokosowy bądź migdałowy. Lecytyna sojowa pełni rolę emulgatora, przez co stanowi ochronę przed uszkodzeniami w trakcie zamrażania. Napój kokosowy jest kolejnym popularnym dodatkiem w recepturze lodów dla wegan. Ze względu na swój skład może być stosowany do podniesienia kaloryczności produktu w związku z zawartością tłuszczu. Z kolei migdały, posiadają wysoką zawartość jednonienasyconych kwasów tłuszczowych (MUFA), które uważa się za pomocne w odchudzaniu i kontrolowaniu masy ciała. Istnieje również znacząca liczba przekonujących dowodów na to, że MUFA przyczynia się do zmniejszenia w organizmie zawartości lipoprotein o niskiej gęstości. Migdały działają również jako ważne źródło różnych składników odżywczych, w tym białek, błonnika, witaminy E i manganu [Vanga i Raghavan 2018]. Ponadto napój migdałowy wpływa pozytywnie na teksturę produktu, nadając bardziej kremowej konsystencji [Aboulfazli i wsp. 2016; Abdullah i wsp. 2018].

Nie tylko składniki, ale też proces produkcyjny ma wpływ na jakość końcową produktu. Dlatego też dość dużą uwagę przywiązuje się do odpowiednio zaprojektowanego procesu produkcyjnego lodów oraz jego parametrów. Proces produkcji lodów wegańskich nie różni się od procesu produkcyjnego lodów mlecznych, jeżeli chodzi o poszczególną kolejność

etapów produkcyjnych, ale na przykład zamienia się tradycyjną homogenizację na homogenizację ultradźwiękową, HPP (High Pressure Processing) lub wprowadza się ultradźwięki w trakcie procesu zamrażania.

Pomimo iż lody traktowane są jako deser, często stanowią niedoceniany produkt spożywczy. Otóż na bazie badań Spence i wsp. [2019] wykazano nowy potencjał tego mrożonego deseru. Ze względu na dynamiczny kontrast w trakcie spożywania lodów, dotyczący wrażliwości na zimno, stanowią one doskonałe medium do transmisji energii chociażby dla osób starszych lub osób niedożywionych. Takie spostrzeżenia dają podstawy do dalszych badań nad szerszym spektrum wykorzystania lodów, nie tylko jako formy deseru.

Podsumowując, na bazie doniesień literaturowych oraz potrzeb obecnego rynku, w prezentowanej pracy podjęto próbę ograniczenia procesu rekrystalizacji w lodach, stosując wybrane stabilizatory oraz modyfikując przebieg procesu technologicznego. Nie tylko podjęto próbę zrozumienia mechanizmu działania iota karagenu i jego hydrolizatów w procesie krystalizacji w lodach, lecz uwzględniono również wpływ kawitacji akustycznej na ten mechanizm. Natomiast aby sprostać oczekiwaniom konsumentów, badania prowadzono jednocześnie na lodach zarówno mlecznych, jak i wegańskich, również po to, żeby wykazać, że sama technologia produkcji i dodatek stabilizatorów nie muszą być inne dla tych dwóch rodzajów lodów. Wiedza ta może ułatwić podjęcie przez producentów decyzji o produkcji lodów na bazie wegańskich składników.

CELE I HIPOTEZY BADAWCZE

W pracy doktorskiej postawione zostały cele badawcze:

1. Określenie wpływu hydrolizatów iota karagenu na ograniczenie procesu rekrystalizacji w układach modelowych oraz próbkach lodów, z uwzględnieniem analizy mechanizmu inhibicji – w oparciu o masę cząsteczkową i budowę chemiczną pozyskanych hydrolizatów.
2. Zbadanie wpływu obróbki wstępnej za pomocą ultradźwięków oraz wybranych dodatków stabilizujących na właściwości fizyczne mieszanki lodowej oraz właściwości fizyczne i strukturę krystaliczną lodów na bazie mleka.
3. Zbadanie wpływu obróbki wstępnej za pomocą ultradźwięków oraz wybranych dodatków stabilizujących na właściwości fizyczne i strukturę krystaliczną lodów na bazie napoju bezmlecznego (napoju migdałowego).

Sformułowane cele badawcze realizowane były na podstawie weryfikacji cząstkowych hipotez badawczych:

H1. Hydrolizaty iota karagenu mają większy wpływ na ograniczenie procesu rekrystalizacji niż sam iota karagen. Polepszenie ich właściwości stabilizujących wynika z odmiennej struktury hydrolizatów a nie tylko z mniejszej masy cząsteczkowej.

H2. Dobór odpowiedniego składu mieszanek lodowych determinuje ich stabilność oraz wpływa na ukształtowanie korzystnej struktury krystalicznej lodów po zamrożeniu.

H3. Zastosowanie homogenizacji ultradźwiękowej polepszy właściwości fizyczne mieszanek lodowych oraz strukturę krystaliczną lodów w porównaniu do mechanicznej metody homogenizacji.

ZAKRES PRACY

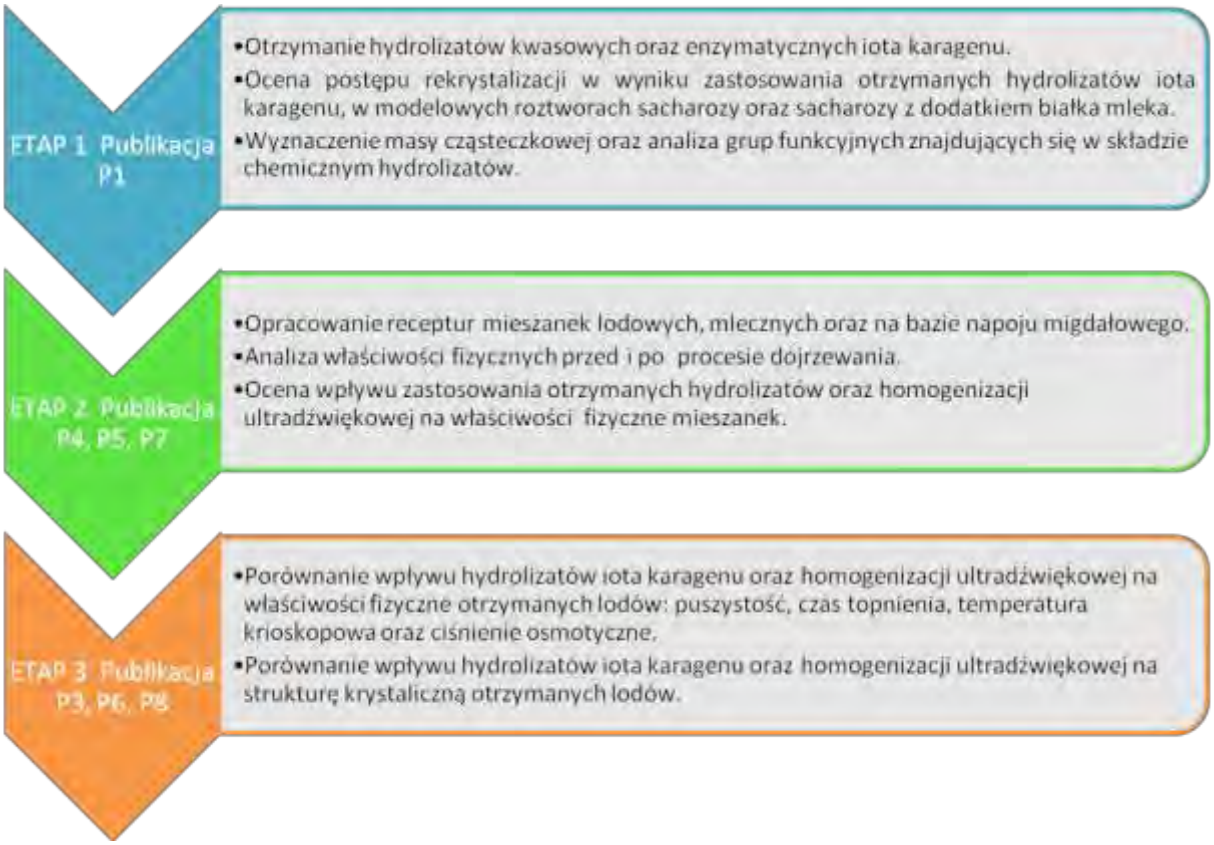
ETAP 1: Pierwszy etap badań dotyczył doboru mieszanek stabilizujących. W tym celu została przeprowadzona hydroliza kwasowa oraz enzymatyczna iota karagenu. Na podstawie analizy struktury krystalicznej zamrożonych roztworów modelowych sacharozy z białkiem i bez białka mleka oraz z dodatkiem iota karagenu i otrzymanymi hydrolizatami iota karagenu oceniono przebieg procesu rekrystalizacji.

ETAP 2: Kolejnym etapem badań było badanie właściwości mieszanek lodowych (przed zamrażaniem) do lodów mlecznych oraz do lodów na bazie napoju migdałowego. Przeprowadzono analizę właściwości fizycznych wszystkich rodzajów mieszanek lodowych (zarówno pod względem surowcowym, jak i wykorzystania wybranych rodzajów homogenizacji – mechanicznej i ultradźwiękowej).

ETAP 3: Ostatni etap badań poświęcony był zbadaniu wpływu dodatku pozyskanych stabilizatorów oraz ultradźwięków na właściwości lodów mlecznych oraz lodów na bazie napoju migdałowego. Przeprowadzono ocenę właściwości fizycznych wszystkich rodzajów badanych lodów oraz analizę struktury krystalicznej po 24 godzinach, po miesiącu oraz po trzech miesiącach przechowywania w stałej temperaturze -18°C.

ORGANIZACJA BADAŃ

Przedstawione w cyklu publikacji badania podzielono na trzy etapy zgodnie z założonymi celami pracy (Rysunek 3).



Rysunek 3. Schemat przeprowadzonych doświadczeń.

ETAP 1 pracy obejmował otrzymanie hydrolizatów iota karagenu dwoma metodami: hydrolizą kwasową z użyciem kwasu solnego oraz hydrolizą enzymatyczną, stosując dwa enzymy: β-galaktozydazę (laktaza wysokooczyszczona) oraz laktazę przemysłową. Po przeprowadzonej hydrolizie sprawdzono masę cząsteczkową otrzymanych hydrolizatów za pomocą chromatografii żelowej SEC (badanie wykonane w Instytucie Nauk Drzewnych i Meblarstwa SGGW), a następnie warianty o najmniejszych masach cząsteczkowych poddano analizie FTIR (badanie wykonane w Zakładzie Biofizyki Molekularnej, Uniwersytetu Przyrodniczego w Lublinie) w celu określenia różnic pomiędzy hydrolizatami jako potencjalnych czynników modelujących mechanizm inhibicji procesu rekrystalizacji lodu. Finalnie przeprowadzono analizę struktury krystalicznej roztworów modelowych sacharozy z białkiem i bez białka mleka (jako modele lodów mlecznych i bezmlecznych) oraz z iota karagenem i otrzymanymi hydrolizatami iota karagenu, po przechowywaniu przez 24, 48, 72 oraz 96 godzin, w stałej temperaturze -8°C. Postęp procesu rekrystalizacji oszacowano na podstawie obrysu kryształów lodu ze zdjęć wykonanych mikroskopem optycznym,

wyposażonym w system chłodzący Linkam Scientific PE 94 oraz kamerę, umożliwiającymi wykonanie zdjęć w temperaturze poniżej 0°C.

ETAP 2 dotyczył badań mieszanek lodowych zarówno przed, jak i po procesie dojrzewania. Analiza fizyczna mieszanek obejmowała badania właściwości reologicznych – krzywe płynięcia i krzywe lepkości emulsji, przy użyciu reometru; rozkładu wielkości cząstek za pomocą analizatora wielkości cząstek, wykorzystującego metodę dyfrakcji laserowej; analizę stabilności emulsji z wykorzystaniem rozproszonego światła wstecznego oraz analizę mikrostruktury za pomocą mikroskopu konfokalnego w przypadku mieszanki mlecznej oraz optycznego w przypadku mieszanki wegańskiej.

ETAP 3 polegał na określeniu wpływu dodatku pozyskanych stabilizatorów oraz wpływu homogenizacji ultradźwiękowej na właściwości lodów mlecznych oraz lodów wegańskich na bazie napoju migdałowego. W tym celu zostały przeprowadzone następujące oznaczenia fizyczne: gęstość, pH, puszystość, czas topnienia, temperatura krioskopowa, ciśnienie osmotyczne oraz analiza struktury krystalicznej po 24 godzinach, miesiącu oraz trzech miesiącach przechowywania w stałej i temperaturze -18°C.

BADANY MATERIAŁ

W zależności od poszczególnych etapów badań materiał stanowiły: iota karagen oraz otrzymane enzymatyczne i kwasowe hydrolizaty iota karagenu; roztwory modelowe: 50% roztwór sacharozy oraz 50% roztwór sacharozy z dodatkiem kazeinianu sodu w ilości 2,6 g na 100 ml roztworu; mleczna i wegańska mieszanka lodowa oraz powstałe na ich bazie lody spożywcze.

W pierwszym etapie badań zostały przebadane otrzymane hydrolizaty jak i czysty iota karagen, jako potencjalne stabilizatory w 50% modelowych roztworach sacharozy oraz 50% roztworach sacharozy z dodatkiem kazeinianu sodu (2,6 g na 100 ml).

Drugi etap badań dotyczył przygotowania mlecznych oraz wegańskich mieszanek lodowych. Do przygotowania receptur mieszanek mlecznych wykorzystano: mleko w proszku, mleko w płynie, inulinę, emulgator E471 (mono- lub diglicerydy kwasów tłuszczowych) oraz układ stabilizujący: iota karagen lub hydrolizaty iota karagenu w połączeniu z mączką chleba świętojańskiego oraz gumą ksantanową (Tabela 1 oraz 3). W wersji wegańskiej receptury oprócz tego samego układu stabilizującego oraz dodatku emulgatora znalazły się: napój migdałowy, syrop migdałowy, białko grochu oraz inulina (Tabela 2 oraz 3).

Metody technologiczne przygotowywania mieszanek lodowych oraz lodów spożywczych

Przygotowanie mieszanek rozpoczęto od naważenia składników (suchych oraz mokrych) zgodnie z podana recepturą, a następnie wymieszanie całości mieszanek za pomocą blendera Bosch Maxo-Mixx 750W (Bosh, Gerlingen, Niemcy). Następnie mieszanki poddano procesowi pasteryzacji wykorzystując termomix Vorwerk (Vorwerk, Wuppertal, Niemcy) w temperaturze 85°C przez 1,5 minuty. Po tym etapie mieszanki poddawano chłodzeniu, aż do osiągnięcia 25°C. W zależności od wariantu mieszanki poddawano procesowi homogenizacji: mechanicznej, wykorzystując homogenizator IKA T 25 digital ULTRA-TURRAX 20obr/min. w ciągu 2,5 minuty lub homogenizację ultradźwiękową, przy zastosowaniu homogenizatora Ultrasonic Liquid Processor VCX 500 (Sonics & Materials, Inc., Newton, CT, USA) w czasie 5 minut przy częstotliwości 20 kHz. Następnie mieszanki lodowe poddawano procesowi dojrzewania, przechowując je w temperaturze 4°C przez 24 godziny (proces dojrzewania) (lodówka, Whirlpool, Polska). Po tym etapie mieszanki lodowe poddawano procesowi zamrażania, wykorzystując maszynkę do lodów Neumaker Gelato 5K SC (Hermer, Niemcy), do momentu osiągnięcia temperatury -7°C (przez 15 minut). Finalnie,

otrzymane próbki lodów przechowywano w plastikowych pojemnikach do 3 miesięcy w temperaturze -18°C (zamrażarka, Whirlpool, Polska).

Tabela 1. Skład mieszanki lodowej mlecznej.

Składnik	**C**	**I**	**A**	**B**	**L**
Mleko 0,5% (Mlekovita)	76,0	75,49	75,495	75,495	75,495
Inulina (Orafti BENEO)	10,0	10,0	10,0	10,0	10,0
Mleko w proszku (Mlekovita)	7,0	7,0	7,0	7,0	7,0
Cukier biały (Diamant)	7,0	7,0	7,0	7,0	7,0
Emulgator E471 (Fooding Shanghai)	0,4	0,4	0,4	0,4	0,4
Mączka chleba świętojańskiego (Fooding Shanghai)	–	0,08	0,08	0,08	0,08
Guma ksantanowa (Fooding Shanghai,)	–	0,02	0,02	0,02	0,02
Iota karagen (Sigma-Aldrich)	–	0,01	–	–	–
Kwasowy hydrolizat iota karagenu	–	–	0,005	–	–
Enzymatyczny hydrolizat iota karagenu po β-galaktozydazie	–	–	–	0,005	–
Enzymatyczny hydrolizat iota karagenu po laktazie	–	–	–	–	0,005

Tabela 2. Skład mieszanki lodowej wegańskiej.

Składnik	**C**	**I**	**A**	**B**	**L**
Napój migdałowy (Enerbio)	66,6	66,49	66,495	66,495	66,495
Syrop migdałowy (Monin)	16,0	16,0	16,0	16,0	16,0
Inulina (Orafti BENEO)	12,0	12,0	12,0	12,0	12,0
Białko grochu (Nuturalys S85plus, Roquette)	5,0	5,0	5,0	5,0	5,0
Emulgator E471 (Fooding Shanghai)	0,4	0,4	0,4	0,4	0,4
Mączka chleba świętojańskiego (Fooding Shanghai)	–	0,08	0,08	0,08	0,08
Guma ksantanowa (Fooding Shanghai)	–	0,02	0,02	0,02	0,02
Iota karagen (Sigma-Aldrich)	–	0,01	–	–	–
Kwasowy hydrolizat iota karagenu	–	–	0,005	–	–
Enzymatyczny hydrolizat iota karagenu po β-galaktozydazie	–	–	–	0,005	–
Enzymatyczny hydrolizat iota karagenu po laktazie	–	–	–	–	0,005

Tabela 3. Stosowane oznaczenia i składniki poszczególnych wariantów mieszanek lodowych/lodów spożywczych.

Nazwa próbki	Rodzaj układu stabilizującego	Rodzaj zastosowanej homogenizacji
C	Próbka kontrolna bez stabilizatorów	-
CH	Próbka kontrolna bez stabilizatorów	Homogenizacja mechaniczna
CU	Próbka kontrolna bez stabilizatorów	Homogenizacja ultradźwiękowa
I	Próbka z dodatkiem iota karagenu, mączki chleba świętojańskiego, gumy ksantanowej	-
IH	Próbka z dodatkiem iota karagenu, mączki chleba świętojańskiego, gumy ksantanowej	Homogenizacja mechaniczna
IU	Próbka z dodatkiem iota karagenu, mączki chleba świętojańskiego, gumy ksantanowej	Homogenizacja ultradźwiękowa
A	Próbka z dodatkiem kwasowego hydrolizatu iota karagenu, mączki chleba świętojańskiego, gumy ksantanowej	–
AH	Próbka z dodatkiem kwasowego hydrolizatu iota karagenu, mączki chleba świętojańskiego, gumy ksantanowej	Homogenizacja mechaniczna
AU	Próbka z dodatkiem kwasowego hydrolizatu iota karagenu, mączki chleba świętojańskiego, gumy ksantanowej	Homogenizacja ultradźwiękowa
B	Próbka z dodatkiem enzymatycznego hydrolizatu iota karagenu po β-galaktozydazie, mączki chleba świętojańskiego, gumy ksantanowej	–
BH	Próbka z dodatkiem enzymatycznego hydrolizatu iota karagenu po β-galaktozydazie, mączki chleba świętojańskiego, gumy ksantanowej	Homogenizacja mechaniczna
BU	Próbka z dodatkiem enzymatycznego hydrolizatu iota karagenu po β-galaktozydazie, mączki chleba świętojańskiego, gumy ksantanowej	Homogenizacja ultradźwiękowa
L	Próbka z dodatkiem enzymatycznego hydrolizatu iota karagenu po laktazie przemysłowej, mączki chleba świętojańskiego, gumy ksantanowej	–
LH	Próbka z dodatkiem enzymatycznego hydrolizatu iota karagenu po laktazie przemysłowej, mączki chleba świętojańskiego, gumy ksantanowej	Homogenizacja mechaniczna
LU	Próbka z dodatkiem enzymatycznego hydrolizatu iota karagenu po laktazie przemysłowej, mączki chleba świętojańskiego, gumy ksantanowej	Homogenizacja ultradźwiękowa

METODY BADAWCZE

W przeprowadzonych badaniach zastosowano następujące metody badawcze:

I ETAP BADAŃ

- **Chromatografia żelowa SEC** – średnia jak również rozkład masy cząsteczkowej zostały określone przy pomocy chromatografii żelowej SEC (Size Exclusion Chromatography). Badanie przeprowadzono na chromatografie cieczowym HPLC firmy Shimadzu [Kamińska-Dwórznicka i wsp. 2015].
- **Analiza FTIR –** pomiary widm ATR-FTIR skorygowanych o tło (25 skanów dla każdej próbki) uzyskano za pomocą płytki kryształowej HATR Ge trough, w temperaturze 20°C, natomiast wyniki zarejestrowano przy pomocy Spektrofotometru IR firmy Agilent [Moniha i wsp. 2018; Moniha i wsp. 2019; Ghani i wsp. 2019].

- **Analiza mikroskopowa kryształów lodu** – po przygotowaniu próbki w temperaturze ujemnej, wykonano zdjęcia przy pomocy mikroskopu Nikon model Alphaphot-2 YS2i kamery Nikon DS.-Fi1. Otrzymany obraz został przeanalizowany za pomocą programu NIS Elements D. Postęp procesu rekrystalizacji został oceniany po 24, 48, 72 oraz 96 godzinach przechowywania [Kamińska-Dwórznicka i wsp. 2015].
- **Metody statystyczne** – analiza wariancji ANOVA, z wykorzystaniem testu Tukey'a na poziomie istotności $\alpha = 0,05$. Analiza statystyczna została wykonana za pomocą programu Statistica 13.1 (Statstof Polska). Ponadto rozkład częstotliwości wielkości kryształów w roztworach modelowych obliczono za pomocą programu Microsoft Excel 2019, wykorzystując analizę danych w postaci histogramu [Kamińska-Dwórznicka i wsp. 2015, 2022].

II ETAP BADAŃ

- **Oznaczenie gęstości mieszanek lodowych** – wykonano za pomocą piknometru próżniowego o pojemności 25 cm^3 [Dłużewska i wsp. 2003].
- **Oznaczenia stabilności mieszanek** – dokonano za pomocą analizatora stabilności Turbiscan Lab Expert [Wang i wsp. 2018].
- **Oznaczenie rozkładu wielkości cząstek** – rozkład wielkości cząstek mieszanek lodowych został wyznaczony za pomocą laserowego analizatora CILAS 1190 [Domian i wsp. 2018].
- **Oznaczenie lepkości mieszanek** – za pomocą reometru Haake MARS. Analizę próbek wykonano przy roboczej szybkości ścinania od 0 do 100s^{-1} w temperaturze 25°C [Kamińska-Dwórznicka i wsp. 2022].
- **Analiza morfologii cząstek mieszanek lodowych** – na podstawie zdjęć mikroskopowych wykonanych za pomocą mikroskopu i kamery Nikon model Alphapot – 2 YS2 lub mikroskopu konfokalnego FLUOVIEW FV300 [Ahn i wsp. 2022].

Wszystkie oznaczenia wykonano bezpośrednio po przygotowaniu mieszanek oraz po 24-godzinnym dojrzewaniu w 4°C.

- **Metody statystyczne** – analiza wariancji ANOVA, z wykorzystaniem testu Tukey'a na poziomie istotności $\alpha = 0,05$. Analiza statystyczna została wykonana za pomocą programu Statistica 13.1 (Statstof Polska).

III ETAP BADAŃ

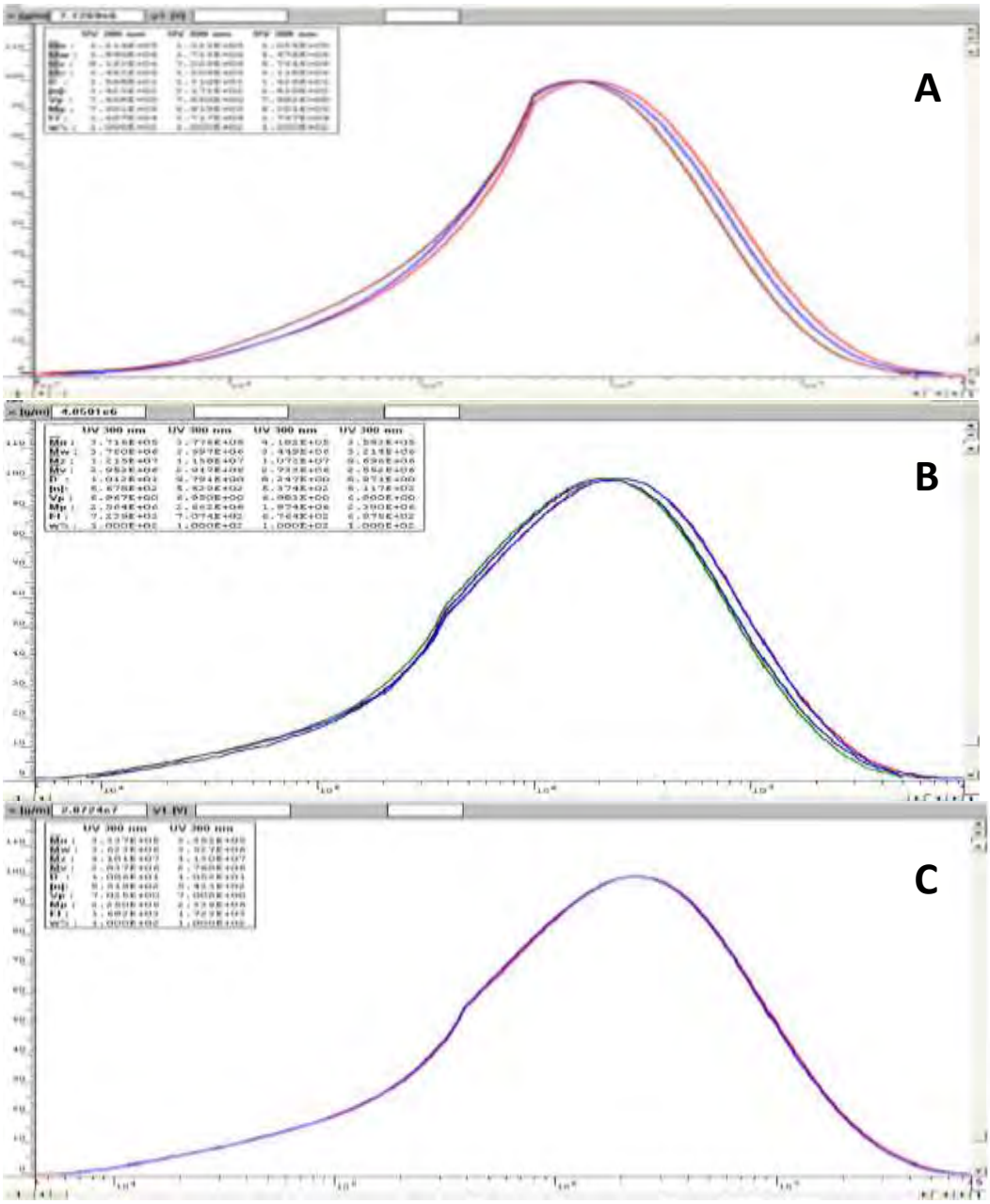
- **Oznaczenie temperatury krioskopowej oraz ciśnienia osmotycznego** – określono za pomocą osmometru Marcel os3000. Dokładność pomiaru temperatury zamarzania wynosiła 0,002°C, a dla osmolalności 1% [Buniowska-Olejnik i wsp. 2023].
- **Oznaczenie puszystości -** określona za pomocą różnic pomiędzy masą mieszanki przed zamrożeniem oraz masą lodów po procesie zamrażania, wykorzystując cylinder miarowy o objętości 25 cm^3 [Góral i wsp. 2018].
- **Oznaczenie czasu topnienia** – oziębiony metalowy walec napełniano świeżo przygotowanymi lodami i przechowywano 24 godziny w temperaturze -18°C. Czas topnienia mierzono od momentu pojawienia się pierwszej kropli [Góral i wsp. 2018; Kamińska-Dwórznicka i wsp. 2022].
- **Analiza mikroskopowa kryształów lodu** – po przygotowaniu próbki w temperaturze ujemnej, wykonano zdjęcia przy pomocy mikroskopu Olympus model BX43F z systemem chłodzącym ciekłym azotem Linkam Scientific Instruments LTD model LNP96-S oraz kamerą Olympus model SC50. Otrzymany obraz został przeanalizowany za pomocą programu Olympus cellSens Dimension Desktop. Postęp procesu rekrystalizacji został oceniany po 24 godzinach, miesiącu oraz trzech miesiącach przechowywania w temp. -18°C [Kamińska-Dwórznicka i wsp. 2015].
- **Metody statystyczne** – analiza wariancji ANOVA, z wykorzystaniem testu Tukey'a na poziomie istotności $\alpha = 0,05$. Analiza statystyczna została wykonana za pomocą programu Statistica 13.1 (Statstof Polska). Ponadto, rozkład częstotliwości wielkości kryształów w lodach, obliczono za pomocą programu Microsoft Excel 2019, wykorzystując analizę danych w postaci histogramu [Kamińska-Dwórznicka i wsp. 2015, 2022].

WYNIKI – omówienie publikacji stanowiących rozprawę doktorską

7.1 Ocena wpływu iota karagenu oraz jego hydrolizatów kwasowych i enzymatycznych na zmiany w strukturze krystalicznej modelowych roztworów sacharozy

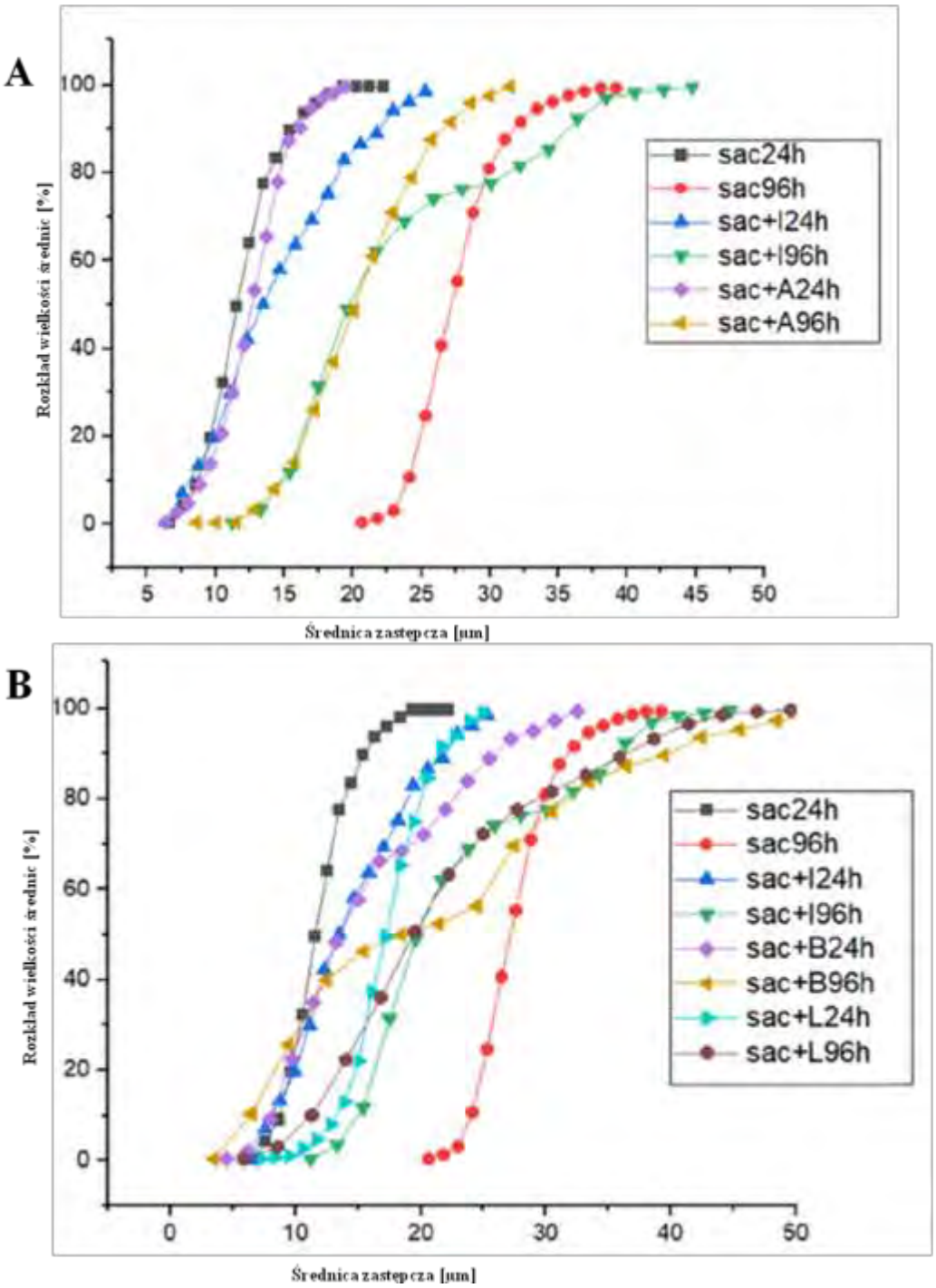
Głównym celem pierwszego etapu badań była ocena wpływu iota karagenu oraz jego hydrolizatów na przebieg procesu rekrystalizacji w roztworach modelowych sacharozy oraz sacharozy z dodatkiem kazeinianu sodu, które miały stanowić model lodów wegańskich oraz analogicznie model lodów mlecznych. Hydrolizaty otrzymano metodą hydrolizy kwasowej (z użyciem kwasu solnego) oraz hydrolizy enzymatycznej (z użyciem enzymu β-galaktozydazy oraz jej bardziej ekonomicznego i łatwiej dostępnego zamiennika – laktazy). Wyniki badań przedstawiono w publikacji **P1 (Effect of ι-carrageenan and its acidic and enzymatic hydrolysates on ice crystal structure changes in model sucrose solution. Colloids and Surfaces A-Physicochemical and Engineering Aspects, 2022, 643, 1-12).**

Badania rozpoczęto od określenia masy cząsteczkowej otrzymanych hydrolizatów, wykorzystując metodę chromatografii cieczowej Size Exclusion Chromatography (Shimadzu) ze wzorcem dekstranu do kalibracji. Z przeprowadzonej analizy wynikało, że hydrolizaty kwasowe po trzygodzinnej hydrolizie charakteryzowały się najniższą masą cząsteczkową $1{,}48 \times 10^6$ Da (Wykres 1A). W porównaniu z masą wyjściową hydrolizatów nastąpiła 24% redukcja masy cząsteczkowej. Masa hydrolizatów enzymatycznych po 72 godzinach hydrolizy z wykorzystaniem enzymu β-galaktozydazy wyniosła $3{,}20 \times 10^6$ Da (Wykres 1B). Po 24 godzinach hydrolizy enzymatycznej z wykorzystaniem laktazy przemysłowej była zbliżona i wyniosła $3{,}50 \times 10^6$ Da (Wykres 1C). Hydroliza enzymatyczna z wykorzystaniem enzymu β-galaktozydazy zredukowała masę cząsteczkową iota karagenu o 16%. Natomiast w przypadku użycia laktazy przemysłowej do hydrolizy enzymatycznej iota karagenu, otrzymano redukcję na poziomie 3%. Na podstawie badań Kamińskiej-Dwórznickiej i wsp. [2016] wykazano, iż przy mniejszej masie hydrolizatów kappa karagenu, efekt inhibicji procesu rekrystalizacji był skuteczniejszy. W oparciu o te badania do dalszych analiz wybrano tylko hydrolizaty po skrajanych czasach hydrolizy (tj. hydrolizat kwasowy po 3 godzinach, hydrolizat enzymatyczny po β-galaktozydazy po 72 godzinach hydrolizy oraz hydrolizat enzymatyczny po laktazie po 24 godzinach hydrolizy), co oznaczało jednocześnie najniższą masę cząsteczkową wybranych stabilizatorów.



Wykres 1. Wyniki analizy SEC po hydrolizie kwasowej (A), po hydrolizie enzymatycznej z użyciem enzymu β-galaktozydazy (B) oraz po hydrolizie enzymatycznej z użyciem laktazy przemysłowej (C).

Kolejnym etapem badań było wykorzystanie iota karagenu oraz wybranych hydrolizatów jako stabilizatorów w badaniu struktury krystalicznej modelowych roztworów sacharozy oraz sacharozy z dodatkiem kazeinianu sodu. Proces rekrystalizacji był oceniany na podstawie uśrednionej wartości średnic kryształów (średnicy zastępczej) ze zdjęć wykonanych po 24, 48, 72 oraz 96 godzinach przechowywania w temperaturze -8°C. Wymierną analizę procesu rekrystalizacji przedstawiono w oparciu o skrajne czasy przechowywania – po 24 i 96 godzinach.



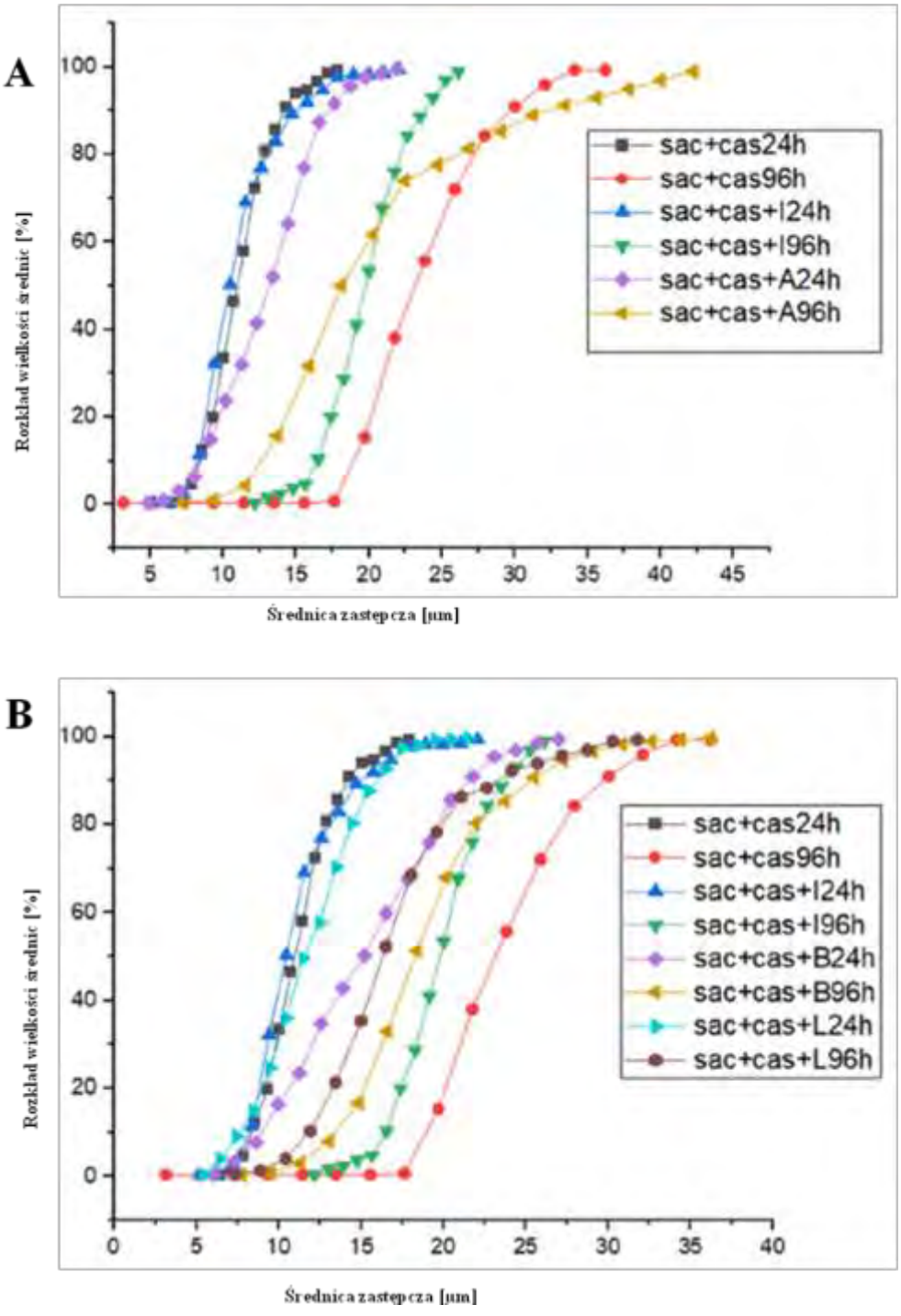
Wykres 2. Wielkości średnic kryształów w modelowych roztworach sacharozy, sacharozy z dodatkiem iota karagenu i hydrolizatami iota karagenu. Wyjaśnienia: sac – roztwór modelowy sacharozy, I – z dodatkiem iota karagenu; A – z dodatkiem hydrolizatu kwasowego po 3h hydrolizy, B – z dodatkiem hydrolizatu enzymatycznego po 72 h hydrolizy enzymem β-galaktozydazą, L – z dodatkiem hydrolizatu enzymatycznego po 24h hydrolizy enzymem laktazą przemysłową.

Analizując roztwory modelowe sacharozy po 24 godzinach przechowywania, wartość parametru X_{50} (wyrażającego uśrednioną wielkość 50% średnic kryształów lodu) nie przekroczyła 18 µm (Wykres 2A i 2B). W przypadku roztworu z dodatkiem iota karagenu

wartość X_{50} utrzymywała się na poziomie 14 μm. Natomiast w przypadku hydrolizatów iota karagenu najmniejszą wartość zanotowano w roztworze sacharozy z dodatkiem hydrolizatu kwasowego. Po 96 godzinach przechowywania średnica kryształów lodu nie przekroczyła 28 μm. Analizując parametr X_{50} (Wykres 2A i 2B), hydrolizaty iota karagenu znacząco wpłynęły na proces rekrystalizacji. Najmniejszą wartość parametru X_{50} stwierdzono w przypadku dodatku hydrolizatu kwasowego (20 μm) oraz hydrolizatów enzymatycznych (21 oraz 22 μm). Z kolei dodatek iota karagenu nie wpłynął znacząco na postęp procesu rekrystalizacji, ponieważ wartość średnicy kryształów była porównywalna z próbką kontrolną.

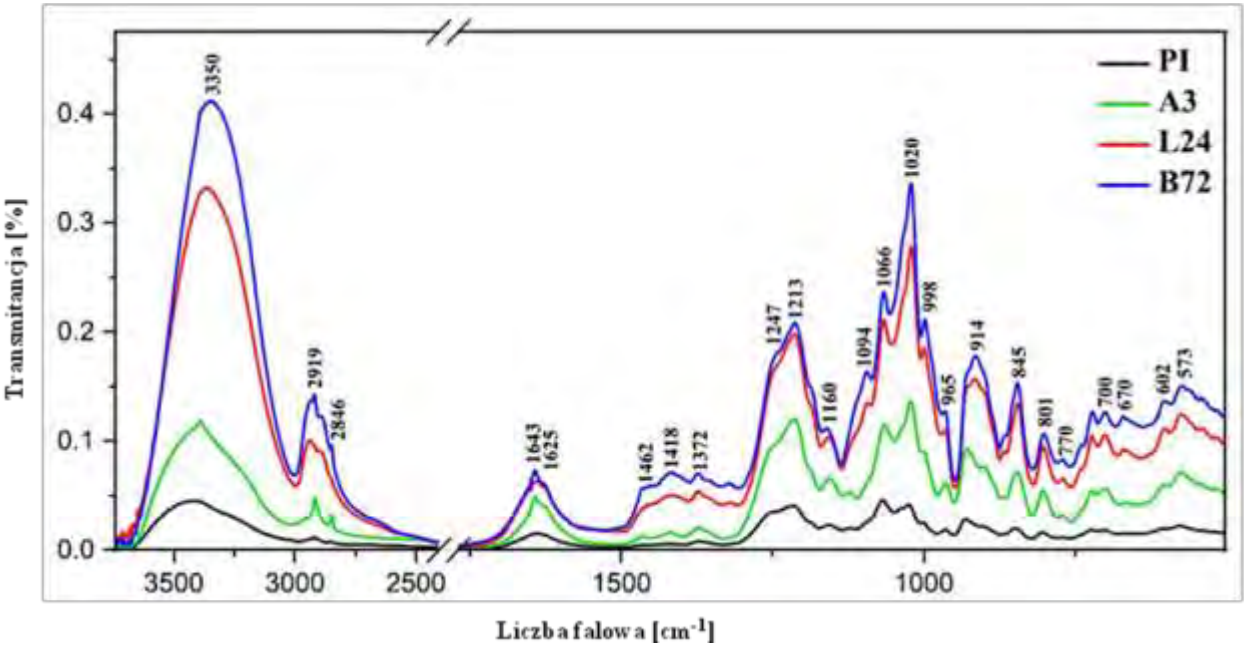
Na podstawie badań Leiter i wsp. [2017] zauważono, iż wzrost jonów sodu po hydrolizie kwasowej kappa karagenu przyczynił się do zmniejszenia aktywności właściwości żelujących tego karagenu. W badaniach Thirmawithana i wsp. [2010] zauważono, iż jony sodu tworzą wiązania jonowe z karagenami w przeciwieństwie do jonów wapnia czy potasu, które znacząco wpływają na właściwości karagenów. W związku z powyższym, również w prezentowanej pracy, możliwa obecność jonów sodu po hydrolizie kwasowej nie zmniejszyła aktywności inhibitującej procesu rekrystalizacji (IRI) hydrolizatu kwasowego.

W roztworach modelowych sacharozy z dodatkiem kazeinianu sodu po 24 godzinach przechowywania wartość parametru X_{50} nie przekroczyła 15 μm (Wykres 3A i 3B). Po dodaniu czystego iota karagenu wyniosła 11 μm. W przypadku wykorzystanych enzymatycznych hydrolizatów iota karagenu wartość parametru X_{50} określono w zakresie od 12 do 15 μm, zaś hydrolizatu kwasowego – około 13 μm. Co warte podkreślenia w próbach dla modelowych z dodatkiem kazeinianu sodu dodatek samego iota karagenu również skutecznie ograniczał postęp procesu rekrystalizacji (X_{50} około 11 μm). Po upływie 96-godzinnego przechowywania roztworów modelowych średnia wartość wyznaczonych średnic kryształów lodu nie przekroczyła 23 μm, zaś najskuteczniejszym inhibitorem okazał się być hydrolizat enzymatyczny iota karagenu po hydrolizie preparatem laktazy (Wykres 3B), a wartość parametru X_{50} nie przekroczyła 18 μm. Najmniej skutecznym efektem w inhibitowaniu procesu rekrystalizacji charakteryzował się dodatek hydrolizatu kwasowego iota karagenu, gdyż parametr X_{50} przekroczył wartość 21 μm. Na podstawie otrzymanych wyników można przypuszczać, iż to nie masa molekularna ma znaczący wpływ na proces inhibitowania rekrystalizacji, lecz struktura oraz mechanizm działania zastosowanych hydrolizatów. Przedstawione wyniki potwierdzają również hipotezy postawione w pracy Kiran-Yildirim i wsp. [2021], stwierdzające, iż ilość, pozycja grup sulfonowych karagenów oraz ich hydrolizatów ma istotny wpływ na inhibicję procesu rekrystalizacji (IRI).



Wykres 3. Wielkości średnic kryształów w modelowych roztworach sacharozy z kazeinianem sodu oraz sacharozy z kazeinianem sodu z dodatkiem iota karagenu i hydrolizatami iota karagenu. Wyjaśnienia: sac+cas – roztwór modelowy sacharozy z dodatkiem kazeinianu sodu; I – z dodatkiem iota-karagenu; A – z dodatkiem hydrolizatu kwasowego po 3h hydrolizy, B – z dodatkiem hydrolizatu enzymatycznego po 72 h hydrolizy enzymem β-galaktozydazą, L – z dodatkiem hydrolizatu enzymatycznego po 24h hydrolizy enzymem laktazą przemysłową.

Ostania analiza dotyczyła wykazania różnic w budowie polisacharydowego łańcucha otrzymanych hydrolizatów za pomocą metody spektroskopii fourierowskiej (FTIR) (Wykres 4). Na podstawie otrzymanych widm wykazano różnice pomiędzy pasmami absorpcji, co może warunkować odmienny wpływ hydrolizatów na inhibitowanie procesu rekrystalizacji. Zauważalne wibracje wystąpiły przy maksimum 3350 cm^{-1}, które odpowiadają grupie –OH. Ponadto występowały charakterystyczne drgania dla struktury iota karagenu przy długości fali 2919, 2886 cm^{-1} co odpowiadało grupie –CH oraz wibracje przy grupie O=S=O, przy długości 1213 cm^{-1}, które również potwierdzono w badaniach Moniha i wsp. [2018]. Biorąc pod uwagę różnicę pomiędzy hydrolizatami po hydrolizie enzymatycznej, zaobserwowano niewielkie przesunięcie w obszarze grupy –OH w próbce B72 (hydrolizat enzymatyczny iota karagenu po 72-godzinnej hydrolizie β-galaktozydazą) oraz L24 (hydrolizat enzymatyczny iota karagenu po 24-godzinnej hydrolizie laktazą przemysłową) (Wykres 4) w porównaniu do czystego iota karagenu. Mogło to wskazywać na obniżenie wibracji w tym zakresie i jednocześnie spadek ich mobilności. W przypadku hydrolizatu kwasowego A3 (hydrolizat kwasowy iota karagenu po 3-godzinnej hydrolizie) wspomniane przesunięcie jest widocznie, lecz zdecydowanie mniej niż w przypadku hydrolizatów enzymatycznych. Ogólnie otrzymane hydrolizaty cechowały się mniejszymi wibracjami w zakresie od 1213 i 914 cm^{-1}, co może wskazywać na ograniczoną (w stosunku do próby kontrolnej) obecność grup sulfonowych w łańcuchu hydrolizatów oraz ich lepszą elastyczność. Ponadto hydroliza enzymatyczna w porównaniu do hydrolizy kwasowej inaczej oddziaływała na łańcuch iota karagenu, ze względu na różnicę w zakresie 1020 i 1063 cm^{-1} oraz poniżej 980 cm^{-1}. Finalnie można zauważyć, iż przebieg widm hydrolizatów enzymatycznych był podobny, co oznacza, że takie hydrolizaty mogą być stosowane zamiennie. Ze względów ekonomicznych wykorzystanie tańszego oraz łatwiej dostępnego enzymu, jakim jest laktaza, wydaje się bardziej korzystne. Otrzymane różnice widm oraz efektywność oddziaływania hydrolizatów na inhibitowanie procesu rekrystalizacji mogą wskazywać, iż struktura hydrolizatów oraz ich grupy funkcyjne powinny być tematem dalszych badań w zakresie analizy mechanizmu działania stabilizatorów na strukturę krystaliczną lodów i że to właśnie budowa polisacharydów, a nie ich masa cząsteczkowa, ma znaczenie przy modyfikacji ich właściwości stabilizujących.



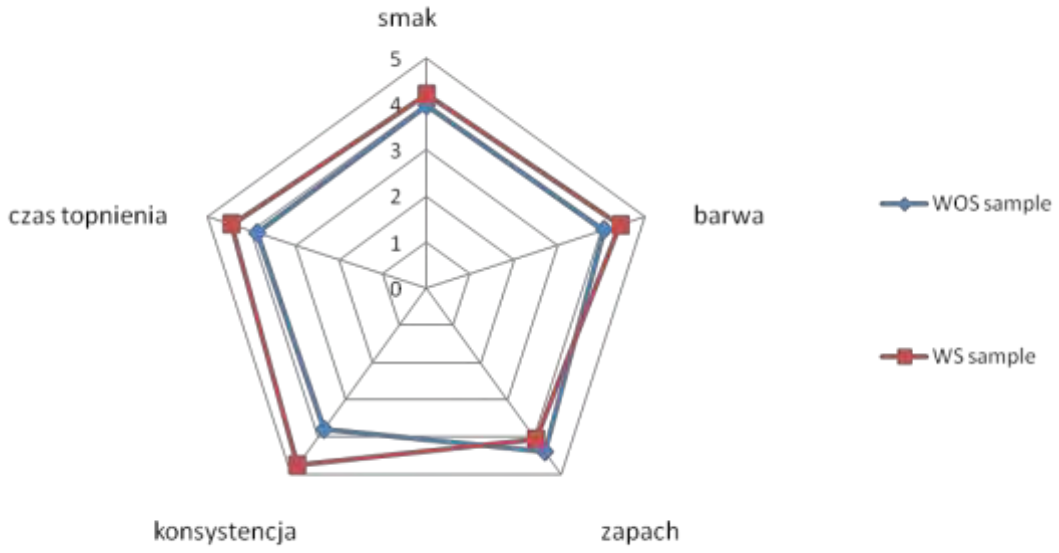
Wykres 4. Widma w podczerwieni FTIR w zakresie widmowym: 500–3740 cm^{-1} dla badanych próbek. Wyjaśnienia: odpowiednio: PI (iota karagen), A3 (hydrolizat kwasowy iota karagenu po 3 h hydrolizy), L24 (hydrolizat enzymatyczny iota karagenu po 24-godzinnej hydrolizie laktazą przemysłową), B72 (hydrolizat enzymatyczny iota karagenu po 72-godzinnej hydrolizie β-galaktozydazą).

7.2 Badania nad właściwościami lodów wegańskich na bazie napoju migdałowego

Badania mieszanek lodowych oraz lodów wegańskich rozpoczęto od opracowania receptury oraz doboru układu stabilizującego. Ponadto została podjęta próba produkcji lodów wegańskich w oparciu o tę samą metodykę, jak przy produkcji lodów mlecznych. Wyniki badań przedstawiono w publikacji **P3 (Study on the properties of vegan ice cream based on almond drink. Zeszyty Problemowe Postępów Nauk Rolniczych, 2020, 600, 21-30).**

Publikacja **P3** rozpoczyna etap badań, dotyczący oceny właściwości fizycznych lodów wegańskich, bez oraz z dodatkiem układu stabilizującego, tj. mączki chleba świętojańskiego oraz gumy ksantanowej. W tej publikacji skupiono się na ocenie takich parametrów jak pH, gęstość mieszanek lodowych oraz czas topnienia, puszystość, struktura krystaliczna i ocena sensoryczna lodów. Ustalono procentowe udziały zastosowanych składników w recepturze lodów wegańskich, tj. inuliny, białka grochu, napoju migdałowego, syropu migdałowego, emulgatora oraz układu stabilizującego. W oparciu o otrzymane wyniki stwierdzono, iż zastosowany dodatek układu stabilizującego (mączka chleba świętojańskiego oraz guma

ksantanowa) nie wpłynął znacząco na właściwości fizyczne mieszanek lodowych. Pomiar pH oraz gęstości mieszanek lodowych wskazywały na brak wpływu zastosowanych stabilizatorów na powyższe parametry. Tylko w przypadku puszystości lodów (26,56%) zanotowano zmniejszenie w porównaniu z próbką kontrolną, bez dodatku stabilizatorów (32,02%). Mniejsza puszystość lodów wegańskich wynikała z braku białek mleka, które są zwykle prekursorami wyższej puszystości lodów. Dodatek stabilizatorów wydłużył czas topnienia do 57 minut (próbka kontrolna 43 minut), głównie ze względu na zdolność stabilizatorów do wiązania wody [BahramParvar i wsp. 2013]. Została również zbadana wielkość kryształów lodu, które są ważnym determinantem jakości lodów spożywczych. Po 24-godzinnym przechowywaniu średnica zastępcza kryształów nie przekroczyła 18 µm w próbce z dodatkiem stabilizatorów. Natomiast w próbce kontrolnej, bez dodatku stabilizatorów, średnica osiągnęła wartość 52 µm. Na podstawie literatury, pożądana a jednocześnie akceptowalna przez konsumentów średnica kryształów, powinna mieścić się w zakresie 10-20 µm [Kamińska-Dwórznicka i wsp. 2015]. W związku z czym zastosowany układ stabilizujący korzystnie wpłynął na ograniczenie procesu rekrystalizacji, podnosząc jednocześnie walory sensoryczne produktu. Wyniki oceny sensorycznej wskazywały na większą pożądalność lodów z dodatkiem stabilizatorów (Wykres 5).



Wykres 5. Wyniki analizy sensorycznej. Wyjaśnienia: WOS sample – lody bez dodatku stabilizatorów; WS sample – lody z dodatkiem stabilizatorów (mączka chleba świętojańskiego, guma ksantanowa).

7.3 Ocena wpływu różnych składników oraz stabilizatorów na właściwości fizyczne wegańskich mieszanek lodowych na bazie napoju migdałowego

Celem publikacji **P4 (Effect of different ingredients and stabilisers on properties of mixes based on almond drink for vegan ice cream production. Sustainability, 2021, 13(21), 1-17)** była ocena właściwości fizycznych wegańskich mieszanek lodowych. Badanie dotyczyło wpływu homogenizacji mechanicznej oraz wybranych stabilizatorów na właściwości mieszanek przed i po procesie dojrzewania. W przypadku metody homogenizacji zastosowano czas 2,5 minuty przy obrotach homogenizatora 20obr/min. Natomiast stabilizatory stosowane do przygotowania mieszanek to iota karagen oraz jego hydrolizat kwasowy lub hydrolizaty enzymatyczne (szerzej opisane w pierwszym etapie badań, publikacja **P1**) (Tabela 2 oraz 3). Po otrzymaniu mieszanek poddano je procesowi homogenizacji, a następnie przeprowadzono następujące oznaczenia: pomiar gęstości, stabilność emulsji, wielkość cząstek, ocenę właściwości reologicznych oraz analizę struktury. Ponadto, aby ocenić skuteczność procesu dojrzewania, oznaczenia powtórzono również po 24-godzinnym dojrzewaniu w temperaturze 4°C.

Na podstawie pomiaru gęstości mieszanek lodowych wykazano, iż zarówno proces homogenizacji, jak i użyte stabilizatory, nie wpływały znacząco na ten parametr. Nie zaobserwowano różnic pomiędzy próbkami. Ponadto po czasie dojrzewania wartość gęstości mieszanek nie zmieniła się w porównaniu do wyniku bezpośrednio po wytworzeniu. Otrzymany wynik mógł wskazywać, iż dojrzewanie wpływało na utrzymanie stabilności emulsji i nie wpływało na powstawanie niepożądanych zmian.

Pomiar stabilności emulsji został oceniony na podstawie wartości indeksu stabilności TSI (Turbiscan Stability Index). Wartość TSI wahała się od 1,7 do 6,5. Najmniejsze wartości, a tym samym większą stabilność, zanotowano w przypadku próbek niepoddanych procesowi homogenizacji, tylko z dodatkiem stabilizatorów, oraz próbki kontrolnej (Tabela 3 oraz 4). Powodem mogła być obecność pęcherzyków powietrza, które w trakcie przechowywania zmieniły swoją lokalizację lub pękały, wpływając na zaburzenie stabilności. Na podstawie wykresów rozproszenia wstecznego światła (Back Scatettering – zamieszczonych w publikacji **P4**) wykazano, że w mieszankach lodowych z dodatkiem stabilizatorów, bez względu na zastosowanie lub brak homogenizacji, wystąpił proces koalescencji. Było to spowodowane tym, iż kuleczki tłuszczowe zwiększały swoje rozmiary w trakcie przechowywania. W przypadku próbki bez dodatku stabilizatorów wystąpiły inne rodzaje destabilizacji emulsji, tj. śmietankowanie oraz sedymentacja. Prawdopodobnie brak

stabilizatorów przyczynił się do pojawienia przestrzeni pomiędzy cząstkami, co umożliwiło przemieszczanie się kuleczek tłuszczowych [Voronin i wsp. 2020]. Ponadto zwykle destabilizowany tłuszcz ma większe zdolności do śmietankowania. Na podstawie badań Berger i White [1971] oraz Berger i wsp. [1972] stwierdzono, że emulsja lodowa jest takim rodzajem emulsji, który może być zaprojektowany do destabilizacji, ponieważ poziom destabilizacji tłuszczu może pozytywnie wpływać na odczucia sensoryczne lodów w trakcie konsumpcji, a w szczególności na ich kremową konsystencję.

Tabela 4. Właściwości fizyczne wegańskich mieszanek lodowych.

Próbka	TSI	D_{50} przed dojrzewaniem	D_{50} po dojrzewaniu
CWH	2,1	$15,65\pm0,62^{c}$	$17,23\pm0,49^{d}$
CH	3,0	$20,54\pm0,45^{d}$	$11,35\pm0,48^{b}$
IWH	2,3	$30,40\pm0,44^{e}$	$28,50\pm0,43^{e}$
IH	4,1	$13,77\pm0,19^{b}$	$11,51\pm0,94^{b}$
AWH	2,1	$38,36\pm0,49^{f}$	$11,41\pm0,97^{b}$
AH	6,4	$13,06\pm0,53^{a}$	$6,33\pm0,12^{a}$
BWH	3,6	$42,15\pm0,19^{g}$	$11,29\pm0,53^{b}$
BH	6,5	$13,42\pm0,40^{b}$	$6,88\pm0,33^{a}$
LWH	1,7	$38,31\pm0,66^{f}$	$13,20\pm0,45^{c}$
LH	5,2	$11,91\pm0,16^{a}$	$6,37\pm0,48^{a}$

Wyjaśnienia: WH – bez homogenizacji; H – z homogenizacją; C – mieszanka bez stabilizatorów; I – mieszanka z iota karagenem; A – mieszanka z hydrolizatem kwasowym; B – mieszanka z hydrolizatem enzymatycznym po β-galaktozydazie; L – mieszanka z hydrolizatem enzymatycznym po laktazie przemysłowej; TSI – Turbiscan Stability Index; D_{50} – mediana. Reprezentatywne dane ± odchylenie standardowe, średnie oznaczone tą samą małą literą oznaczają grupy homogeniczne nieróżniące się przy poziomie istotności $\alpha = 0,05$.

Pomiar wielkości cząstek kuleczek tłuszczowych został oceniony na podstawie mediany D_{50} oraz rozkładu wielkości cząstek. Mediana cząstek przed procesem dojrzewania mieściła się w zakresie od 11,91 do 42,15 μm. Najniższe wartości mediany przed procesem dojrzewania, zanotowano w przypadku próbek po procesie homogenizacji oraz próbek z dodatkiem stabilizatorów (Tabela 3 oraz 4). Próbki niepoddane procesowi homogenizacji

charakteryzowały się zdecydowanie większymi wielkościami cząstek. Również w badaniach Innocente i wsp. [2009] lub Biasutti i wsp. [2013], w których wykorzystywano metodę homogenizacji wysokociśnieniowej (HPH) w mieszankach lodowych, zanotowano zmniejszenie wielkości cząstek w próbkach poddanych temu procesowi. Stwierdzono jednak, że próbka kontrolna po procesie homogenizacji, jak i bez, miała mniejsze wartości w porównaniu do próbek ze stabilizatorami (CWH oraz CH, Tabela 4). Po procesie dojrzewania we wszystkich próbkach nastąpiła redukcja wielkości cząstek, ponieważ jej zakres wyniósł od 6,33 do 28,50 μm. Największą wielkość cząstek zanotowano w przypadku próbki z dodatkiem iota karagenu zarówno z, jak i bez homogenizacji. Mogło to być wynikiem mniejszej plastyczności iota karagenu niż jego hydrolizatów, które w trakcie dojrzewania wpłynęły na polepszenie struktury emulsji (wyniki FTIR, omówione w pierwszym etapie badań – publikacja **P1**).

Analizując rozkład wielkości cząstek w próbkach bezpośrednio po wytworzeniu zaobserwowano, że wystąpił dwu- lub trimodalny rozkład, zaś po procesie dojrzewania trimodalny rozkład wielkości cząstek w badanych mieszankach lodowych. Pierwszy pik pojawił się w zakresie 7-8 μm, co świadczyło o małych rozmiarach kuleczek tłuszczowych, drugi pik w przedziale 30-40 lub 60-70 μm, co mogło być wynikiem obecności aglomeratów tłuszczu oraz jednocześnie jego destabilizacji. Wielkość największych cząstek mieściła się w zakresie 70-80 μm, co świadczyło o obecności aglomeratów kuleczek tłuszczowych lub wpływu mono- i diglicerydów kwasów tłuszczowych, które zostały użyte jako emulgator w recepturze mieszanek lodowych. Na przykładzie badań przeprowadzonych przez Mendez-Velasco i Goff [2012] potwierdzono, iż obecność tych nienasyconych monoglicerydów kwasów tłuszczowych może przyczyniać się do formowania większych i bardziej stabilnych cząstek tłuszczu, podczas gdy nasycone monoglicerydy kwasów tłuszczowych mogą oddziaływać na formowanie w lodach mniejszych kuleczek tłuszczowych.

Właściwości reologiczne mieszanek lodowych analizowano na podstawie modelu Herschel-Bulkley, który został dobrany spośród innych modeli matematycznych ze względu na najlepiej dopasowane wartości R oraz Chi^2. Parametry, które zostały wzięte pod uwagę, to indeks płynięcia, współczynnik konsystencji oraz lepkość pozorna. Na podstawie badań stwierdzono, iż wszystkie mieszanki lodowe charakteryzowały się pseudoplastycznym (nieniutoniskim charakterem), ponieważ indeks płynięcia wszystkich przygotowanych próbek był mniejszy niż 1 [Mostafavi i wsp. 2017]. Najniższą wartość indeksu płynięcia zaobserwowano w próbkach z dodatkiem iota karagenu, bez względu na stosowanie homogenizacji (IWH 0,633-0,653 oraz IH 0,742-0,757). Ponadto w próbkach poddanych

homogenizacji (BH oraz LH) zauważono niższe wartości indeksu płynięcia niż w próbkach z tymi samymi stabilizatorami, lecz bez homogenizacji (LWH oraz BWH). Było to dowodem, iż rodzaj stabilizatora mógł znacząco oddziaływać na zachowanie się cieczy, co mogło być powiązane z jego zdolnością do wiązania wody. W przypadku współczynnika konsystencji (K) oraz lepkości pozornej (τ_0) zaobserwowano ich wzrost po dodaniu stabilizatorów. Mieszanka lodowa z dodatkiem iota karagenu (IH oraz IWH) cechowała się największą wartością współczynnika konsystencji (255,6-462,3 m Pas^n). Po czasie dojrzewania mieszanki odnotowano wyższe wartości współczynnika K, co było wynikiem polepszenia właściwości wiązania cząsteczek wody, tłuszczu oraz białek [Dogan i Kayacier 2007]. W przypadku naprężenia ścinającego również mieszanka z dodatkiem iota karagenu (IH, IWH) charakteryzowała się najniższymi wartościami, co umożliwiło temu stabilizatorowi utworzenie stabilnej i solidnej struktury emulsyjnej.

Analizując strukturę mikroskopową, można było zaobserwować obecność pęcherzyków powietrza, które również były odpowiedzialne za destabilizację układu emulsyjnego w mieszankach lodowych. Redukcja wielkości kuleczek tłuszczowych została potwierdzona przez analizę Cilas (pomiar wielkości cząstek). Z drugiej strony obecność aglomeratów tłuszczu, również widoczna na zdjęciach (umieszczonych w publikacji **P4**), była efektem koalescencji, śmietankowania oraz sedymentacji. Natomiast nie zauważono jednolitości w strukturze prezentowanych mieszanek zarówno przed, jak i po procesie dojrzewania.

7.4 Badania nad wpływem homogenizacji ultradźwiękowej na właściwości fizyczne wegańskich mieszanek lodowych

Celem kolejnych badań była ocena wpływu homogenizacji ultradźwiękowej na właściwości fizyczne mieszanek lodowych. W przypadku metody homogenizacji ultradźwiękowej zastosowano czas 5 minut przy częstotliwości 20 kHz. Zastosowane parametry homogenizacji ultradźwiękowej, ustalono na podstawie aktualnej literatury [O'Sullivan i wsp. 2016b; da Silva i wsp. 2019] oraz własnych badań wstępnych. Natomiast stabilizatory wykorzystane do przygotowania mieszanek to iota karagen oraz jego hydrolizat kwasowy lub hydrolizaty enzymatyczne (Publikacja **P1**) (Tabela 2 oraz 3). Po otrzymaniu mieszanek lodowych poddano je procesowi homogenizacji ultradźwiękowej, a następnie przeprowadzono oznaczenia: stabilności emulsji, wielkości cząstek, oceny właściwości reologicznych oraz analizy struktury. Ponadto oceniono wpływ procesu dojrzewania,

powtarzając wymienione oznaczenia przed oraz po 24-godzinnym dojrzewaniu w temperaturze 4°C. Wyniki badań przedstawiono w publikacji **P5 (Study on the influence of ultrasound homogenisation on the physical properties of vegan ice cream mixes. Applied Sciences, 2022, 12(17), 8492).**

Analizując właściwości fizyczne mieszanek lodowych, wykazano, iż wartości indeksu stabilności TSI (Turbiscan Stability Index) mieściły się w zakresie od 1,7 do 4,2. Wpływ homogenizacji ultradźwiękowej przyczynił się do zmniejszenia stabilności mieszanek lodowych, w porównaniu do próbek tylko z dodatkiem stabilizatorów. Najlepszą stabilnością w trakcie dojrzewania cechowała się mieszanka z dodatkiem enzymatycznego hydrolizatu iota karagenu po laktazie przemysłowej (L), a najmniejszą stabilnością próbka z dodatkiem hydrolizatu enzymatycznego iota karagenu po β-galaktozydazie (B) (Tabela 3 oraz 5). Pomimo iż we wspomnianych wcześniej badaniach zostały potwierdzone niewielkie różnice w budowie tych hydrolizatów, w przypadku stabilności zdecydowanie lepiej sprawdziłby się pierwszy wspomniany hydrolizat. Odnosząc się do wcześniej opisanej publikacji (**P4**), homogenizacja mechaniczna zmniejszyła stabilność emulsji w większym stopniu niż homogenizacja ultradźwiękowa. W badaniach O'Sullivan i wsp. [2015] również wykazano, iż homogenizacja ultradźwiękowa przyczyniła się do poprawy właściwości stabilizujących emulsji na bazie białka grochu. Poprawa stabilności emulsji w wyniku działania kawitacji akustycznej mogła być wynikiem zmian w warstwie międzyfazowej w emulsji. Ponadto wpływ działania ultradźwięków na stabilność był również uzależniony od czasu ich działania oraz metody emulgowania. Analizując wykresy rozproszenia wstecznego (Back Scatettering – zamieszczonych w publikacji **P5**), zanotowano dwa rodzaje destabilizacji – sedymentację oraz koalescencję. Sedymentacja pojawiła się w próbce bez stabilizatorów, natomiast w przypadku dodatku stabilizatorów wystąpiła koalescencja.

Na podstawie analizy mediany D_{50} po procesie dojrzewania określono przedział od 9,76 do 28,50 μm, przy czym najmniejsze wartości zauważono w próbkach z dodatkiem hydrolizatu enzymatycznego iota karagenu po β-galaktozydazie oraz po zastosowaniu ultradźwięków (BU). W próbkach, w których zastosowano jednocześnie stabilizatory oraz homogenizację ultradźwiękową, mediana D_{50} była mniejsza niż w próbkach bez homogenizacji. Po procesie dojrzewania zaobserwowano zmniejszenie wielkości cząstek. W mieszankach lodowych, które były poddane działaniu ultradźwięków, efekt redukcji wielkości cząstek po dojrzewaniu był bardziej widoczny niż w próbkach z dodatkiem stabilizatorów, bez obróbki ultradźwiękowej. Przykładowo, w mieszance B oraz BU, po procesie dojrzewania wielkość cząstek wynosiła odpowiednio 11,29 oraz 9,76 μm (Tabela 3

oraz 5). Ponadto dowodem na skuteczność działania ultradźwięków jest chociażby wynik prób kontrolnych (bez dodatku stabilizatorów), w których wystąpiły różnice istotne statystycznie. W porównaniu do działania homogenizacji mechanicznej mieszanek lodowych (omówionej w publikacji **P4**), homogenizacja ultradźwiękowa nie zredukowała wielkości kuleczek tłuszczowych tak skutecznie jak mechaniczna. Z drugiej strony rozmiar wielkości kuleczek nie odgrywa tak znaczącej roli w przypadku lodów, jak wielkość kryształów lodu po zamrożeniu [Firouz 2021]. Dlatego też w przypadku mieszanek lodowych większą uwagę skupia się na stabilności oraz jednolitości struktury emulsji.

Tabela 5. Właściwości fizyczne mieszanek lodowych.

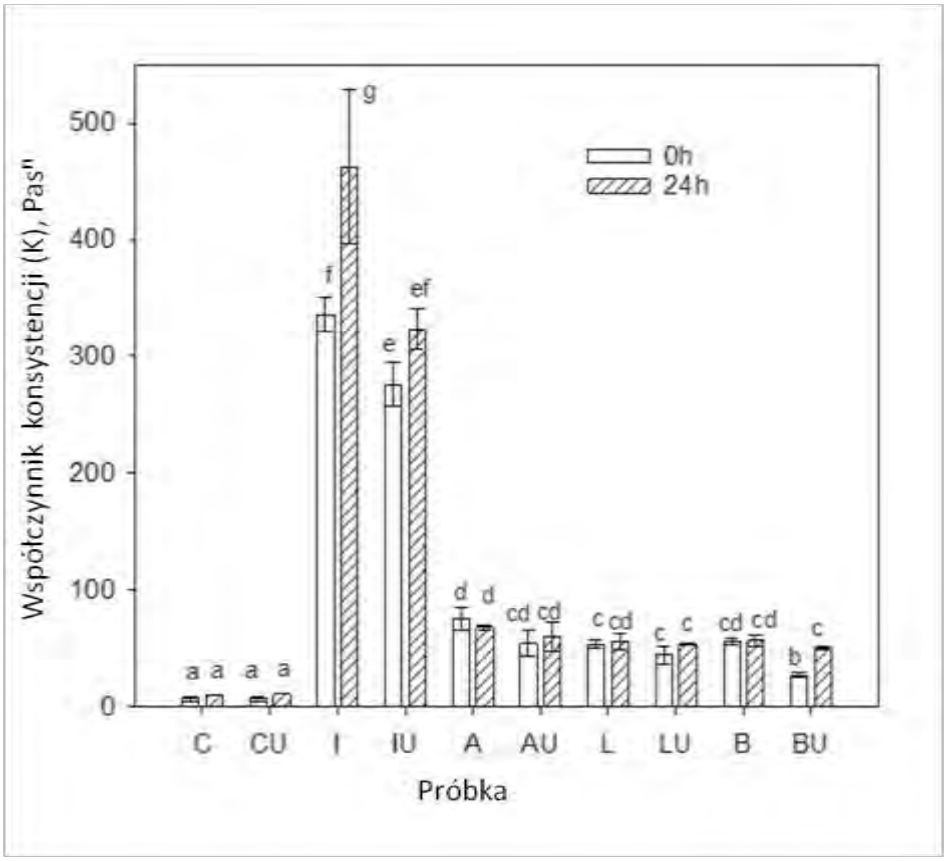
Próbka	TSI	D_{50} przed dojrzewaniem	D_{50} po dojrzewaniu
C	2,1	$15,65\pm0,62^{a}$	$17,23\pm0,49^{c}$
CU	2,7	$14,68\pm0,17^{a}$	$9,97\pm0,23^{a}$
I	2,3	$30,40\pm0,44^{e}$	$28,50\pm0,43^{e}$
IU	3,9	$28,70\pm0,16^{d}$	$23,73\pm0,62^{d}$
A	2,1	$38,36\pm0,49^{c}$	$11,41\pm0,97^{ab}$
AU	3,3	$35,70\pm0,50^{b}$	$11,24\pm1,41^{a}$
B	3,6	$42,15\pm0,19^{g}$	$11,29\pm0,53^{ab}$
BU	4,2	$34,29\pm0,14^{f}$	$9,76\pm0,36^{a}$
L	1,7	$38,31\pm0,66^{c}$	$13,20\pm0,45^{b}$
LU	2,2	$36,92\pm0,56^{b}$	$10,59\pm0,09^{a}$

Wyjaśnienia: U – homogenizacja ultradźwiękowa, C – próbka kontrolna, I – iota karagen, A – hydrolizat po hydrolizie kwasowej, B – hydrolizat po hydrolizie enzymatycznej β-galaktozydazą, L – hydrolizat po hydrolizie enzymatycznej laktazą przemysłową. TSI – Turbiscan Stability Index; D_{50} – mediana. Reprezentatywne dane ± odchylenie standardowe, średnie oznaczone tą samą małą literą oznaczają grupy homogeniczne nieróżniące się przy poziomie istotności $\alpha = 0,05$.

Na podstawie rozkładu wielkości cząstek przed procesem dojrzewania zaobserwowano tri- lub czteromodalny rozkład. Po procesie maturacji (dojrzewania)

wystąpiły cztery charakterystyczne piki, które świadczyły o czteromodalnym rozkładzie wielkości cząstek. Wielkość małych kuleczek tłuszczowych została oceniona w zakresie 7-8 μm, drugi pik w przedziale 30-40 lub 60-70 μm, co było to wynikiem obecności aglomeratów tłuszczu, zaś przedział 70-80 μm to również aglomeraty kuleczek tłuszczowych lub mono- i diglicerydy kwasów tłuszczowych, które zostały użyte jako stabilizator w recepturze mieszanek lodowych (co zostało już wspomniane przy omawianiu publikacji **P4**, na podstawie badań Mendez-Velasco i Goff [2012]).

Analiza reologiczna, określona na podstawie modelu Herschley-Bulkley umożliwiła ocenę takich parametrów jak współczynnik konsystencji, naprężenie ścinające oraz indeks płynięcia. Na podstawie wartości indeksu płynięcia ($n < 1$) wszystkie otrzymane mieszanki lodowe można było zaliczyć do cieczy nieniutonowskich, rozrzedzanych ścinaniem. Analizując współczynnik konsystencji (Wykres 6), zauważono, iż próbki z dodatkiem iota karagenu charakteryzowały się prawie 3- a nawet 4-krotnie większymi wartościami współczynnika konsystencji, od pozostałych próbek. Również po procesie dojrzewania mieszanka z iota karagenem cechowała się większymi wartościami współczynnika konsystencji (K) oraz naprężeniem ścinającym (około 100 mPas), co mogło wskazywać na mniejszą homogeniczność próbki. Potwierdził to również rozkład wielkości cząstek, który w przypadku próbki z iota karagenem odbiegał od pozostałych wariantów mieszanek. Natomiast ogólnie można wnioskować, iż wpływ ultradźwięków przyczynił się do zmniejszenia współczynnika konsystencji w mieszankach lodowych. Ponadto naprężenie ścinające mieszanek lodowych znacząco zwiększyło się po dodaniu stabilizatorów, w szczególności iota karagenu, co mogło wpływać na osiągnięcie stabilniejszej struktury emulsji [Rao 2007].



Wykres 6. Współczynnik konsystencji mieszanek lodowych. Wyjaśnienia: U – homogenizacja ultradźwiękowa, C – próbka kontrolna, I – iota karagen, A - hydrolizat po hydrolizie kwasowej, B – hydrolizat po hydrolizie enzymatycznej β-galaktozydazą, L – hydrolizat po hydrolizie enzymatycznej laktazą przemysłową.

Analizując zdjęcia mikroskopowe mieszanek lodowych przed i po procesie dojrzewania, zauważono nieregularne rozmieszczenie kuleczek tłuszczowych oraz ich aglomeraty. Również w badaniach Voronin i wsp. [2020] potwierdzono obecność aglomeratów kuleczek tłuszczowych, co wynikało z destabilizacji mlecznych mieszanek lodowych. Ponadto potwierdzono, iż próbka bez dodatku stabilizatorów odbiegała wizualnie od próbek z zastosowanym układem stabilizującym, co wykazała również analiza TSI oraz pomiar wielkości cząstek (inny rodzaj destabilizacji oraz większe kuleczki tłuszczowe). Analiza reologiczna również pokazała, iż mieszanki lodowe przypominają bardziej ciecze niutonowskie z większą stabilnością niż próbki z dodatkiem stabilizatorów. Dodatkowo proces dojrzewania przyczynił się do zmian właściwości mieszanek lodowych, czyli redukcji wielkości cząstek oraz pojawienia się destabilizacji. Uwzględniając wpływ metody homogenizacji ultradźwiękowej, zauważono większą redukcję wielkości cząstek po procesie dojrzewania, ale również i destabilizację.

7.5 Ocena wpływu homogenizacji ultradźwiękowej na proces rekrystalizacji w trakcie przechowywania lodów wegańskich

W publikacji **P6 (The influence of ultrasound homogenization on recrystallization during storage of vegan ice cream. Journal of Food Process Engineering, e14472)** zaprezentowano badania, które były podsumowaniem prowadzonych prac nad recepturą wegańskich mieszanek lodowych, ze szczególnym uwzględnieniem dodatku stabilizatorów oraz modyfikacji na poziomie technologicznym (Tabela 2 oraz 3). W pracy przebadano właściwości fizyczne lodów wegańskich, tj. temperaturę krioskopową, ciśnienie osmotyczne, puszystość, czas topnienia oraz strukturę krystaliczną po trzech etapach przechowywania w stałej temperaturze -18°C (24 godziny, 1 miesiąc oraz 3 miesiące).

Temperatura krioskopowa mieściła się w zakresie od -1,918 do -1,757°C (Tabela 6). Na podstawie analizy statystycznej nie wykazano różnic pomiędzy próbkami lodów zarówno pod względem stosowanych dodatków stabilizujących, jak i metody homogenizacji. Porównując wyniki pomiędzy dwoma metodami homogenizacji, zauważono nieznaczny wzrost temperatury po zastosowaniu homogenizacji ultradźwiękowej. Na podstawie źródeł literaturowych [Chow i wsp. 2005; Cheng i wsp. 2015a] kawitacja akustyczna wytwarzana w trakcie działania ultradźwięków nie tylko może powodować przyspieszenie przepływu masy i energii, ale również może zwiększać temperaturę krioskopową zarodkowania kryształów w trakcie zamrażania. W związku z czym otrzymany wynik w prezentowanej pracy jest kolejnym dowodem, iż ultradźwięki nawet już na etapie homogenizacji zwiększały temperaturę krioskopową mieszanki lodowej, a w konsekwencji prowadziło to do przyspieszania zarodkowania kryształów i otrzymania znacząco mniejszych ich rozmiarów. Dodatkowo niewielkie różnice w wynikach pomiędzy stabilizatorami mogły wynikać z założenia, iż stabilizatory wpływają na punkt zamrażania lodów. W oparciu o dane literaturowe [Tecson i wsp. 2021] wykazano chociażby, iż karageny z większą masą cząsteczkową były mniej odporne na działanie kawitacji akustycznej niż krótsze polimerowe łańcuchy hydrolizatów.

Tabela 6. Właściwości fizyczne wegańskich lodów spożywczych.

Próbka	Temperatura krioskopowa, °C	Ciśnienie osmotyczne, mOsm/kg	Czas topnienia, min.	Puszystość, %
C	-1,852±0,042^{a}	997±4^{efg}	26,24±0,14^{bcde}	11,44±0,30^{ab}
CH	-1,861±0,012^{a}	1002±6^{fg}	24,38±1,50^{bcd}	12,06±0,86^{ab}
CU	-1,834±0,041^{a}	988±4^{def}	29,54±1,39^{de}	10,86±2,49^{a}
I	-1,918±0,115^{a}	969±3^{c}	32,10±1,45^{e}	32,36±1,41^{e}
IH	-1,856±0,005^{a}	999±3^{fg}	20,18±0,17^{ab}	8,15±1,20^{a}
IU	-1,813±0,029^{a}	976±1^{cd}	24,36±1,08^{bcd}	14,05±1,28^{abc}
A	-1,824±0,018^{a}	982±3^{cde}	24,07±1,86^{bcd}	22,13±0,84^{cd}
AH	-1,858±0,011^{a}	1000±7^{fg}	16,19±1,26^{a}	15,19±0,05^{abcd}
AU	-1,839±0,013^{a}	999±4^{efg}	22,31±1,23^{abc}	14,6±2,97^{abc}
B	-1,804±0,010^{a}	969±4^{bc}	23,25±1,41^{bcd}	32,21±2,76^{e}
BH	-1,844±0,027^{a}	993±2^{efg}	15,37±1,32^{a}	16,39±3,31^{abcd}
BU	-1,771±0,011^{a}	953±7^{ab}	26,12±3,80^{bcde}	19,55±2,57^{bcd}
L	-1,894±0,004^{a}	1020±2^{h}	24,10±0,14^{bcd}	23,09±2,96^{d}
LH	-1,873±0,011^{a}	1008±6^{gh}	29,22±3,14^{cde}	9,22±3,13^{a}
LU	-1,757±0,042^{a}	946±3^{a}	33,05±2,16^{e}	11,08±0,59^{a}

Wyjaśnienia: H – próbka po homogenizacji mechanicznej; U – próbka po homogenizacji ultradźwiękowej; C - próbka kontrolna, I – iota karagen, A – hydrolizat po hydrolizie kwasowej, B – hydrolizat po hydrolizie enzymatycznej β-galaktozydazą, L – hydrolizat po hydrolizie enzymatycznej laktazą przemysłową; mOsm/kg – osomolalność. Reprezentatywne dane ± odchylenie standardowe, średnie oznaczone tą samą małą literą oznaczają grupy homogeniczne nieróżniące się przy poziomie istotności $\alpha = 0,05$.

W przypadku ciśnienia osmotycznego wartości wahały się w przedziale od 946 do 1020 mOsm/kg (Tabela 6). Ciśnienie osmotyczne jest zależne od rodzaju oraz liczby substancji rozpuszczonych, w związku z czym w prezentowanych lodach różnice okazały się być nieznaczące, ponieważ nie dokonywano zmian w recepturze pomiędzy próbkami lodów. Zwrócono jednak uwagę, iż metoda homogenizacji mogła wpływać na opisywany parametr. Otóż w przypadku homogenizacji mechanicznej wartości ciśnienia osmotycznego były wyższe niż w lodach po stosowaniu homogenizacji ultradźwiękowej. Prawdopodobnie ultradźwięki obniżyły zdolność stabilizatorów do wiązania wody, co potwierdziła również ocena właściwości reologicznych w publikacji **P5**, dotycząca zmniejszenia wartości współczynnika konsystencji mieszanek lodowych (K).

Na podstawie badania czasu topnienia lodów wykazano, iż ten parametr nie przekroczył 34 minut (Tabela 6). Ponadto zauważono, że po zastosowaniu homogenizacji ultradźwiękowej czas topnienia lodów wydłużył się. Może to być uwarunkowane utworzeniem bardziej pożądanej struktury emulsji, czyli emulsji z mniejszymi cząstkami tłuszczu oraz odpowiednią ilością pęcherzyków powietrza. Już na tym etapie takie wyniki dają podstawy do założeń o bardziej korzystnej strukturze krystalicznej produktu finalnego [Adapa i wsp. 2000]. W przypadku zastosowanych stabilizatorów czas topnienia był zbliżony przy zastosowaniu wybranych hydrolizatów iota karagenu – około 24 minut, zaś dłuższy w przypadku samego dodatku iota karagenu – ponad 31 minut. Wynik taki świadczy o tworzeniu bardziej stabilnej struktury przez iota karagen, skutkującej ograniczeniem topnienia lodów. Potwierdziła to również analiza reologiczna, na podstawie której wykazano, iż iota karagen może tworzyć bardziej stabilną strukturę niż jego hydrolizaty (Publikacja **P4** oraz **P5**). Można wnioskować, iż pomimo krótszego łańcucha hydrolizatów oraz ich lepszej plastyczności, tworzone z nimi mieszanki lodowe nie są stabilne. Na uwagę zasługuje dodatek inuliny w recepturze lodów. Badania wskazują na dużą zdolność inuliny do wiązania wody i w przypadku lodów do wydłużania czasu topnienia [Akın i wsp. 2007; Góral i wsp. 2018]. Ponadto na parametry czasu topnienia, ma dość duży wpływ udział tłuszczu w strukturze lodów. Przez niską zawartość tego składnika w prezentowanych badaniach ogólny czas topnienia może być krótszy [Regand i Goff 2003].

Puszystość lodów określono na poziomie nie większym niż 33%. Na podstawie testu statystycznego ANOVA wykazano istotny wpływ stosowanych stabilizatorów oraz metody homogenizacji na ten parametr. Dodatek stabilizatorów zwiększył puszystość lodów, zaś najlepszym dodatkiem okazał się być iota karagen oraz jego hydrolizat enzymatyczny po β-galaktozydazie (Tabela 6). Ogólnie nie wykazano jednoznacznej tendencji odnośnie zastosowania homogenizacji ultradźwiękowej. To znaczy, że w zależności od kombinacji

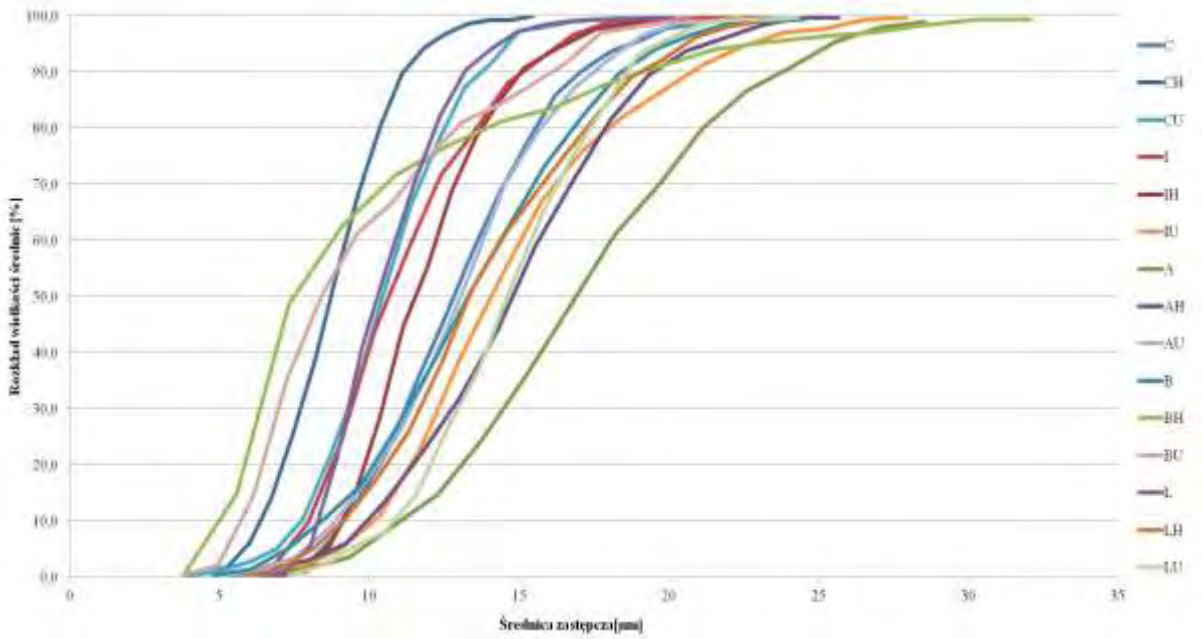
rodzaju homogenizacji z rodzajem stabilizatora, wielkość ta zmieniała swoje wartości. I tak połączenie hydrolizatu kwasowego z homogenizacją ultradźwiękową było mniej korzystne niż połączenie z homogenizacją mechaniczną. Finalnie puszystość lodów wegańskich zwykle osiąga niższe wartości niż lodów mlecznych ze względu na brak obecności białek zwierzęcych. Również wspomniana niska zawartość tłuszczu może przyczyniać się do takich wartości. Tłuszcz pełni rolę bariery dla obecnych pęcherzyków powietrza, jednocześnie stabilizując ich układ [Flores i Goff 1999]. Ponadto również inulina ma zdolności do pogarszania puszystości lodów. Na podstawie badań Góral i wsp. [2018] już poziom 4-procentowy poziom inuliny w lodach kokosowych oddziaływał na pogorszenie puszystości lodów wegańskich.

Postęp procesu rekrystalizacji w lodach wegańskich określono na podstawie analizy krystalicznej lodów po 24 godzinach, miesiącu oraz trzech miesiącach przechowywania. W prezentowanej pracy omówiono tylko wyniki 24-godzinnego oraz trzymiesięcznego przechowywania.

Po czasie 24 godzin przechowywania w temperaturze -18°C, biorąc pod uwagę wpływ zastosowanych stabilizatorów, tj. iota karagenu oraz jego hydrolizatów, najmniejszą wartość parametru X_{50}, wynoszącą 11 μm, odnotowano w przypadku próbki lodów L z dodatkiem hydrolizatu enzymatycznego iota karagenu po działaniu przemysłową laktazą. Natomiast największą wartość parametru X_{50} (17 μm) osiągnęła próbka lodów z dodatkiem hydrolizatu kwasowego iota karagenu (A) (Wykres 7). W badaniach Kamińskiej-Dwórznickiej i wsp. [2023], wykazano, że wykorzystując karageny jako stabilizatory w lodach bezmlecznych (sorbet mango) oraz stosując zamrażanie wspomagane ultradźwiękami, jest możliwe osiągnięcie uformowanie jednolitej struktury krystalicznej o średnicy kryształów właśnie na poziomie – 17 μm.

Po trzech miesiącach przechowywania, analizując rodzaj zastosowanych stabilizatorów, najmniejszą wartością parametru X_{50} charakteryzowała się próbka z dodatkiem hydrolizatu enzymatycznego iota karagenu po użyciu enzymu β-galaktozydazy (B) - 17 μm, a największą próbka lodów z dodatkiem iota karagenu (I) – 20 μm (Wykres 8). Natomiast na przykładzie wspomnianych próbek L oraz A, po 24-godzinnym przechowywaniu, zaobserwowano postęp procesu rekrystalizacji. Wartość parametru X_{50} w przypadku próbki L (z dodatkiem hydrolizatu enzymatycznego iota karagenu po działaniu przemysłową laktazą) wyniosła 19 μm a próbki A (z dodatkiem hydrolizatu kwasowego iota karagenu) wyniosła 20 μm. Analizując wpływ zastosowanego układu stabilizującego na postęp procesu rekrystalizacji, stwierdzono, iż najkorzystniejszym stabilizatorem

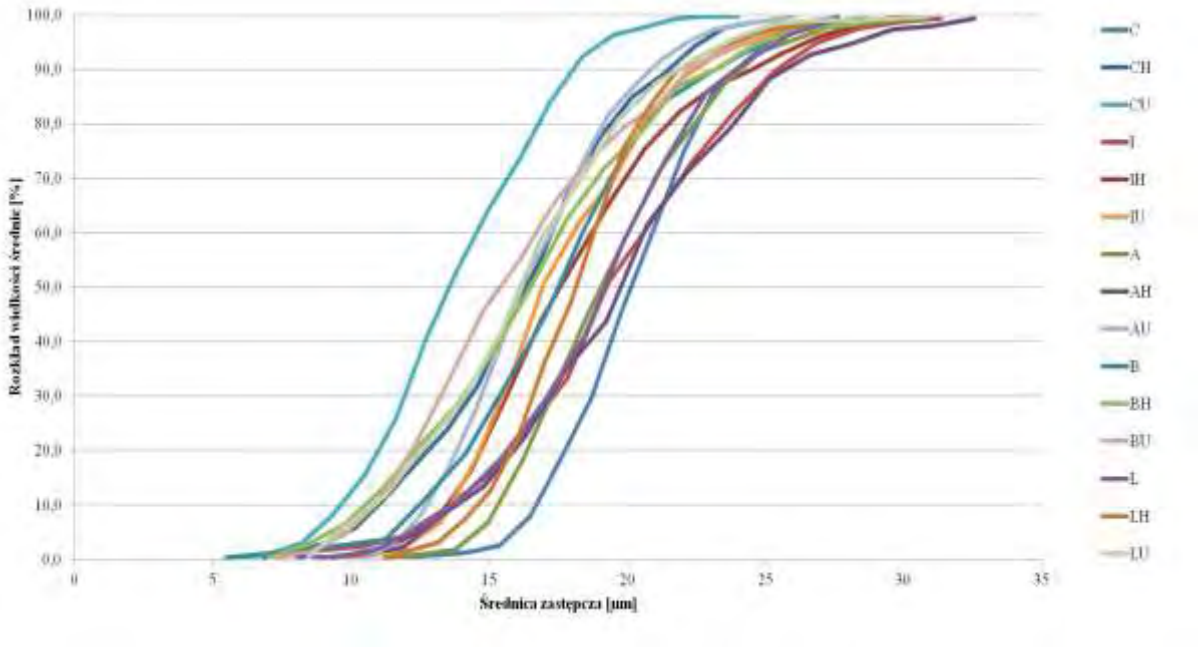
w przypadku wielkości kryształów był hydrolizat enzymatyczny iota karagenu po β-galaktozydazie (B). Taki efekt może być związany z odmiennymi, w tym przypadku lepszymi, zdolnościami wiązania wody przez ten hydrolizat w porównaniu z czystym iota karagenem czy jego hydrolizatem kwasowym. Ponadto, w publikacji **P1** na podstawie wyników FTIR otrzymanych hydrolizatów, również wykazano, iż hydrolizaty enzymatyczne wykazywały większe drgania w zakresie grupy –OH, co warunkowało prawdopodobnie ich większą elastyczność.



Wykres 7. Rozkład wielkości średnic kryształów w lodach wegańskich po 24 godzinach przechowywania w temp. -18°C. Wyjaśnienia: H – próbka po homogenizacji mechanicznej; U – próbka po homogenizacji ultradźwiękowej; C - próbka kontrolna, I – iota karagen, A – hydrolizat po hydrolizie kwasowej, B – hydrolizat po hydrolizie enzymatycznej β-galaktozydazą, L – hydrolizat po hydrolizie enzymatycznej laktazą przemysłową.

W prezentowanej pracy, w oparciu o postawione hipotezy, analizowano również wpływ metody homogenizacji na postęp procesu rekrystalizacji, tj. homogenizacji tradycyjnej (mechanicznej) oraz homogenizacji wspomaganej ultradźwiękami. Po 24-godzinnym przechowywaniu próbek lodów wegańskich, najmniejszą wartość parametru X_{50} (11 µm) zanotowano w próbce kontrolnej bez dodatku stabilizatorów oraz po stosowaniu ultradźwięków (CU), a największą (15 µm) w próbce AH z dodatkiem hydrolizatu kwasowego oraz po homogenizacji mechanicznej (Wykres 7). Ponadto tylko w próbce kontrolnej na tym etapie przechowywania homogenizacja ultradźwiękowa przyczyniła się do osiągnięcia mniejszych kryształów lodu. Natomiast w próbkach z użyciem stabilizatorów i po homogenizacji mechanicznej średnice kryształów były mniejsze niż w próbkach z tymi samymi stabilizatorami, ale po zastosowaniu ultradźwięków.

Po trzech miesiącach przechowywania najniższą wartość parametru X_{50} zanotowano w próbce kontrolnej po stosowaniu ultradźwięków (CU) – 14 μm (Wykres 8). Natomiast największą średnicę (20 μm) w próbce lodów z dodatkiem hydrolizatu kwasowego iota karagenu i po stosowaniu homogenizacji mechanicznej (AH). Warto zaznaczyć, że w każdej próbce, gdzie zastosowano homogenizację ultradźwiękową, średnica kryształów była mniejsza niż po homogenizacji mechanicznej czy w przypadku dodatku tylko stabilizatorów. Ultradźwięki mogły przyspieszyć proces zarodkowania kryształów lodu po procesie zamrażania [Cheng i wsp. 2015b; Akdeniz i Akalin 2019]. Prawdopodobnie wpłynęły również na utworzenie jednolitej struktury, złożonej z drobnych kryształów położonych blisko siebie, co ograniczyło możliwość ich wzrostu w trakcie procesu przechowywania. W badaniach Cheng i wsp. [2015b] udowodniono, iż siły generowane prze ultradźwięki mogą oddziaływać na większe kryształy, rozbijając je na mniejsze nawet w trakcie przechowywania lodów. Co więcej, w badaniach Islam i wsp. [2015] czy Xu i wsp. [2015] stosowanie ultradźwięków przyczyniło się do zmniejszenia wielkości kryształów lodu w porównaniu do próbek bez udziału kawitacji akustycznej.



Wykres 8. Rozkład wielkości średnic kryształów w lodach wegańskich po 3 miesiącach przechowywania w temp. -18°C. Wyjaśnienia: H – próbka po homogenizacji mechanicznej; U – próbka po homogenizacji ultradźwiękowej; C – próbka kontrolna, I - iota karagen, A – hydrolizat po hydrolizie kwasowej, B –hydrolizat po hydrolizie enzymatycznej β-galaktozydazą, L – hydrolizat po hydrolizie enzymatycznej laktazą przemysłową.

Analizując strukturę morfologiczną otrzymanych kryształów w lodach wegańskich, ogólnie określono ich kształt jako regularny i owalny. Nie występowały znaczące wizualnie różnice pomiędzy próbkami poddanymi działaniu homogenizacji ultradźwiękowej oraz homogenizacji mechanicznej. Zaś widoczny był postęp procesu rekrystalizacji, porównując zdjęcia po 24-godzinnym przechowywaniu i trzech miesiącach przechowywania, co potwierdził przyrost wartości parametru X_{50} (Wykresy 7 oraz 8).

7.6 Badanie wpływu iota karagenu oraz jego hydrolizatów na stabilność mlecznych mieszanek lodowych

W publikacji **P7 (The effect of iota carrageenan and its hydrolysates on the stability of milk ice cream mixes. Polish Journal of Food and Nutrition Science, 2023, 73(2), 196-204)** zaprezentowane zostały zmiany właściwości fizycznych mlecznych mieszanek lodowych ze względu na rodzaj zastosowanego stabilizatora. W tym celu wykorzystany został iota karagen oraz jego hydrolizaty: kwasowy i dwa enzymatyczne, po użyciu β-galaktozydazy oraz laktazy przemysłowej (Tabela 1 oraz 3). Pomiar stabilności, rozkładu cząstek oraz mediany D_{50}, właściwości reologicznych, mikrostruktury mieszanek został wykonany bezpośrednio po wytworzeniu i po procesie 24-godzinnego dojrzewania w temperaturze 4°C.

Wartości indeksu TSI (Turbiscan Stability Index) wahały się od wartości 1,9 do 2,8 (Tabela 7). Najlepszą stabilność mieszanki zauważono przy dodatku hydrolizatu kwasowego (A), zaś najmniejszą stabilność w trakcie dojrzewania zanotowano w przypadku próbki z dodatkiem iota karagenu (I). Natomiast otrzymane hydrolizaty enzymatyczne iota karagenu uzyskały wartość na poziomie próbki kontrolnej wynoszącą – 2,2. Również w badaniach Seo i Oh [2022] wartości indeksu TSI mieszanek lodowych, uzyskanych z wykorzystaniem kappa karagenu oraz koniugatów kappa karagenu powstałych w reakcji Maillarda, mieściły się w podobnym zakresie (około 1,8). W przypadku rodzaju destabilizacji zaobserwowano koalescencję/flokulację wszystkich mieszanek lodowych oraz dodatkowo sedymentację w próbce z dodatkiem hydrolizatu kwasowego (A). Jest to jednocześnie potwierdzenie wyższej destabilizacji (wartości indeksu TSI) w tej mieszance.

Wartość mediany D_{50} wielkości kuleczek tłuszczowych przed procesem dojrzewania mieściła się w zakresie od 17,56 do 45,50 μm. Największą wartość zanotowano w przypadku próbki z dodatkiem hydrolizatu kwasowego (A) jako stabilizatora. Również ta próbka charakteryzowała się najmniejszą stabilnością spośród wszystkich mieszanek lodowych.

Prawdopodobnie wynika to z większej ilości aglomeratów tłuszczu, które są odpowiedzialne za jego destabilizację. Po procesie dojrzewania niewielkie zmniejszenie mediany D_{50} wystąpiło w mieszankach z dodatkiem iota karagenu oraz hydrolizatów enzymatycznych. Wzrost D_{50} był obserwowany w próbce kontrolnej oraz w mieszance z dodatkiem hydrolizatu kwasowego (A). Ogólnie wartość mediany nie przekroczyła 47 µm. W porównaniu z wcześniej prezentowanymi wegańskimi mieszankami lodowymi (Publikacja **P4** oraz **P5**) analizowane wartości były znacząco większe. Powodem była większa obecność tłuszczu, wynikająca z receptury mieszanek lodowych i przez to większy stopień destabilizacji tego składnika (Tabela 2 oraz 3).

Tabela 7. Właściwości fizyczne mieszanek lodowych.

Próbka	TSI	Przed dojrzewaniem				Po dojrzewaniu			
		D_{50} (µm)	K, $10^{-3}\cdot Pas^n$	n	R	D_{50} (µm)	K, $10^{-3}\cdot Pas^n$	n	R
C	2,2	17,56±0,31^{c}	0,009±0,001^{d}	0,900±0,001^{a}	0,99	23,24±2,40^{c}	0,012±0,012^{b}	0,838±0,007^{a}	0,99
I	2,8	40,60±2,97^{ab}	0,061±0,005^{b}	0,738±0,022^{c}	0,99	35,57±2,63^{b}	0,059±0,058^{ab}	0,761±0,011^{ab}	0,99
A	1,9	45,50±2,37^{a}	0,059±0,005^{bc}	0,765±0,018^{bc}	0,99	46,73±0,19^{a}	0,058±0,057^{ab}	0,771±0,031^{ab}	0,99
B	2,2	37,05±2,34^{b}	0,048±0,001^{c}	0,798±0,005^{b}	0,99	34,73±0,90^{b}	0,050±0,049^{b}	0,803±0,016^{ab}	0,99
L	2,2	41,08±1,79^{ab}	0,083±0,006^{a}	0,727±0,014^{c}	0,99	35,16±1,23^{b}	0,104±0,103^{a}	0,702±0,083^{b}	0,99

Wyjaśnienia: C – mieszanka bez stabilizatorów; I – mieszanka z iota karagenem; A – mieszanka hydrolizatem kwasowym; B – mieszanka z hydrolizatem enzymatycznym po β-galaktozydazie; L – mieszanka z hydrolizatem enzymatycznym po laktazie przemysłowej; TSI – Turbiscan Stability Index; D_{50} – mediana. Reprezentatywne dane ± odchylenie standardowe, średnie oznaczone tą samą małą literą oznaczają grupy homogeniczne nieróżniące się przy poziomie istotności $\alpha = 0,05$.

Analizując rozkład wielkości cząstek, trzy lub cztery piki występowały zarówno przed, jak i po procesie dojrzewania. Pierwszy zakres cząstek zanotowano na poziomie 2-5 µm, drugi zakres około 15-30 µm, następnie 70-80 µm i największe wielkości cząstek około 100 µm. Dodatkowo na tym etapie, analizując zdjęcia wykonane przed, jak i po procesie dojrzewania na mikroskopie konfokalnym, zaobserwowano także obecność cząstek o niewielkich rozmiarach. Ponadto fakt, iż nie nastąpiła znacząca redukcja wielkości cząstek, potwierdziły również wartości mediany D_{50}. W związku z czym można było wnioskować, że

pojedyncze cząstki występowały w przedziale 5 μm, zaś kolejne wartości to aglomeraty cząsteczek tłuszczu. W przytoczonych wcześniej badaniach (w punkcie omawiania mieszanek wegańskich – publikacja **P4** oraz **P5**), przeprowadzonych przez Mendez-Velasco i Goff [2012], potwierdzono, iż obecność nienasyconych monoglicerydów kwasów tłuszczowych jako emulgatora w mieszankach lodowych może przyczyniać się do formowania większych cząstek tłuszczu, co w prezentowej pracy mogło spowodować wysokie wartości mediany i jednocześnie destabilizację układu. Ponadto zdjęcia mikroskopowe potwierdzają obecność aglomeratów tłuszczu i chociażby procesu koalescencji.

Właściwości reologiczne mieszanek opisano na podstawie modelu Ostwalda de Waele, biorąc pod uwagę indeks płynięcia oraz współczynnik konsystencji (Tabela 7). Przed procesem dojrzewania wartość współczynnika konsystencji K mieściła się w zakresie od 0,009 do 0,083. Ogólnie dodatek stabilizatorów przyczynił się do zwiększenia wartości tego parametru. Najwyższą wartość współczynnika konsystencji zanotowano w przypadku dodatku hydrolizatu enzymatycznego iota karagenu po przemysłowej laktazie (L). W związku z czym można wyróżnić ten stabilizator jako ten, który daje największą stabilność mieszanki lodowej. Różnice wartości współczynnika konsystencji (K) mogą wynikać z innej zdolności do tworzenia żelu pomiędzy stosowanymi stabilizatorami. Również Soukoulis i wsp. [2008] wykazali, iż połączenie kappa karagenu z różnymi hydrokolidami miało wpływ na wartość współczynnika konsystencji. Wartości indeksu stabilności (TSI) także wskazują na dobrą stabilność mieszanki lodowej z dodatkiem hydrolizatu enzymatycznego iota karagenu po laktazie (L) w porównaniu z pozostałymi mieszankami. Po procesie dojrzewania zanotowano zwiększenie współczynnika konsystencji (K), co wynikało z kształtowania struktury mieszanki, np. pęcznienia białek, stabilizatorów oraz destabilizacji tłuszczu [Alvarez i wsp. 2005]. Największą wartość, na poziomie 0,104, charakteryzowała się próbka L, tak jak przed procesem dojrzewania. Wartości indeksu płynięcia przed i po procesie dojrzewania były poniżej 1, co wskazywało na pseudoplastyczny, nieniutonowski charakter cieczy i było wynikiem obecności aglomeratów tłuszczu oraz polisacharydowych stabilizatorów [Akbari i wsp. 2019]. Analiza mikroskopowa została przeprowadzona w oparciu o zdjęcia z mikroskopu konfokalnego, poprzez wybarwienie cząstek tłuszczu barwnikiem Red Nil. Wyniki z mikroskopu potwierdziły obecność aglomeratów kuleczek tłuszczowych, co było dowodem na destabilizację układu mieszanki lodowej, zaobserwowanej również w wynikach TSI. Ponadto wielkości kuleczek tłuszczowych na zdjęciach potwierdziły zakres wielkości cząstek z rozkładu wielkości cząstek zarówno przed, jak i po procesie dojrzewania.

7.7 Badanie wpływu homogenizacji ultradźwiękowej na proces rekrystalizacji w lodach mlecznych

Publikacja ta była podsumowaniem analizy właściwości mlecznych lodów, otrzymanych z wykorzystaniem stabilizatorów (iota karagenu oraz jego hydrolizatów) oraz zmiany homogenizacji mechanicznej na homogenizację ultradźwiękową (Tabela 1 oraz 3). W pracy zbadano właściwości fizyczne lodów mlecznych, tj. temperaturę krioskopową, ciśnienie osmotyczne, puszystość, czas topnienia oraz strukturę krystaliczną w trakcie przechowywania przez 24 godziny, miesiąc oraz trzy miesiące w temperaturze -18°C. Wpływ stabilizatorów analizowano osobno oraz w połączeniu z homogenizacją mechaniczną lub z homogenizacją ultradźwiękową. Wyniki badań przedstawiono w publikacji **P8 (The effectiveness of ultrasound homogenisation on the recrystallisation process in milk ice cream. Applied Sciences, 2023, 13(13), 7561, 1-16).**

Temperatura krioskopowa mieściła się w przedziale od -2,921 do -2,486°C (Tabela 8). Analiza statystyczna wykazała istotny wpływ stosowanych stabilizatorów oraz metody homogenizacji. Najmniejszą temperaturę zanotowano w przypadku próbki AH (z dodatkiem hydrolizatu kwasowego oraz po homogenizacji mechanicznej), zaś największą próbki I (z dodatkiem iota karagenu). Ponadto zauważono tendencję zmniejszania się temperatury krioskopowej po zastosowaniu homogenizacji mechanicznej, a zwiększania po działaniu homogenizacji ultradźwiękowej. Powodem mogła być zdolność ultradźwięków do przyspieszania przepływu masy i energii przez kawitację akustyczną, co mogło korzystnie wpływać na formującą się strukturę krystaliczną [Chemat i wsp. 2011]. Niezależnie od metody homogenizacji stabilizatory oddziaływały na zakres temperatury krioskopowej. Według Hagiwara i Hartel [1996] masa molekularna substancji słodzących warunkowała zmienność omawianego parametru. Na podstawie tego można wnioskować, że hydrolizaty iota karagenu ze względu na małą masę molekularną a większą plastyczność, miały zdolność do podwyższenia temperatury krioskopowej. Wartości ciśnienia osmotycznego, mieściły się w zakresie pd 1346 do 1572 mOsm/kg. W przypadku ciśnienia osmotycznego większy wpływ miał rodzaj substancji rozpuszczonych niż rodzaj stosowanej obróbki. W prezentowanych badaniach zauważono również zależność pomiędzy temperaturą krioskopową a ciśnieniem osmotycznym. W próbach o większej temperaturze krioskopowej otrzymano mniejsze wartości ciśnienia osmotycznego. W badaniach Buniowskiej-Olejnik i wsp. [2023] również odnotowano taką zależność w mlecznych mieszankach lodowych. Powodem takiej korelacji jest to, iż wartość temperatury krioskopowej, jest zależna od rodzaju oraz stężenia użytych

substancji – im większe stężenie i mniejsza masa cząsteczkowa rozpuszczonych związków tym niższa temperatura krioskopowa. W konsekwencji, rodzaj zastosowanych w badaniach stabilizatorów oraz ich zdolności wiązania cząsteczek wody, wpłynęły na proces budowania struktury krystalicznej [Patel i wsp. 2006].

Tabela 8. Właściwości fizyczne mlecznych lodów spożywczych.

Próbka	Temperatura krioskopowa, °C	Ciśnienie osmotyczne, mOsm/kg	Puszystość, %	Czas topnienia, min.
C	-2,502±0,006^{fg}	1347±4^{ab}	15,35±3,52^{bcde}	23,19±2,23^{cde}
CH	-2,549±0,005^{defg}	1372±3^{d}	8,3±0,49^{a}	22,25±3,05^{bcde}
CU	-2,510±0,018^{efg}	1355±5^{ab}	17,53±2,16^{de}	22,37±3,01^{cde}
I	-2,486±0,013^{g}	1358±0^{bc}	24,40±0^{f}	25,14±0,97^{de}
IH	-2,531±0,033^{defg}	1346±7^{a}	10,9±1,94^{abcd}	27,46±1,34^{de}
IU	-2,519±0,013^{defg}	1348±4^{ab}	20,59±0^{ef}	26,23±2,09^{de}
A	-2,611±0,044^{de}	1422±0^{f}	18,86±3,77^{ef}	28,26±2,36^{de}
AH	-2,921±0,008^{a}	1572±4^{i}	9,05±0^{ab}	27,33±0de
AU	-2,512±0,066^{efg}	1377±0de	15,72±0,05^{bcde}	30,34±0^{e}
B	-2,721±0,022^{bc}	1456±0^{g}	31,79±0,86^{g}	12,34±0^{abc}
BH	-2,608±0,025^{def}	1413±0^{f}	16,06±0,37^{cde}	12,17±5,06^{ab}
BU	-2,754±0,025^{b}	1490±0^{h}	18,27±0^{ef}	11,23±0,45^{a}
L	-2,578±0,000^{defg}	1388±0^{e}	14,32±0,99^{abcde}	18,15±1,85^{abcd}
LH	-2,622±0,026^{cd}	1421±0^{f}	10,63±2,33^{abc}	20,13±1,85^{abcd}
LU	-2,535±0,017^{defg}	1368±4^{cd}	20,43±1,10^{ef}	23,19±5,89^{de}

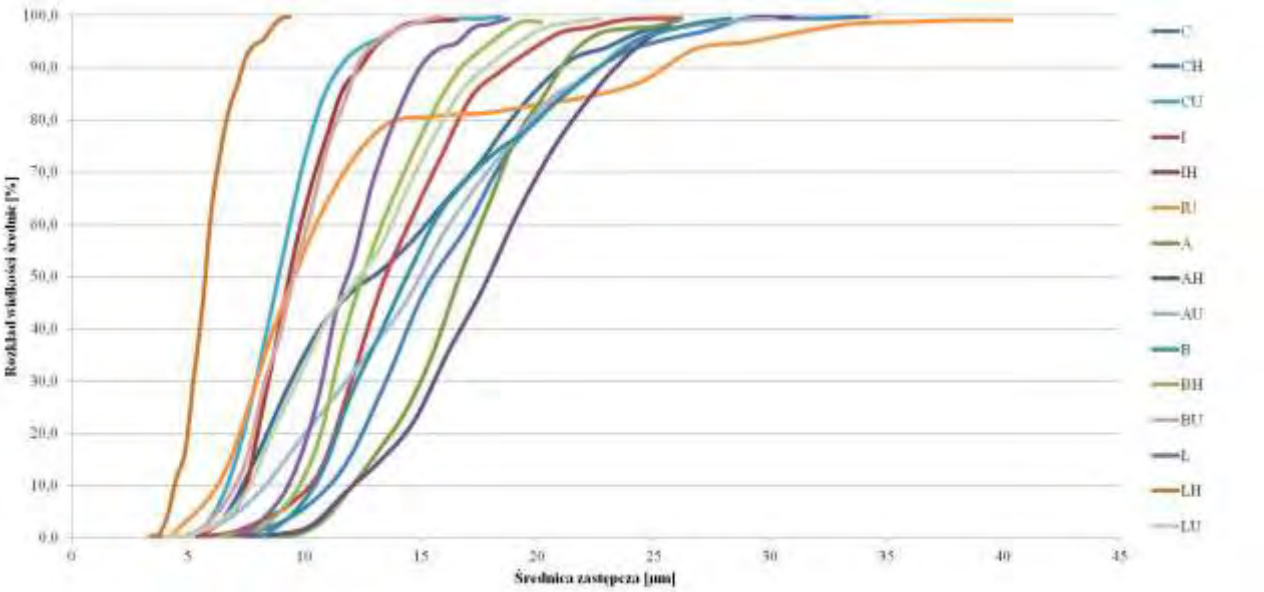
Wyjaśnienia: H – próbka po homogenizacji mechanicznej; U – próbka po homogenizacji ultradźwiękowej; C – próbka kontrolna, I – iota karagen, A – hydrolizat po hydrolizie kwasowej, B – hydrolizat po hydrolizie enzymatycznej β-galaktozydazą, L – hydrolizat po hydrolizie enzymatycznej laktazą przemysłową; mOsm/kg – osomolalność. Reprezentatywne dane ± odchylenie standardowe, średnie oznaczone tą samą małą literą oznaczają grupy homogeniczne nieróżniące się przy poziomie istotności $\alpha = 0,05$.

Puszystość badanych lodów mlecznych nie przekroczyła wartości 32% (Tabela 8). Niska wartość tego parametru jak na lody mleczne, kojarzone zwykle z wysokimi wartościami puszystości, mogła być wynikiem obecności inuliny oraz małej zawartości tłuszczu w recepturze lodów. W badaniach Mahidan i Karazihan [2013] oraz Ismali i wsp. [2013] również wykazano, iż dodatek inuliny w lodach przyczynił się do pogorszenia ich puszystości. Inulina posiada dobre właściwości wiązania cząsteczek wody, przez co może powodować wzrost lepkości, a w konsekwencji pogorszenie topliwości lodów. Analiza

statystyczna wykazała, różnice zarówno w przypadku stosowania stabilizatorów, jak i metody homogenizacji. Najmniejszą puszystość odnotowano w przypadku próbki kontrolnej bez dodatku stabilizatorów i po procesie homogenizacji mechanicznej (CH), a największą w próbce z hydrolizatem enzymatycznym iota karagenu po β-galaktozydazie (B). Ponadto w próbkach, w których zastosowano dodatek stabilizatorów, lecz nie poddano ich żadnej metodzie homogenizacji (I, A, B oraz L), wartości puszystości były większe. Należy również zwrócić uwagę na fakt, iż w wyniku połączenia stabilizatorów z homogenizacją ultradźwiękową uzyskano wartości puszystości w przedziale od 15,72 do 20,43%, zaś w przypadku połączenia z homogenizacją mechaniczną od 9,05 do 16,06%.

Czas topnienia lodów oceniono w zakresie od 11,23 do 30,34 minuty (Tabela 8). Większy wpływ na wartość tego parametru miał rodzaj zastosowanych stabilizatorów niż zastosowana metoda homogenizacji. Przykładowo, próbka z dodatkiem iota karagenu charakteryzowała się czasem topnienia 25,24 minut (I), w połączeniu z homogenizacją mechaniczną – 27,46 minut (IH), a z homogenizacją ultradźwiękową – 26,23 minuty (IU). W związku z powyższym nie występowały znaczące różnice pomiędzy stosowaną metodą homogenizacji. Pomiędzy próbkami z dodatkiem stabilizatorów różnice były znaczące (około 14 minut), jak np. pomiędzy próbką z dodatkiem hydrolizatu enzymatycznego iota karagenu po β-galaktozydazie (B) a próbką z dodatkiem hydrolizatu kwasowego iota karagenu (A). Krótkie czasy topnienia mogły być wynikiem małej zawartości tłuszczu w recepturze lodów, który stanowi barierę ochronną dla pęcherzyków powietrza, prowadząc do lepszej stabilności przy zmiennej temperaturze. Inulina jako dodatek do lodów również mogła wpłynąć na zmniejszenie wartości tego parametru. W badaniach Mahdian i Karazihan [2013] lub Góral i wsp. [2018] zanotowano, że przy zwiększaniu dodatku inuliny, czas topnienia lodów był zdecydowanie krótszy.

Na podstawie analizy struktury krystalicznej oceniono postęp procesu rekrystalizacji w lodach mlecznych po 24 godzinach, miesiącu oraz trzech miesiącach przechowywania. Poniżej omówiono najważniejsze wyniki, wybierając 24-godzinny oraz trzymiesięczny czas przechowywania, natomiast wyniki po miesiącu zostały szczegółowo omówione w Publikacji **P8**.



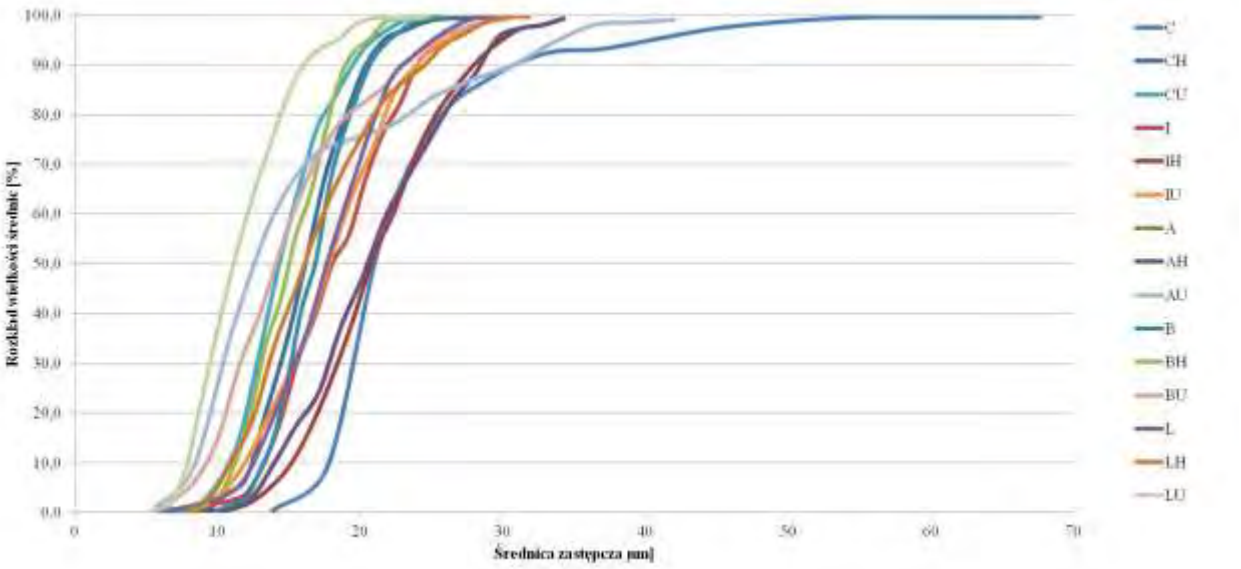
Wykres 9. Rozkład wielkości średnic kryształów w lodach mlecznych po 24 godzinach przechowywania w temp. -18°C. Wyjaśnienia: H – próbka po homogenizacji mechanicznej; U – próbka po homogenizacji ultradźwiękowej; C – próbka kontrolna, I – iota karagen, A – hydrolizat po hydrolizie kwasowej, B – hydrolizat po hydrolizie enzymatycznej β-galaktozydazą, L – hydrolizat po hydrolizie enzymatycznej laktazą przemysłową.

Biorąc pod uwagę rodzaj zastosowanych stabilizatorów, tj. iota karagenu oraz jego hydrolizatów po 24 godzinach przechowywania, na podstawie parametru X_{50} (Wykres 9), wyrażającego uśrednioną wielkość 50% średnic kryształów lodu, próbka z dodatkiem enzymatycznego hydrolizatu iota karagenu po użyciu przemysłowej laktazy (L) charakteryzowała się najmniejszą wartością średnicy zastępczej – 12 µm. Największą wartość średnicy zastępczej zanotowano w przypadku próbki z dodatkiem kwasowego hydrolizatu iota karagenu (A) – 17 µm (Wykres 9). Podobne wartości średnic kryształów zaobserwowano również w próbce kontrolnej (C) oraz próbce z dodatkiem enzymatycznego hydrolizatu iota karagenu po hydrolizie β-galaktozydazą (B) ponad 16 µm. W lodach mlecznych z dodatkiem serwatki przy użyciu iota karagenu jako stabilizatora wartość parametru X_{50} również była na zbliżonym poziomie – 15 µm [Kamińska-Dwórznicka i wsp. 2022]. Po 3 miesiącach przechowywania zaobserwowano postęp procesu rekrystalizacji. W próbce z dodatkiem enzymatycznego hydrolizatu iota karagenu po użyciu przemysłowej laktazy (L) zanotowano zwiększenie wartości średnic kryształów lodu od 12 µm (po 24 godzinach przechowywania) do ponad 17 µm (Wykres 10). Najmniejszą wartość parametru X_{50} zanotowano w przypadku próbki z dodatkiem enzymatycznego hydrolizatu iota karagenu po hydrolizie β-galaktozydazą (B) – 17 µm, co oznaczało brak postępu w procesie rekrystalizacji (zbliżoną wartość odnotowano 24 h po procesie produkcji). Największą wartość X_{50} (21 µm) wyznaczono w próbce z dodatkiem kwasowego hydrolizatu iota karagenu (A), co stanowi dowód postępu

procesu rekrystalizacji, ponieważ po 24 godzinach wartość ta wynosiła 17 μm (Wykres 9). Podobne wynki otrzymano już na etapie przygotowywnia roztworów modelowych lodów mlecznych, czyli roztworów sacharozy z dodatkiem kazienanianu sodu, opisanych w pierwszym etapie badań (Publikacja **P1**). Z tego względu, iż po 96 godzinach przechowywania, również hydrolizaty enzymatyczne oraz sam iota karagen osiągnęły niższe wartości średnic zastępczych w porównaniu z hydrolizatem kwasowym iota karagenu.

Kolejnym badanym czynnikiem, mającym wpływ (w odniesieniu do stawianych hipotez badawczych) na postęp procesu rekrystalizacji w lodach mlecznych, był rodzaj zastosowanej homogenizacji: mechanicznej oraz wspomaganej ultradźwiękami. W związku z czym, porównując wpływ tych dwóch metod homogenizacji, po 24-godzinnym przechowywaniu, najmniejszą wartość parametru X_{50} (9 μm) zaobserwowano w lodach bez dodatku stabilizatorów i po działaniu ultradźwiękami (CU), na poziomie (Wykres 9). Natomiast największą wartość średnicy – 18 μm, w próbce AH, czyli w lodach z dodatkiem hydrolizatu kwasowego iota karagenu oraz po homogenizacji mechanicznej. Biorąc pod uwagę tylko wpływ homogenizacji, należy zwrócić uwagę na próbkę kontrolną poddaną działaniu homogenizacji, lecz bez dodatku stabilizatorów. Zastosowanie homogenizacji ultradźwiękowej już na tym etapie przechowywania przyczyniło się do osiągnięcia mniejszych rozmiarów kryształów lodu – po 24 godzinach wartość parametru X_{50} dla wspomnianej próbki CU wynosiła 9 μm, a próbki kontrolnej, po homogenizacji mechanicznej (CH) – 14 μm.

Po trzech miesiącach przechowywania, tak jak w próbkach ze stabilizatorami, również zaobserwowano postęp procesu rekrystalizacji. Najmniejszą wartość parametru X_{50} (15 μm) zanotowano w przypadku lodów bez dodatku stabilizatorów i po działaniu homogenizacji ultradźwiękowej (CU) (Wykres 10). Oznacza to, że nastąpił wzrost kryształów, jednak ich średnice nie przekraczały 20 μm. Największą wartość X_{50} odnotowano w próbce lodów po homogenizacji mechanicznej oraz z dodatkiem iota karagenu (IH) – 22 μm (Wykres 10). Widoczna efektywność ultradźwięków w inhibitowaniu procesu rekrystalizacji mogła być spowodowana zjawiskiem kawitacji akustycznej, mającym wpływ na podział dużych kryształów lodu w próbce, w wyniku czego po kilku miesiącach przechowywania proces rekrystalizacji postępował w nieznacznym stopniu lub całkowicie zostaje wyeliminowany w porównaniu z próbkami po homogenizacji mechanicznej [Chow i wsp. 2005].



Wykres 10. Rozkład wielkości średnic kryształów w lodach mlecznych po 3 miesiącach przechowywania w temp. -18°C. Wyjaśnienia: H – próbka po homogenizacji mechanicznej; U – próbka po homogenizacji ultradźwiękowej; C – próbka kontrolna, I – iota karagen, A – hydrolizat po hydrolizie kwasowej, B – hydrolizat po hydrolizie enzymatycznej β-galaktozydazą, L – hydrolizat po hydrolizie enzymatycznej laktazą przemysłową.

Analizując strukturę morfologiczną w lodów mlecznych, stwierdzono regularny oraz owalny kształt kryształów lodu. Ponadto pomimo różnic wartości średnicy zastępczej czy parametru X_{50}, wizualnie nie stwierdzono występowania różnic pomiędzy próbkami poddanymi homogenizacji ultradźwiękowej oraz homogenizacji mechanicznej. Porównując zdjęcia po 24-godzinnym przechowywaniu oraz po trzech miesiącach przechowywania, można zaobserwować jedynie wzrost kryształów, co świadczy o postępie procesu rekrystalizacji. Podsumowując analizę struktury krystalicznej otrzymanych próbek lodów mlecznych, spośród zastosowanych stabilizatorów, najkorzystniejszym okazał się być enzymatyczny hydrolizat iota karagenu po hydrolizie β-galaktozydazą. Natomiast porównując metody homogenizacji, skuteczniejszą obróbką mieszanki lodowej, na podstawie wielkości średnic kryształów, była homogenizacja wspomagana ultradźwiękami.

PODSUMOWANIE I WNIOSKI

Na podstawie wyników przeprowadzonych badań, opublikowanych w formie publikacji naukowych, zweryfikowano hipotezy badawcze, co pozwoliło na sformułowanie następujących wniosków:

- **Wniosek 1**

Wykazano, że substancje stabilizujące, które zostały pozyskane na drodze hydrolizy kwasowej oraz enzymatycznej iota karagenu, mogły korzystnie ograniczać proces rekrystalizacji w modelowych roztworach sacharozy. Zarówno hydrolizat kwasowy (masa cząsteczkowa na poziomie $1{,}48 \times 10^6$Da), jak i enzymatyczne hydrolizaty (po użyciu enzymu β-galaktozydazy – masa cząsteczkowa $3{,}20 \times 10^6$Da oraz po użyciu enzymu laktazy – masa cząsteczkowa $3{,}50 \times 10^6$Da), niezależnie od redukcji masy cząsteczkowej iota karagenu, skutecznie ograniczyły proces rekrystalizacji w modelowych roztworach sacharozy (model lodów wegańskich). Średnia wartość średnicy zastępczej nie przekraczała 22 μm po 96 godzinach przechowywania w temperaturze -8°C. W roztworach modelowych z dodatkiem kazeinianu sodu (model lodów mlecznych) efekt inhibicji był nawet bardziej widoczny, a najlepsze rezultaty osiągnięto w przypadku próbki z dodatkiem hydrolizatu po laktazie przemysłowej (średnica kryształów nie przekraczała 17 μm). W odniesieniu do hipotezy 1. przeprowadzone analizy, tj. SEC i FTIR, potwierdziły, że na aktywność inhibicyjną w procesie rekrystalizacji ma wpływ struktura hydrolizatów oraz obecne grupy funkcyjne, a nie tylko sama redukcja ich masy cząsteczkowej.

- **Wniosek 2**

W odniesieniu do hipotezy 2 przeprowadzono badania wstępne lodowych mieszanek wegańskich, które pozwoliły na ustalenie receptury i wykazanie, że proces technologiczny wykorzystywany przy produkcji lodów tradycyjnych był odpowiedni również przy produkcji lodów wegańskich.

- **Wniosek 3**

Wykazano wpływ wybranych stabilizatorów oraz dwóch rodzajów homogenizacji na stabilność i właściwości fizyczne wegańskich mieszanek lodowych na bazie napoju migdałowego.

Najmniejszą wartość indeksu płynięcia zaobserwowano w próbkach z dodatkiem iota karagenu, bez względu na stosowanie procesu homogenizacji. Natomiast w próbkach poddanych homogenizacji (BH oraz LH) zanotowano mniejsze wartości indeksu płynięcia niż w próbkach z tymi samymi stabilizatorami, lecz bez homogenizacji (LWH oraz BWH). W odniesieniu do tych wyników można stwierdzić, że rodzaj stabilizatora mógł znacząco oddziaływać na zachowanie się cieczy. Wykorzystanie stabilizatorów skutkowało redukcją wielkości kuleczek tłuszczowych, a najlepsze rezultaty wykazywał dodatek hydrolizatów iota karagenu, co mogło świadczyć o ich większej plastyczności niż samego iota karagenu. Otrzymane wyniki są potwierdzeniem pierwszej i drugiej hipotezy badawczej.

Zastosowanie homogenizacji ultradźwiękowej przyczyniło się do obniżenia stabilności mieszanek lodowych w porównaniu z próbkami tylko z dodatkiem stabilizatorów. Połączenie homogenizacji ultradźwiękowej oraz wybranych stabilizatorów wpłynęło na redukcję wielkości kuleczek tłuszczowych, co było szczególnie widoczne po procesie dojrzewania (najmniejsza wartość mediany D_{50} – 9,76 μm – próbki z dodatkiem hydrolizatu enzymatycznego iota karagenu po β–galaktozydazie i po homogenizacji ultradźwiękowej).

- **Wniosek 4**

Zastosowanie wybranych stabilizatorów oraz homogenizacji ultradźwiękowej wpłynęło na zmianę struktury krystalicznej lodów wegańskich. Po okresie trzech miesięcy przechowywania stwierdzono, że najskuteczniejszym stabilizatorem w ograniczaniu procesu rekrystalizacji był hydrolizat enzymatyczny iota karagenu po β–galaktozydazie (B). Taki efekt mógł być związany z odmiennymi, w tym przypadku lepszymi, zdolnościami wiązania wody przez ten hydrolizat w porównaniu z czystym iota karagenem czy jego hydrolizatem kwasowym.

W oparciu o postawione hipotezy (2 i 3) przeanalizowano również wpływ metody homogenizacji na postęp procesu rekrystalizacji, tj. homogenizacji tradycyjnej (mechanicznej) oraz homogenizacji wspomaganej ultradźwiękami. Po 3 miesiącach przechowywania najniższą wartość parametru X_{50} (uśredniona wielkość 50% średnic kryształów lodu) – 13 μm – zanotowano dla próbki kontrolnej po zastosowaniu homogenizacji ultradźwiękowej (CU). Należy podkreślić, że w każdej próbce poddanej obróbce homogenizacji ultradźwiękowej, średnica kryształów była mniejsza niż po homogenizacji mechanicznej.

- **Wniosek 5**

Wykazano wpływ iota karagenu i jego hydrolizatów na stabilność mieszanek lodów mlecznych. Hydrolizaty iota karagenu poprawiały stabilność mieszanek lodowych w trakcie

procesu dojrzewania. Zarówno przed, jak i po procesie dojrzewania, wielkość kuleczek tłuszczowych mogła wskazywać na aglomerację tłuszczu i destabilizację próbek. W porównaniu z wegańskimi mieszankami lodowymi wartości były znacząco większe. Na podstawie zdjęć wykonanych przed i po procesie dojrzewania, można było wnioskować, że część kuleczek tłuszczu charakteryzowała się niewielkimi rozmiarami, ok. 2-5 μm. Obecność emulgatora w mieszankach lodowych mogła przyczynić się do formowania większych cząstek tłuszczu, co spowodowało wysokie wartości mediany (D_{50}) i jednocześnie destabilizację układu. Dodatek enzymatycznych hydrolizatów iota karagenu, szczególnie hydrolizatu po przemysłowej laktazie, korzystnie wpłynął na poprawę właściwości reologicznych mieszanek lodów mlecznych (największa wartość współczynnika konsystencji przed procesem dojrzewania 0,083 i po procesie dojrzewania 0,104). Uzyskane wyniki potwierdzają postawioną hipotezę 2.

- **Wniosek 6**

Zarówno dodatek stabilizatorów, jak i rodzaj homogenizacji, miały wpływ na kształtowanie struktury krystalicznej lodów mlecznych. Po 3 miesiącach przechowywania najniższą wartość parametru X_{50} (uśredniona wielkość dla 50% średnic kryształów lodu) zanotowano dla próbki z dodatkiem enzymatycznego hydrolizatu iota karagenu po hydrolizie β-galaktozydazą (B) – 17 μm, co w przypadku tej próbki oznaczało brak postępu w procesie rekrystalizacji. Jeśli chodzi o wpływ rodzaju homogenizacji, najmniejszą wartość parametru X_{50} zanotowano dla lodów bez dodatku stabilizatorów i po działaniu ultradźwiękami (CU), na poziomie 15 μm. Widoczna efektywność ultradźwięków w inhibitowaniu procesu rekrystalizacji mogła być spowodowana zjawiskiem kawitacji akustycznej mającym wpływ na podział dużych kryształów lodu w próbce.

Podsumowując, w prezentowanej pracy wszystkie założone cele badawcze zostały zrealizowane. Zgodnie z założeniami pierwszego celu badawczego określono wpływ iota karagenu oraz otrzymanych hydrolizatów na postęp procesu rekrystalizacji w układach modelowych oraz lodach spożywczych. Dwa kolejne cele dotyczyły wpływu homogenizacji ultradźwiękowej oraz wybranych stabilizatorów na właściwości fizyczne mieszanki lodowej oraz właściwości fizyczne i strukturę krystaliczną lodów mlecznych oraz bezmlecznych (wegańskich). Ich realizacja pozwoliła na określenie zależności pomiędzy właściwościami mieszanek lodowych a właściwościami produktu finalnego, czyli lodów spożywczych.

Sformułowane i zrealizowane cele badawcze umożliwiły weryfikację postawionych hipotez. Założenia hipotezy pierwszej, dotyczącej mechanizmu działania hydrolizatów iota karagenu na ograniczenie procesu rekrystalizacji, zostały zweryfikowane pozytywnie.

Hydrolizat kwasowy iota karagenu najskuteczniej ograniczał proces rekrystalizacji w roztworach modelowych sacharozy, a hydrolizat enzymatyczny po użyciu β-galatozydazy w roztworach sacharozy z kazeinianem sodu. Wpływ na skuteczność inhibitowania rekrystalizacji miała budowa otrzymanych hydrolizatów, a nie ich masa cząsteczkowa. Hipoteza druga, związana z wpływem składu mieszanek lodowych, tj. wybranych stabilizatorów, na stabilność oraz strukturę krystaliczną również została zweryfikowana pozytywnie. Dodatek iota karagenu oraz jego hydrolizatów przyczynił się do poprawy stabilności oraz właściwości reologicznych mieszanek lodowych. W przypadku produktu finalnego stabilizatory pozytywnie wpływały na puszystość lodów mlecznych oraz wegańskich. Natomiast w przypadku pozytywnej weryfikacji hipotezy trzeciej, dotyczącej wpływu ultradźwięków na właściwości mieszanek i lodów, należy wziąć pod uwagę rodzaj lodów oraz tylko wybrane właściwości fizyczne. Homogenizacja ultradźwiękowa wykazywała lepszy wpływ na stabilność niż homogenizacja mechaniczna, a w połączeniu z czasem dojrzewania również zmniejszyła wielkość kuleczek tłuszczowych. Zarówno w lodach mlecznych, jak i wegańskich, homogenizacja ultradźwiękowa skutecznie inhibitowała proces rekrystalizacji oraz wydłużała czas topnienia tych lodów.

Finalnie otrzymany produkt – lody wegańskie – mogą być alternatywą dla lodów mlecznych. Zostały wyprodukowane tą samą metodą, co ogranicza koszty związane ze zmianą technologii oraz są dobrą alternatywą dla ludzi z ograniczeniami chorobowymi (np. alergia na mleko krowie lub nietolerancja laktozy). Ponadto wykazano, że dodatek wybranych stabilizatorów w połączeniu z obróbką ultradźwiękową może korzystnie wpłynąć na optymalizację produkcji lodów spożywczych – pod względem ekonomicznym oraz środowiskowym. Niemniej jednak w dalszym ciągu istnieje potrzeba określenia dokładniejszego wpływu mechanizmu działania stabilizatorów (hydrolizatów iota karagenu) oraz ultradźwięków na strukturę krystaliczną lodów spożywczych, czy wpływu składników oraz ich proporcji w recepturze lodów.

LITERATURA

1. Abdullah, N., Wahab, N., Saruan, N., Matias-Peralta, H., Xavier, N., Muhammad, N., Talip, B.A., Bakar, M.F.A. (2018). Effect of replacing coconut milk with almond milk in spicy coconut gravy on its sensorial, nutritional and physical properties. *Materials Today: Proceedings,* 5 (10), 21919-21925.
2. Aboulfazli, F., Shori, A.B., Baba, A.S. (2016). Effects of the replacement of cow milk with vegetable milk on probiotics and nutritional profile of fermented ice cream. *LWT – Food Science and Technology,* 70 (7), 261- 270.
3. Adapa, S., Schmidt, K.A., Jeon, I.J., Herald, T.J., Flores, R.A. (2000). Mechanisms of ice crystallization and recrystallization in ice cream: a review. *Food Reviews International,* 16 (3), 259-271.
4. Ahn, N., Park, J-H., Chai, Ch., Imm, J-Y. (2022). The interaction of milk sphingomyelin and proteins on stability and microstructure of dairy emulsions. *Journal of Dairy Science*, 105(5), 3832-3845.
5. Akbari, M., Eskandari, M.H., Davoudi, Z. (2019). Application and functions of fat replacers in low-fat ice cream: A review". *Trends in Food Science & Technology,* 86, 34-40.
6. Akdeniz, V., Akalin, A. (2019). New approach for yoghurt and ice cream production: High-intensity ultrasound. *Trends in Food Science & Technology,* 86, 392–398.
7. Akın, M.B., Akın, M.S., Kırmacı, Z. (2007). Effects of inulin and sugar levels on the viability of yoghurt and probiotic bacteria and the physical and sensory characteristics in probiotic ice-cream. *Food Chemistry,* 104, 93–99.
8. Alvarez, V.B., Wolters, C.L., Vodovotz, Y., Ji, T. (2005). Physical properties of ice cream containing milk protein concentrates. *Journal of Dairy Science,* 88(3), 862-871.
9. BahramParvar, M., Tehrani, M.M., Razavi, S.M.A. (2013). Effects of a novel stabilizer blend and presence of κ-carrageenan on some properties of vanilla ice cream during storage. *Food Bioscience,* 3, 10–18.
10. Berger, K.G., White, G.W. (1971). An electron microscopical investigation of fat destabilization in ice cream. *International Journal of Food Science & Technology,* 6, 285–294.
11. Berger, K.G., Bullimore, B.K., White, G.W., Wright, W.B. (1972). The structure of ice cream. *Dairy Industries,* 419–425, 493–497.

12. Biasutti, M., Venir, E., Marino, M., Maifreni, M., Innocente, N. (2013). Effects of high pressure homogenisation of ice cream mix on the physical and structural properties of ice cream. *International Dairy Journal,* 32, 40–45.
13. Buniowska-Olejnik, M., Mykhalevych, A., Polishchuk, G., Sapiga, V., Znamirowska-Piotrowska, A., Kot, A., Kamińska-Dwórznicka, A. (2023). Study of water freezing in low-fat milky ice cream with oat-glucan and its influence on quality indicators. *Molecules*, 28, 2924.
14. Clarke, C. 2004. The science of ice cream. Cambridge, UK : The Royal Society of Chemistry.
15. Chemat, F., Zill-e-Huma, Khan, M.K. (2011). Application of ultrasound in food technology: Processing, preservation and extraction. *Ultrasonic Sonochemistry,* 18, 813–835.
16. Cheng, J., Ma, Y., Li, X., Yan, T., Cui, J. (2015b). Effects of milk protein-poly-saccharide interactions on the stability of ice cream model systems. *Food Hydrocolloids,* 45, 327-336.
17. Cheng, X., Zhang, M., Xu, B., Adhikari, B., Sun, J. (2015a). The principles of ultrasound and its application in freezing related processes of food materials: A review, *Ultrasound Sonochememistry*, 27, 576-585.
18. Chow, R., Blindt, R., Chivers, R., Povey, M. (2005). A study on the primary and secondary nucleation of ice by power ultrasound. *Ultrasonics*, 43, 227–230.
19. da Silva, L.F.R., da Silva Gomes, A., Castro, D.R.G., das Chagas do Amaral Souza, F., Mar, J.M., Souza da Silva, L., Sanches, E.A., de Araújo Bezerra, J., Bakry, A.M., Campelo, P.H. (2019). Ultrasound-assisted homogenisation and gum Arabic combined to physicochemical quality of cupuaçu juice. *Journal of Food Processing and Preservation,* 43, e14072.
20. Dłużewska, E., Gazda, B., Leszczyński, K. (2003). Wpływ wybranych hydrokoloidów polisacharydowych na jakość koncentratów lodów owocowych. *Acta Scientarium Polonorum Technologia Alimentaria*, 2, 97–107.
21. Dogan, M., Kayacier, A. (2007). The effect of ageing at a low temperature on the rheological properties of Kahramanmaras-type ice cream mix. *International Journal of Food Properties*, 10, 19–24.
22. Domian, E., Cenkier, J., Górska, A., Brynda-Kopytkowska, A. (2018). Effect of oil content and drying method on bulk properties and stability of powdered emulsions with OSA starch and linseed oil. *LWT-Food Science and Technology*, 88, 95-102.

23. Fiol, C., Prado, D., Romero, C., Laburu, N., Mora, M., Alava Inaki, J. (2017). Introduction of a new family of ice creams. *International Journal of Gastronomy and Food Science,* 7, 5-10.
24. Firouz, M.S. (2021). Application of high-intensity ultrasound in food processing for improvement of food quality. In design and optimization of innovative food processing techniques assisted by ultrasound. Developing healthier and sustainable food products; Barba, F., Cravotto, G., Chemat, F., Lorenzo Rodriguez, J.M., Sichetti Munekata, P.E., Eds.; Elsevier Inc.: Amsterdam, The Netherlands, 155–159.
25. Flores, A.A., Goff, H.D. (1999). Ice crystals size distribution in dynamically frozen model solutions and ice cream as affected by stabilisers. *Journal of Dairy Science,* 82, 1399.
26. Gaukel, V., Leiter, A., Spiess, W.E.L. (2014). Synergism of different fish antifreeze proteins and hydrocolloids on recrystallization inhibition of ice in sucrose solutions. *Journal of Food Engineering,* 141, 44-50.
27. Gelin, J.L., Poyen, L., Courthaudon, J.L., Meste, M.Le., Lorient, D. (1994). Structural changes in oil-water emulsions during the manufacture of ice cream. *Food Hydrocolloids,* 8(3-4), 299-308.
28. Ghani, N.A.A., Othaman, R., Ahmad, A., Anuar, F.H., Hassan, N.H. (2019). Impact of purification on iota carrageenan as solid polymer electrolyte. *Arabian Journal of Chemistry,* 12 (3), 370–376.
29. Goff, H.D., Kinsella, J.E., Jordan, W.K. (1989). Influence of various milk protein isolates on ice cream emulsion stability. *Journal of Dairy Science*, 72(2), 385-397.
30. Goff, H.D. (1997). Colloidal aspects of ice cream – A review. *International Dairy Journal*, 7(6-7), 363-373.
31. Goff, H.D., Verespej, E., Smith, A.K. (1999). A study of fat and air structures in ice cream. *International Dairy Journal*, 9, 817-829.
32. Góral, M., Kozłowicz, K., Pankiewicz, U., Góral, D., Kluza, F., Wójtowicz, A. (2018). Impact of stabilizers on the freezing process and physicochemical and organoleptic properties of coconut milk-based ice cream. *LWT-Food Science and Technology*, 92, 516–522.
33. Hagiwara, T., Hartel, R.W. (1996). Effect of sweetener, stabilizer, and storage temperature on ice recrystallization in ice cream. *Journal of Dairy Science,* 79, 735–744.

34. Innocente, N., Biasutti, M., Venir, E., Spaziani, M., Marchesini, G. (2009). Effect of high-pressure homogenization on droplet size distribution and rheological properties of ice cream mixes. *Journal of Dairy Science*, 92, 1864–1875.
35. Islam, Md. N., Zhang, M., Fang, Z., Sun, J. (2015). Direct contact ultrasound assisted freezing of mushroom (*Agaricus bisporus*): Growth and size distribution of ice crystals. *International Journal of Refigeration,* 57, 46-53.
36. Ismail, E.A., Al-Saleh, A.A., Metwalli, A.A.M. (2013). Effect of inulin supplementation on rheological properties of low-fat ice cream. *Life Science Journal*, 10, 1742–1746.
37. Kamińska-Dwórznicka, A., Matusiak, M., Samborska, K., Witrowa-Rajchert, D., Gondek, E., Jakubczyk, E. (2015). The influence of kappa carrageenan and its hydrolysates on the recrystallization process in sorbet (Part B). *Journal of Food Engineering*, 167, 162–165.
38. Kamińska-Dwórznicka, A., Skrzypczak, P., Gondek, E. (2016). Modification of kappa carrageenan by β-galactosidase as a new method to inhibit recrystallization. *Food Hydrocolloids,* 61, 31-35.
39. Kamińska-Dwórznicka, A., Gondek, E., Łaba, S., Jakubczyk, E., Samborska, K. (2019). Characteristics of instrumental methods to describe and assess the recrystallization process in the ice cream systems. *Foods*, 8, 117.
40. Kamińska-Dwórznicka, A., Łaba, S., Jakubczyk, E. (2022). The effects of selected stabilizers addition on physical properties and change in crystal structure of whey ice cream. *LWT-Food Science and Technology*, 154, 112841.
41. Kamińska-Dwórznicka, A., Kot, A., Jakubczyk, E., Buniowska-Olejnik, M., Nowacka, M. (2023). Effect of ultrasound-assisted freezing on the crystal structure of mango sorbet. *Crystal,* 13, 396.
42. Karlsson, A., Singh, S.K. (1999). Acid hydrolysis of sulphated polysaccharides. Delsulphation and the effect on molecular mass, *Carbohydrate Polymers,* 38, 7-15.
43. Kiran-Yildirim, B., Hale, J., Wefers, D., Gaukel, V. (2021). Ice recrystallisation inhibition of commercial κ-, ι-, and λ-carrageenans, *Journal of Food Engineering*, 290, 110269.
44. Konstanas, A., Stamford, L., Azapagic, A. (2019). Enviromental impacts of ice cream. *Journal of Cleaner Production,* 209, 259-272.
45. Koxholt, M.M.R., Eisenmann, B., Hinrichs, J. (2001). Effect of the fat globule sizes on the meltdown of ice cream. *Journal of Dairy Sciences*, 84(1), 31-37.

46. Leiter, A., Ludwig, A., Gaukel, V. (2017). Influence of heating temperature, pH and ions on recrystallization inhibition activity of κ-carrageenan in sucrose solution. *Journal of Food Engineering*, 195, 14–20.
47. Lomolino, G., Zannoni, S., Zabara, A., Da Lio, M., De Iseppi, A. (2020). Ice recrystallisation and melting in ice cream with different proteins levels and subjected to thermal fluctuation. *International Dairy Journal,* 100, 104557.
48. Mahdian, E., Karazhian, R. (2013). Effect of fat replacers and stabilizers on rheological, physicochemical and sensory properties of reduced-fat ice cream. *Journal of Agriculture, Science and Technology*, 15, 1163–1174.
49. Mendez-Velasco, C., Goff, H.D. (2012). Fat structures as affected by unsaturated or saturated monoglyceride and their effect on ice cream structure, texture and stability. *International Dairy Journal*, 24(1), 33-39.
50. Moniha, V., Alagar, M., Selvasekarapandian, S., Sundaresan, B., Boopathi, G. (2018). Conductive bio-polymer electrolyte iota-carrageenan with ammonium nitrate for application in electrochemical devices. *Journal of Non-Crystalline Solids*, 481, 424–434.
51. Moniha, V., Alagar, M., Selvasekarapandian, S., Sundaresan, B., Hemalatha, R. (2019). Development and characterization of bio-polymer electrolyte iota-carrageenan with ammonium salt for: Electrochemical Application. *Materials Today Proceeding,* 8, 449–455.
52. Mostafavi, F.S., Tehrani, M.M., Mohebbi, M. (2017). Rheological and sensory properties of fat reduced vanilla ice creams containing milk protein concentrate (MPC). *Journal of Food Measurement and Characterization,* 11, 567–575.
53. Nazarewicz, S., Kozłowicz, K., Kobus, Z., Gładyszewska, B., Matwijczuk, A., Ślusarczyk, L., Skrzypek, T., Sujka, M., Kozłowicz, N. (2022). The use of ultrasound in shaping the properties of ice cream with oleogel based on oil extracted from tomato seeds. *Applied Sciences*, 12, 9165.
54. Ndoye, T. F., Alvarez, G. (2015). Characterization of ice recrystallization in ice cream during storage using the focused beam reflectance measurement. *Journal of Food Engineering,* 148, 24-34.
55. Pal, R. (2019). Modeling of sedimentation and creaming in suspensions and pickering emulsions. *Fluids*, 4(4), 186.
56. Patel, M.R., Baer, R.J., Acharaya, M.R. (2006). Increasing the protein content of ice cream. *Journal of Dairy Science*, 89, 1400–1406.

57. Regand, A., Goff, H.D. (2003). Structure and ice recrystallization in frozen stabilized ice cream model systems. *Food Hydrocolloids,* 17, 95–102.
58. Rao, M.A. (2007). Rheology of fluid and semisolid foods: Principles and applications; Springer Science & Business Media: New York, NY, USA.
59. Seo, C.W., Oh, N.S. (2022). Functional application of Maillard conjugate derived from a κ-carrageenan/milk protein isolate mixture as a stabilizer in ice cream. *LWT-Food Science and Technology,* 161, 113406.
60. Soukoulis, Ch., Chandrinos, I., Tzia, C. (2008). Study of the functionality of selected hydrocolloids and their blends with κ-carrageenan on storage quality of vanilla ice cream. *LWT-Food Science and Technology,* 41(10), 1816-1827.
61. Spence, Ch., Navarraa, J., Youssef, J. (2019). Using ice-cream as an effective vehicle for energy/nutrient delivery in the elderly. *International Journal of Gastronomy and Food Science,* 16, 100140.
62. Taha, A., Hu, T., Zhang, Z., Bakry, A., Khalifa, I., Pan, S., Hu, H. (2018). Effect of different oils and ultrasound emulsification conditions on the physicochemical properties of emulsions stabilized by soy protein isolate. *Ultrasonics Sonochemistry,* 49, 283-293.
63. Tecson, M.G., Abad, L.V., Ebajo, V.D., Jr., Camacho, D.H. (2021). Ultrasound-assisted depolymerization of kappa-carrageenan and characterization of a degradation product. *Ultrasonics Sonochemistry*, 73, 105540.
64. Thrimawithana, T., Young, S., Dunstan, D., Alany, R. (2010). Texture and rheological characterisation of kappa and iota carrageenan in the presence of counter ions. *Carbohydrate Polymers*, 82 (1), 69–77.
65. Tüker, D.A., Dogan, M. (2021). Effect of ultrasound homogenization on the structural and sensorial attributes of ice cream: optimization with Taguchi and data envelopment analysis. *Journal of Food Measurement and Characterization,* 15, 4888-4898.
66. O'Sullivan, J., Beevers, J., Park, M., Greenwood, R., Norton, I. (2015). Comparative assessment of the effect of ultrasound treatment on protein functionality pre- and post-emulsification. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 484, 89–98.
67. O'Sullivan, J., B. Murray, C. Flynn, I. Norton. (2016a). The effect of ultrasound treatment on the structural, physical and emulsifying of animal and vegetable protein. *Food Hydrocolloids,* 53, 141-154.

68. O'Sullivan, J., Park, M., Beevers, J. (2016b). The effect of ultrasound upon the physicochemical and emulsifying properties of wheat and soy protein isolates. *Journal of Cereal Science*, 69, 77–84.
69. Zhu, Z., Zhou, Q., Sun, D-W. (2019). Measuring and controlling in frozen foods: A review of recent developments. *Trends in Food Science & Technology,* 90, 13-25.
70. Wang, K., Li, G., Zhang, B. (2018). Opposite results of emulsion stability evaluated by the TSI and the phase separation proportion. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 558, 402-409.
71. Vanga, K.S., Raghavan, V. (2018). How well do plant based alternatives fare nutritionally compared to cow's milk?. *Journal of Food Science and Technology,* 55(1), 10-20.
72. Voronin, G.L., Roberts, R., Felix, T.L., Coupland, J.N., Harte, F.M. (2020). Effect of high-pressure-jest processing on the physicochemical properties of low-fat ice cream mix. *Journal of Dairy Science*, 103, 6003–6014.
73. Xu, B-g., Zhang, M., Bhandari, B., Cheng, X-f., Sun, J. (2015). Effect of ultrasound immersion freezing on the quality attributes and water distributions of wrapped red radish. *Food Bioprocess Technology,* 8, 1366-1376.
74. Internet1:https://www.grandviewresearch.com/industryanalysis/icecreammarket?fbclid=IwAR1TwreI9IFlQ0PqP_0qA0Mlky5y1Ai6FDUlLCYy5MwFHYAKwYHT4jGrOk [dostęp: 25.08.2022].
75. Internet 2: https://ec.europa.eu/eurostat/web/products-eurostat-news/w/ddn-20230814-1 [dostęp 26.09.2023].

Życiorys naukowy

Dane personalne: Anna Kot

Wykształcenie:

10/2019- 09/2023	Szkoła Doktorska SGGW **Studia stacjonarne III°** Dyscyplina: Technologia żywności i żywienie
10/2019-12/2019	Polski Instytut Dietetyki **Dietetyk**
02/2016-07/2017	Szkoła Główna Gospodarstwa Wiejskiego w Warszawie Kierunek: technologia żywności i żywienie człowieka **Studia stacjonarne II°**
10/2012-02/2016	Szkoła Główna Gospodarstwa Wiejskiego w Warszawie Kierunek: technologia żywności i żywienie człowieka **Studia stacjonarne I°**

Publikacje naukowe:

1. **Kot, A.,** Szulc K., Kamińska-Dwórznicka, A. (2019). Właściwości sproszkowanej żywności dla dzieci. *Przemysł Spożywczy,* 73, 22-26.
2. Kamińska-Dwórznicka, A., Janczewska-Dupczyk, A., **Kot, A.,** Łaba, S., Samborska, K. (2019). The impact of iota and kappa carrageenan addition on freezing process and ice crystals structure of strawberry sorbet frozen by various methods. *Journal of Food Science,* 85(1), 50-56.
3. **Kot, A.,** Kamińska-Dwórznicka, A., Barańska, A. (2020). Study of the properties of vegan ice cream based on almond drink. *Zeszyty Problemowe Postępów Nauk Rolniczych*, 600, 21-30.
4. Barańska, A., **Kot, A.,** Samborska, K. (2020). Ultrafiltration as a method to obtain sugar reduced cloudy juices – a research on juice's properties. *Zeszyty Problemowe Postępów Nauk Rolniczych*, 600, 3-11.
5. Kamińska-Dwórznicka, A., **Kot, A.,** Samborska, K. (2021). Ice recrystallization in model glucose/fructose and honey powder solutions as affected by selected stabilizers. *Journal Of Food Processing And Preservation*, 45 (10), 1-10.

6. **Kot, A.,** Kamińska-Dwórznicka, A., Galus, S., Jakubczyk, E. (2021). Effects of different ingredients and stabilisers on properties of mixes based on almond drink for vegan ice cream production. *Sustainability,* 13 (21), 1-17.

7. **Kot, A.,** Kamińska-Dwórznicka, A. Antczak, A., Jakubczyk, E. Matwijczuk, A. (2022). Effect of ι-carrageenan and its acid hydrolysates on ice crystal structure changes in model sucrose solution. *Colloids and Surfaces A-Physicochemical and Engineering Aspects,* 643, 1-12.

8. Kot, A., Kamińska-Dwórznicka, A., Jakubczyk, E. (2022). Study on the influence of ultrasound homogenisation on the physical properties of vegan ice cream mixes. *Applied Sciences,* 12(17), 8492.

9. Jakubczyk. E., Kamińska-Dwórznicka. A., **Kot. A.** (2022). The rheological properties and texture of agar gels with canola oil effect of mixing rate and addition of lecithin. *Gels,* 8 (11), 1-15.

10. Kot, A., Kamińska-Dwórznicka, A. (2022). Vegan ice cream: A review. *Przemysł Spożywczy,* 12(76), 30-34.

11. Kamińska-Dwórznicka, A., **Kot, A.,** Jakubczyk, E., Buniowska-Olejnik, M., Nowacka, M. (2023). Effect of ultrasound-assisted freezing on the crystal structure of mango sorbet, *Crystals,* 13 (3), 396, 1-13.

12. Buniowska-Olejnik, M., Mykhalevyvh, A., Polishchuk, G., Sapiga, V., Znamirowska-Piotrowska, A., **Kot, A.,** Kamińska-Dwórznicka, A. (2023). Study of water freezing in low-fat milky ice cream with oat β-glucan and its influence on quality indicators. *Molecules,* 28 (7), 2924, 1-17.

13. Kot, A., Jakubczyk, E., Buniowska-Olejnik, M., Kamińska-Dwórznicka, A. (2023). The effect of iota carrageenan and its hydrolysates on the stability of milk ice cream mixes. *Polish Journal of Food and Nutrition Science*, 73(2), 196-204

14. Kot, A., Kamińska-Dwórznicka, A., Jakubczyk, E. (2023). The effectiveness of ultrasound homogenisation on the recrystallisation process in milk ice cream. *Applied Sciences*, 13(13), 7561,1-16.

15. Kot, A., Kamińska-Dwórznicka, A. (2023). The effectiveness of ultrasound homogenization on the recrystallisation during the storage of vegan ice cream. *Journal of Food Process Engineering,* e14472, 1-15.

<u>Sumaryczny IF: 37,289, Sumaryczna punktacja MEIN: 990 pkt, h-index: 4</u>

Udział w konferencjach:

1. Proceedings of the 8th International Conference On The Quality And Safety On Food Production Chain, Wroclaw, 20-21.06.2018. E-poster: The physicochemical properties of spray-dried infant milk (Karolina Szulc, **Anna Kot**).
2. Biologically Active Compounds in Food, 3rd International Conference, 19-20.09.2019, Łódź. Poster: Characteristic of freezing kinetics of model glucose-fructose and natural honey solutions with the addition of chosen hydrocolloids (Anna Kamińska-Dwórznicka, Marta Błaszczak, **Anna Kot,** Katarzyna Samborska, Ewa Gondek)
3. Żywność. Żywienie. Rynek. Innowacje w nauce i praktyce"- Wydział Żywienia Człowieka, SGGW 14.11.2019r, Warszawa. Poster: Badanie właściwości fizyko-chemicznych i struktury krystalicznej lodów wegańskich na bazie napoju migdałowego (**Anna Kot,** Anna Kamińska-Dwórznicka, Karolina Świderek, Alicja Barańska).

   Poster: Badanie wpływu rodzaju nośnika oraz rozpuszczalnika na proces suszenia rozpyłowego miodu wielokwiatowego (Alicja Barańska, Magda Konachowicz, **Anna Kot,** Aleksandra Jedlińska, Katarzyna Samborska).
4. „Techniki, technologie, badania w przemyśle rolno-spożywczym" –Sieć Badawcza Łukasiewicz –Przemysłowy Instytut Maszyn Rolniczych, 05.12.2019r., Poznań. Poster: Wpływ zmiennej temperatury na rekrystalizację lodu w przechowywanych lodach przemysłowych (**Anna Kot,** Anna Kamińska-Dwórznicka, Alicja Barańska, Kinga Zakrzewska).

   Poster: Badanie wpływu lepkości roztworu na przebieg procesu suszenia rozpyłowego oraz właściwości otrzymanych proszków (Alicja Barańska, Monika Kropidłowska, **Anna Kot,** Aleksandra Jedlińska, Katarzyna Samborska).
5. IV edycji Ogólnopolskiej Konferencji Naukowej „Nauka Okiem Młodego Naukowca",06.06.2020 r., e-konferencja Łódź. Poster: Comparison of the influence of the iota and kappa carrageenan on the recrystallization processes in ice cream based on almond drink (**Anna Kot,** Anna Kamińska-Dwórznicka, Karolina Świderek, Alicja Barańska).

   Poster: Low temperature honey spray drying with milk powder as a carrier agent to obtain clean label product (Alicja Barańska**,** Aleksandra Jedlińska, **Anna Kot,** Małgorzata Ignaczewska, Katarzyna Samborska).

6. XXV Jubileuszowa Sesja Naukowa Sekcji Młodej Kadry Naukowej PTTŻ „PRZYSZŁOŚĆ W ŻYWNOŚCI –ŻYWNOŚĆ W PRZYSZŁOŚCI”, 21-22.05.2021, e-konferencja Wrocław. Prezentacja: Wpływ homogenizacji mechanicznej oraz ultradźwiękowej na właściwości wegańskiej mieszanki lodziarskiej (**Anna Kot,** Anna Kamińska-Dwórznicka).
7. Euro-Aliment 2021, THE 10TH INTERNATIONAL SYMPOSIUM 7-8.10.2021 r., Galați, Romania, (online). Prezentacja: Analysis of the emulsion properties on the example of a milk ice cream mix for ice cream production (**Anna Kot,** Anna Kamińska-Dwórznicka).
8. XXVI Sesja naukowa Młodej Kadry Naukowej „Żywność dzisiaj-lokalna czy globalna”.21-22.05.2022 r., Poznań. Prezentacja: The influence of iota carrageenan on the physical properties of milk ice cream mixes (**Anna Kot,** Anna Kamińska-Dwórznicka).
9. Sympozjum Inżynierii Żywności 29-30.06.2022, **Poster:** Badanie właściwości stabilizujących enzymatycznych hydrolizatów iota karagenu (Anna Kamińska-Dwórznicka, **Anna Kot,** Paulina Zwierzchowska).
10. XX Konferencja Naukowo-Techniczna Budowa i Eksploatacja Maszyn Przemysłu Spożywczego „BEMS 2022”, 20 – 23.09.2022 r., Pułtusk, Prezentacja: Wpływ zamrażania wspomaganego ultradźwiękami na zmiany struktury krystalicznej sorbetu z mango (Anna Kamińska-Dwórznicka, **Anna Kot**, Wojciech Tymofijewicz).
11. XXIX Konferencja Naukowa z cyklu „Postęp Naukowo-Techniczny i Organizacyjny w Rolnictwie”, 6-10.02.2023 r., Zakopane. Prezentacja: Wpływ beta glukanu na właściwości fizyczne i strukturę krystaliczną lodów na bazie napoju migdałowego (Anna Kamińska-Dwórznicka, **Anna Kot,** Magdalena Buniowska-Olejnik).
12. Food Symposium 3.0 - LSU AgCenter / MENDELU / SGGW, 20-24.03.2023 r., Baton Rouge, Louisiana (online). **Poster:** The effectiveness of ultrasound homogenisation on the recrystallisation process in vegan ice cream (**Anna Kot,** Anna Kamińska-Dwórznicka).
13. XXVII Sesji Naukowej Sekcji Młodej Kadry Naukowej „Żywność. Nauka. Technologia. Jakość”, 11-12.05.2023 r., Warszawa, Prezentacja: The effect of ultrasound homogenization on the recrystallization process in milk ice cream (**Anna Kot**, Anna Kamińska-Dwórznicka).

Działalność organizacyjna:

- Członkostwo w polskim Towarzystwie Technologów Żywności PTTŻ - od 2019 do chwili obecnej
- Członkostwo w IFT Student Association, w latach 2019-2020
- IT FOOD RIS Summer School Targeted Nutrition (online) 09-10.2021
- Pomoc w realizacji prac dyplomowych pod kierunkiem dr hab. Anny Kamińskiej-Dwórznickiej, prof. SGGW
- Uczestnictwo w Dniach SGGW 2022
- Uczestnictwo w Dniach Otwartych SGGW 2023

Nagrody:

- Nagroda Rektora – Zespołowa Stopnia III za osiągnięcia badawcze – 04.10.2023 r.

PUBLIKACJE WCHODZĄCE W SKŁAD MONOTEMATYCZNEGO CYKLU PT.

Wpływ składu surowcowego, obróbki ultradźwiękami i nowych dodatków stabilizujących na właściwości fizyczne mieszanek lodowych oraz strukturę krystaliczną lodów spożywczych

Colloids and Surfaces A: Physicochemical and Engineering Aspects 643 (2022) 128744

Contents lists available at ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

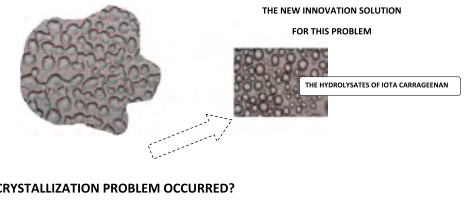
journal homepage: www.elsevier.com/locate/colsurfa

Effect of ι-carrageenan and its acidic and enzymatic hydrolysates on ice crystal structure changes in model sucrose solution

Anna Kot [a,*], Anna Kamińska-Dwórznicka [a], Andrzej Antczak [b], Ewa Jakubczyk [a], Arkadiusz Matwijczuk [c]

[a] Department of Food Engineering and Process Management, Institute of Food Sciences, Warsaw University of Life Sciences (WULS-SGGW), Nowoursynowska 159C, 02-776 Warsaw, Poland
[b] Department of Wood Science and Wood Preservation, Institute of Wood Sciences and Furniture, Warsaw University of Life Sciences (WULS-SGGW), Nowoursynowska 159, 02-776 Warsaw, Poland
[c] Department of Biophysics, Faculty of Environmental Biology, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
ι-carrageenan
Acid hydrolysis
Enzymatic hydrolysis
Recrystallisation
Model solutions
Fourier Transform Infrared Spectroscopy FTIR

ABSTRACT

The aim of the study was to compare the effects of ι-carrageenan and its hydrolysates on ice recrystallisation inhibition (IRI) in model solutions. Acid hydrolysis of ι-carrageenan was carried out using hydrochloric acid (HCl). Enzymatic hydrolysis was conducted using the enzymes β-galactosidase and commercial lactase. Hydrolysates were used as a stabiliser in model system solutions. The recrystallisation process was analysed on the basis of images of ice crystals in 50% sucrose and 50% sucrose with the addition of sodium caseinate after 24, 48, 72 and 96 h of storage at − 8 °C. The molecular mass after hydrolysis of ι-carrageenan was decreased by around 16% after hydrolysis with β-galactosidase use, around 3% after hydrolysis with commercial lactase use and 24% after acid hydrolysis. Furthermore, using the hydrolysates of ι-carrageenan had a strong impact on the recrystallisation process. Significant retardation of recrystallisation in the sucrose solution was observed for the addition of both types of hydrolysates (after acid and enzymatic hydrolysis). However, in sucrose with the addition of sodium caseinate, the oligosaccharides obtained after hydrolysis with commercial lactase use were more effective in recrystallisation inhibition – the average diameter of ice crystals was 16.92 μm. The spectra obtained with Fourier Transform Infrared Spectroscopy (FTIR) show changes in the absorption bands, which confirms that functional groups changes could lead to the IRI activity of gained hydrolysates.

* Correspondence author.
E-mail address: anna_kot1@sggw.edu.pl (A. Kot).

https://doi.org/10.1016/j.colsurfa.2022.128744
Received 11 December 2021; Received in revised form 23 February 2022; Accepted 8 March 2022
Available online 10 March 2022
0927-7757/© 2022 Elsevier B.V. All rights reserved.

1. Introduction

Recrystallisation is an issue that generates significant concern in the ice cream industry. It may be caused by fluctuating temperatures and additionally during long-term storage above the glass transition point [1,2]. Recrystallisation leads to many ice crystal modifications, for instance, changes in shape, number or an increase in them. This undesirable process is predominantly caused by two mechanisms: accretion and migration. Accretion consists of combining two or more adjacent ice crystals to form a large, single one. Simultaneously, migration is based on the melting of smaller ice crystals and the movement of the melted liquid to the surface of larger ice crystals [3–5]. The mean size of ice crystals around 10–20 μm is pivotal to provide the product with its eligible texture. For instance, ice crystals estimated at more than 50 μm may be detected during consumption as a kind of sandiness. Hence, it is vital to reduce or eliminate these phenomena, so it is advisable to use hydrocolloid stabilisers [6,7]. Hydrocolloid stabilisers have little impact on the initial size distribution in ice cream production, and sometimes no effect is detectable. Nonetheless, the stabilisers limit the growth of ice crystals during the recrystallisation process. Moreover, in the ice cream industry, the functions of stabilisers are connected with producing smoothness in texture during eating, reducing the rate of meltdown and slowing down moisture migration out of ice cream during storage. Moreover, stabilisers may control the incorporation of air in the factory freezer and help produce a stable foam [3,5,6]. Also, in the dairy industry crystallisation and recrystallisation of lactose create a big problem. This process can also be controlled by the addition of stabilisers. The difference between ice and lactose crystals can be detected during consumption when the ice crystals are disappearing while lactose crystals are still perceptible [8]. Moreover, the rate of crystallisation is determined by numerous effects such as crystalliser design, parameters or impurities on the kinetics of the process. The major issue of industrial lactose crystallisation is to obtain small crystals and limit the separation of the downstream process, to achieve low recovery [9].

Carrageenans as sulphated polysaccharides are widely used due to their remarkable gelling capacities. Carrageenans are classified into different groups depending on the number and position of sulphates esters. Three primary forms of carrageenan have been identified: kappa, iota and lambda [10,11]. Carrageenans contain D-galactose and 3, 6-anhydro-D-galactose units which are bonded together with α-1,3 and β-1,4 linkages, in attendance of sulphate groups [1,12,13].

The influence of carrageenans as hydrocolloids on ice recrystallisation is still being investigated and generates controversial discussion. A plethora of studies shows that these hydrocolloids limit the rate of ice crystal growth during the recrystallisation process. Nonetheless, in other research no or only a small effect of hydrocolloids on the recrystallisation process was observed. Moreover, it is considered that ice recrystallisation inhibition activity (IRI) of hydrocolloids is associated with a decrease in the mobility of water molecules because of water-binding or steric hindrance [2,5,14]. Additionally, according to Bahramparvar and Mzaheri Tehrani (2011), the IRI activity of hydrocolloids that were used as stabilisers in ice cream might be explained by viscosity and molecular mobility, hydrocolloid phase separation or cryo-gelation formation [1, 15]. ι-carrageenan contains two sulphate groups per unit and its properties depend on it. For instance, the gelation properties are different in all forms of carrageenans due to the number and location in the linkage of sulphate groups. Additionally, the presence of the 3,6-anhydro-bridge in κ- and ι- carrageenan contribute to forming a strong gel [1,12,13]. Based on the gelation process of polymers, it has been shown that ι-carrageenan is capable of forming a soft elastic gel in the presence of calcium ions. Calcium is a divalent cation that has the ability to create intra-molecular combinations between the sulphate groups of anhydro-D-galactose and D-galactose of ι-carrageenan. Additionally, ι-carrageenan hydrates at ambient temperature in water, but hydration with the addition of salts may raise the gel point. The solution is converted into the gel with distinctive yield strength. Regarding syneresis properties, iota gels do not exhibit such capacity [1,16].

In the native form, the molecular weight of carrageenans is estimated at over 100 kDa. Due to the chemical or heat hydrolysis and specific enzymes, these polysaccharides are degraded into smaller fragments [13]. The degraded carrageenans may exhibit thermal, oxidative and hydrolytic abilities. Furthermore, the functionality of these new oligosaccharides, such as gel-forming or viscosity-enhancing, may help to create a new product [17].

Hydrolysis of carrageenans is an immensely desirable process that allows control of physical properties of solutions or gels, which are determined by molecular mass distribution [18]. Modifying the repeating unit in the composition is possible during the acid hydrolysis for sulphated polysaccharides such as carrageenans. Thus, reducing the sulphate groups will cause more flexibility and less extension to a polymer. It may suggest that such a desulphated polysaccharide molecule can behave efficaciously in solution compared to different molecules with the same degree of polymerisation and untouched sulphate groups [17]. Additionally, according to research by Kiran-Yildirim et al. [1], the structural features of carrageenans such as the amount and position of the sulphate groups may be important for the IRI activity. For example, κ-carrageenan showed a higher IRI activity in comparison to ι-carrageenan samples [1]. On the other hand, enzymatic hydrolysis of carrageenans, which is conducted by specific enzymes, may be more lucrative than acid hydrolysis. Because enzymes are highly specific to substrates, they generate oligosaccharides equivalent in molecular mass. The enzyme which degrades ι-carrageenan is called ι-carrageenase. It belongs to the group of endohydrolase enzymes, by which the internal β(1−4) linkages are cleaved into oligo-carrageenans. This degradation is provided by breaking these linkages instead of hydrolysing units from the ends. As a result, such enzymatic hydrolysis generates a group of homologous and even-number oligosaccharides [12,19].

The aim of this study was to perform acid and enzymatic hydrolysis of ι-carrageenan. The next step was to investigate their properties that inhibit the recrystallisation process in model sucrose solutions. Pure sucrose and sucrose with sodium caseinate were used to study the stabilizing properties in the presence of milk proteins. According to the satisfactory results of hydrolysis of κ-carrageenan described by Kamińska-Dwórznicka et al. [2,20], the same method was used. Due to the fact that iota-carrageenase is not available for purchase, different and alternative enzymes were used to conduct the hydrolysis. The commercial lactase and in comparison, β-galactosidase were applied as the cheapest and more available substitute. In order to confirm that IRI activity depends on the functional groups position changes, as well as the spectroscopic changes observed in the samples, FTIR infrared spectroscopy was also used. A shift in the absorption bands (e.g. 3350, 1020, 914 cm^{-1}) as well as a change in their intensity confirm our assumptions.

2. Materials and methods

2.1. Materials

ι-carrageenan powder samples were obtained from Sigma-Aldrich. The enzymes for enzymatic hydrolysis were: β-galactosidase which was obtained from Sigma-Aldrich and commercial lactase from Serowar s.c. Szczecin. For acid hydrolysis, 0.1 M hydrochloric acid and 0.1 M sodium hydroxide obtained from Chempur were used.

2.2. Sample preparation

2.2.1. Enzymatic hydrolysis of ι-carrageenan

ι-carrageenan was first dissolved in distilled water to obtain a 0.4 mg/ml solution and heated up to 40 °C. This hydrolysis was carried out using two different enzymes. The first one was β-galactosidase (1000 U/mg, from *Escherichia coli*, soluble in glycerol and TRIS buffer (pH 7.4)). It was conducted for 2, 24, 48 and 72 h, at 37 °C. Reference samples

(without incubation) and samples of degraded ι-carrageenan were taken, neutralised at 48 °C for 5 min and then cooled to stop any further reactions (Sigma-Aldrich – Product Information; Kamińska-Dwórznicka et al. [2]). After repeating the solution of ι-carrageenan, the second enzyme was used – commercial lactase (activity: min. 5200 NLU/g; 1 ml/1 l solution; 1 drop/50 ml solution). It was conducted for 24 h, at 5 °C. Reference samples (after time 0) and samples of degraded ι-carrageenan were taken, neutralised at 48 °C for 5 min and then cooled to stop any further reactions (Serowar- Product Information). For both treatments samples were stored frozen at − 18 °C and thawed just before analysis.

2.2.2. Acid hydrolysis of ι-carrageenan

ι-carrageenan was first dissolved in distilled water to obtain a 10 mg/ml solution and heated up to 40 °C. This prepared solution was mixed with 0.1 M hydrochloric acid to reach a pH level of 3, measured by an ELMETRON CPC-501 pHmeter. In order to prepare a reference sample (after time 0), 25 ml of the prepared solution (10 mg/ml) was taken and quickly neutralised with a 0.1 M NaOH solution to obtain a pH level at 7. The rest of the solution was placed on a mechanical heater (RCT Basic IKAMAG) preheated to 60 °C, with a magnetic stirrer (2 rpm). Then, samples were taken after 1 and 3 h, neutralised (the same as in the reference sample) and cooled. Finally, samples were stored frozen at − 18 °C and thawed prior to analysis [20].

2.2.3. SEC analysis

The experiments of all types of hydrolysis and each time were repeated three times and before chromatography three samples from each variant and each repeat (including reference samples) were prepared. The molecular mass distribution was estimated through size-exclusion chromatography (SEC) after hydrolysis. Polymer degradation was followed by SEC on a Shimadzu liquid chromatography system equipped with a RID-10A detector, an LC-20 CE pump, a CTO-20A heater and a Phenomenex column (Phenomenex USA). Narrow molecular mass distribution dextran standards were used in the calibration from Polymer Laboratories Ltd (Minneapolis, MN, USA). Results of molecular mass are presented as mean ± standard deviations (Table 1, Figs. 1, 2 and 3). The influence of hydrolysis type and time on the molecular mass was used to establish the significance of differences among the mean values by one-way analysis of variance (ANOVA) and Tukey's test. The data were analysed using the Statgraphics Centurion XV program, version 15.01.02 (StatPoint Inc., Warrenton, USA).

Table 1
Average molecular mass of ι-carrageenan and its hydrolysates after hydrolysis process.

Type of hydrolysis	Time of hydrolysis (h)	Molecular mass (Da)
Acid hydrolysis	0	$1.94 \times 10^6 \pm 0.31 \times 10^6$ a
	1	$1.73 \times 10^6 \pm 0.29 \times 10^6$ a
	3	$1.48 \times 10^6 \pm 0.20 \times 10^6$ b
Enyzmatic B-galactosidase hydrolysis	0	$3.80 \times 10^6 \pm 0.63 \times 10^5$ a
	24	$3.70 \times 10^6 \pm 0.75 \times 10^5$ a
	48	$3.40 \times 10^6 \pm 0.10 \times 10^6$ b
	72	$3.20 \times 10^6 \pm 0.45 \times 10^5$ b
Enyzmatic commercial lactase hydrolysis	0	$3.60 \times 10^6 \pm 0.44 \times 10^5$ a
	24	$3.50 \times 10^6 \pm 0.40 \times 10^5$ a

Values represent means ± standard deviations. Mean values followed by the same small letter (in the column, for the same type of hydrolysis) do not differ significantly at $\alpha = 0.05$.

2.2.4. Preparation of model sucrose solutions

50 ml of model 50% sucrose solutions and 50% sucrose with the addition of sodium caseinate were prepared (the amount of sodium caseinate used was 2.6 g/100 ml) without any addition and with the addition of:

- 0.01% ι-carrageenan
- 0.005% of hydrolysates after 3 h of ι-carrageenan acid hydrolysis
- 0.005% hydrolysates after 72 h of ι-carrageenan enzymatic hydrolysis of β-galactosidase
- 0.005% hydrolysates after 24 h of ι-carrageenan enzymatic hydrolysis of commercial lactase

According to the previous research conducted on κ-carrageenan by Kamińska-Dwórznicka et al. [2], Gaukel et al. [5], Kamińska and Gaukel [21], concentrations of ι-carrageenan (0.01%) and its hydrolysates (0.005%) were prepared. 0.01% of κ-carrageenan is the minimal concentration that could influence the recrystallisation process based on previous studies [2]. The experiment by Kamińska-Dwórznicka et al. [2] was provided with the addition of 0.005% κ-carrageenan hydrolysates (concentration obtained after preparation processes). Based on that it was assumed that the minimal amount of ι-carrageenan also could influence the recrystallisation process in examined samples. Also, assuming the stronger effect of the hydrolysates of ι-carrageenan, such an amount was used.

Sodium caseinate was used instead of for example skim milk powder because of the more effective capacity of sodium caseinate to link with water molecules. Due to the specific functionalities of sodium caseinate, such as thickening and gel formation, caseinate sodium was used to obtain the desired structure and sensory characteristics of the product. Additionally, sodium caseinate may constitute a highly dense and viscoelastic network. In ice cream, it was reported that the use of sodium caseinate may positively influence whipping time and also increase the initial overrun [22,23].

2.3. Microscopic analysis of the recrystallisation process in model sucrose solutions

Forty microliters of each sample were dropped on the space between two microscope coverslips (fixed earlier to the object slide), covered with an additional microscope coverslip and sealed with silicone. Subsequently, they were subjected to fast freezing at − 70 °C in a quick air freezer (National Lab ProfiMaster), for one hour. This kind of fast freezing transformed the aqua solution into a glassy state. It was found that at the temperature of − 70 °C 1 million small crystals (diameter of 2–3 μm) formed in 1 cm^3 during 1 h [24].

Then the object slides were stored at − 8 °C for 24 h in the storage chamber. Under this condition, the resulting ice volume fraction is sufficient for observation of ice crystal changes during storage [5,25]. The solution was prepared for each of the treatments in triplicate, according to previous studies [5,20,26].

Recrystallisation was then analysed based on the images of ice crystals taken after 24, 48, 72 and 96 h of storage at − 8 °C. A microscope (Nikon Alpha Phot-2) with the cooling system Linkam Scientific PE 94 and a camera (Nikon DS-Fi1) were used. Images were taken at − 8 °C (cooling system). The obtained images were analysed using NIS Elements D software. Three hundred crystals were marked for a particular sample, and area, equivalent diameter and standard deviation were calculated using the NIS Elements D Imaging software (ver. 3.00, Nikon). Then the frequency distribution of crystal size was computed using Microsoft Excel 2011 macro data analysis. The relative frequency of any class interval was calculated as the number of crystals in the class (class frequency) divided by the total number of crystals and expressed as percentages (Fig. 4, Fig. 5, Fig. 6, Fig. 7). The mean diameter (D_A) and

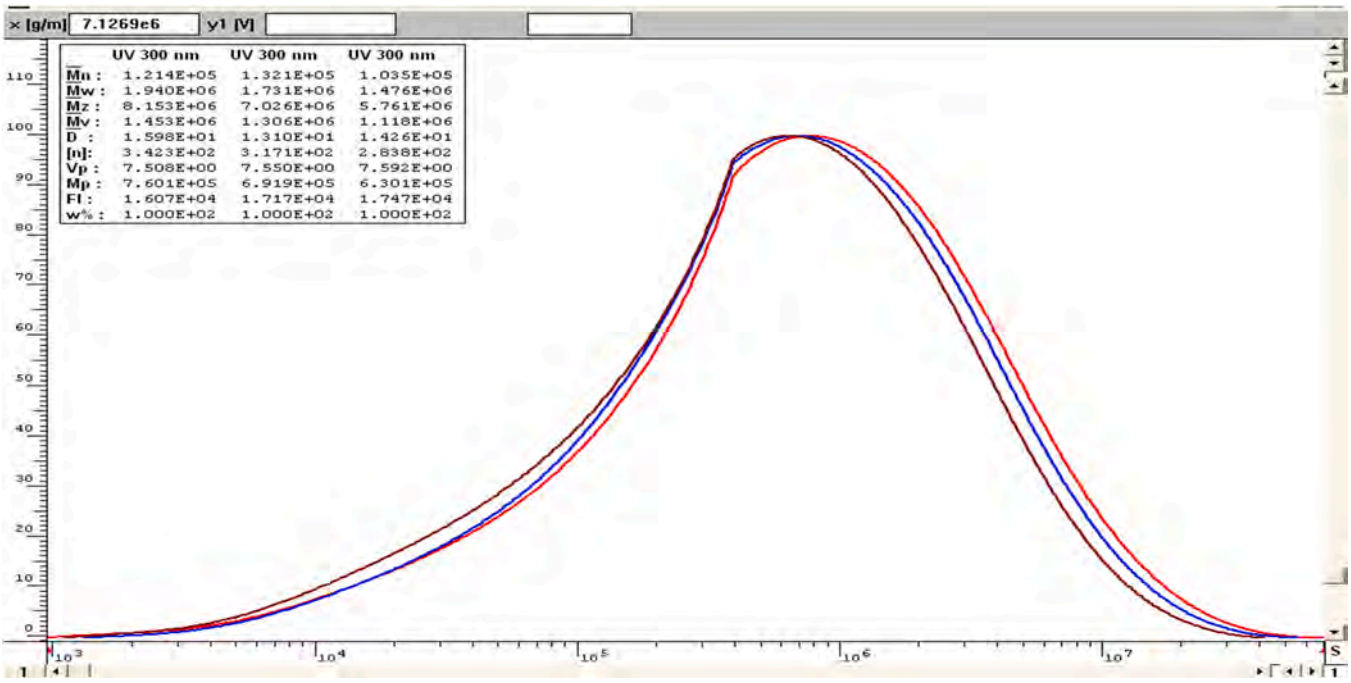


Fig. 1. Results of SEC analysis after acid hydrolysis of ι-carrageenan.

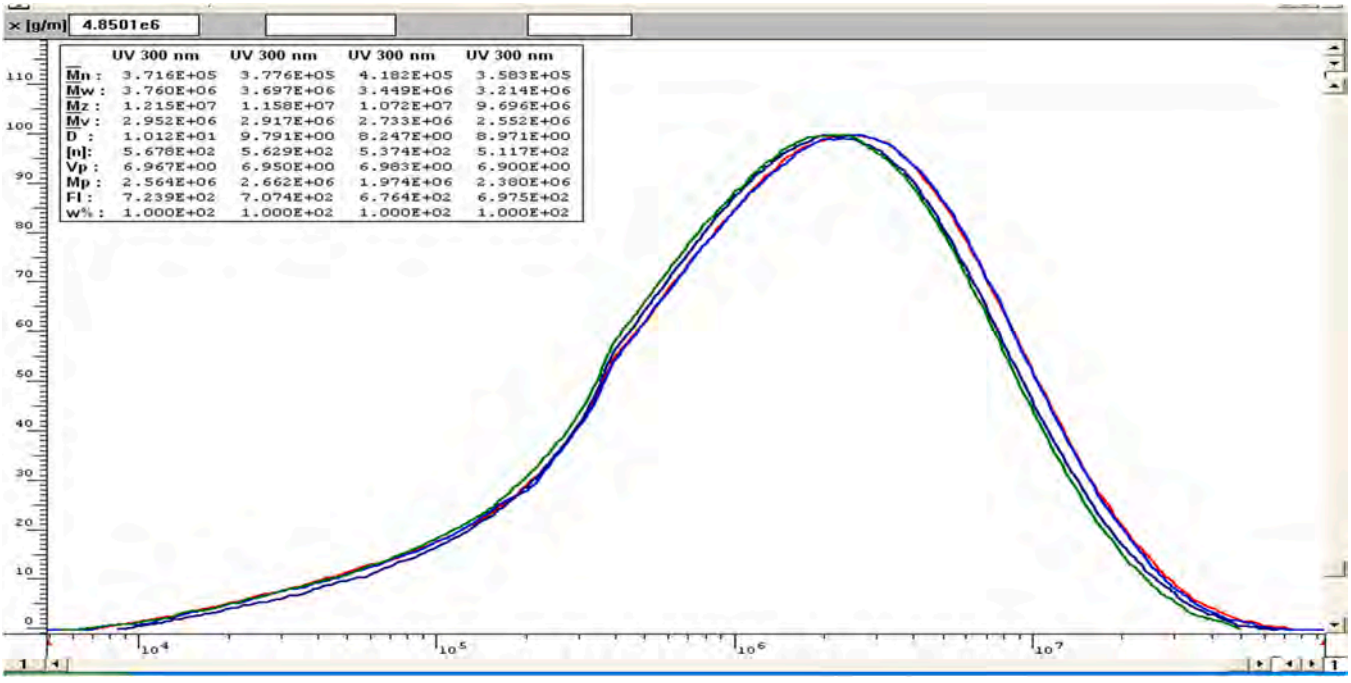


Fig. 2. Results of SEC analysis after enzymatic hydrolysis of ι- carrageenan by β-galactosidase.

standard deviation (S_D) of each class were also calculated, according to the method described by Kamińska-Dwórznicka et al. [26] and Kamińska-Dwórznicka et al. [20] (Table 2; Table 3). The ice crystal size distribution in model sucrose solutions was characterised based on the model with the cumulative distribution of equivalent diameters (Figs. 4–7), which were prepared in OrginLab 2022. Based on that, the parameter X_{50} was analysed as a value of ice crystal diameter at 50% of the cumulative distribution of the sample [6].

2.4. FTIR analysis

Samples after hydrolysis and also pure carrageenan solution were stored frozen at − 18 °C. The materials were then freeze-dried for 24 h with the application of an Alpha 1–4 freeze-dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) under the pressure 63 Pa and at a shelf temperature of 30 °C.

Measurements of ATR-FTIR spectra corrected for the background (25 scans for each sample) were obtained with the use of a HATR Ge trough (45° cut, yielding 10 internal reflections) crystal plate at 20 °C, and were recorded with a 670-IR spectrometer (Agilent, USA). The instrument

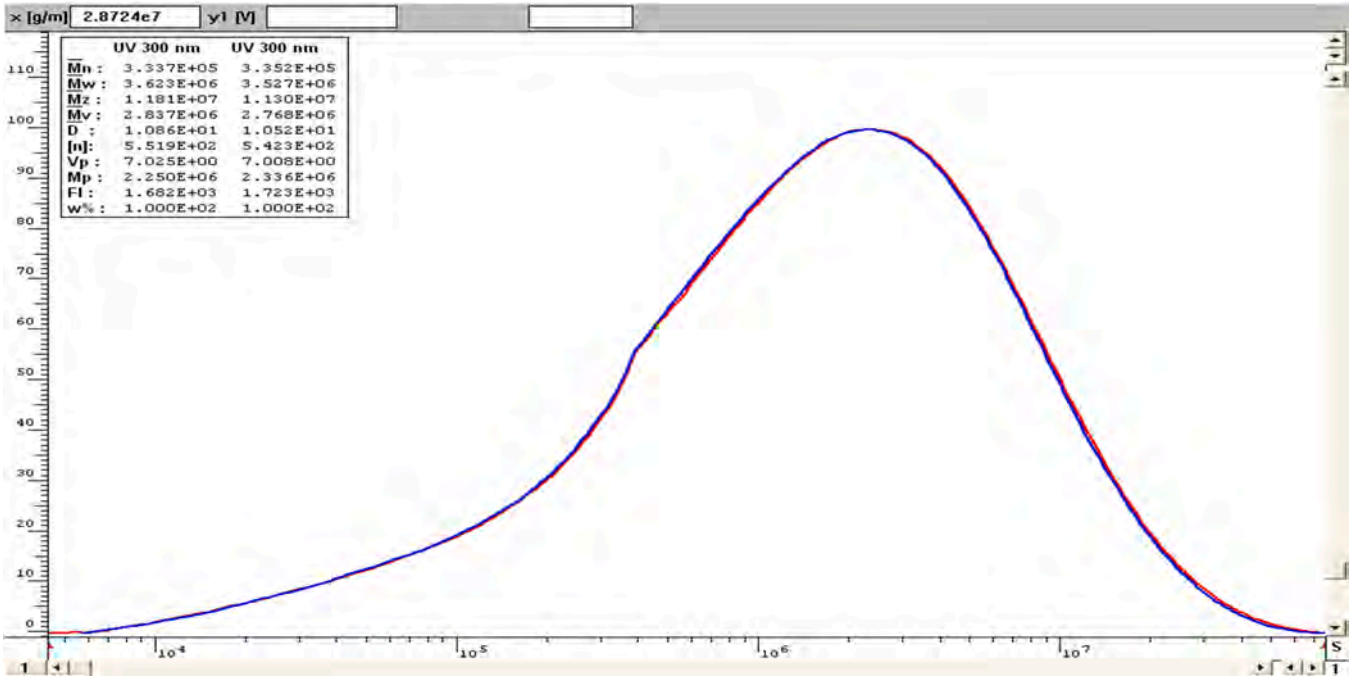


Fig. 3. Results of SEC analysis after enzymatic hydrolysis of ι- carrageenan by commercial lactase.

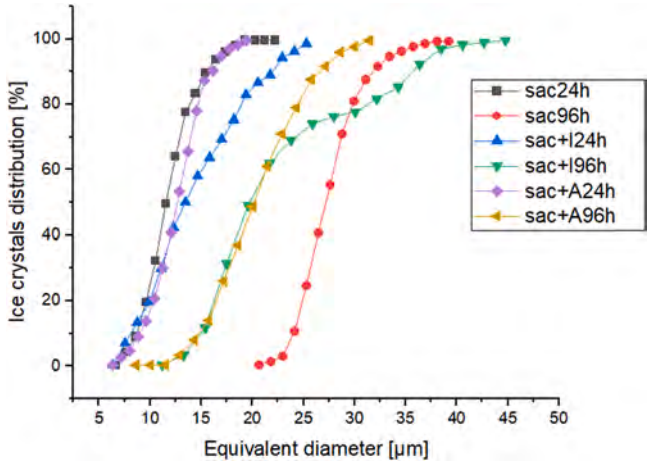


Fig. 4. Ice crystal size distribution in model sucrose solutions with ι-carrageenan and its acid hydrolysates: after 24 h and after 96 h of storage at − 8 °C. Explanatory notes: 50% suc+I – 50% sucrose solution with the addition of ι-carrageenan; 50% suc+A – 50% sucrose solution with the addition of the acid hydrolysates of ι-carrageenan.

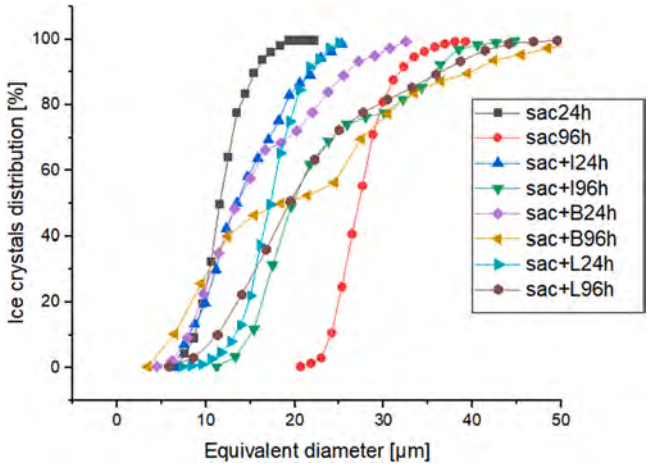


Fig. 5. Ice crystal size distribution in model sucrose solutions with ι-carrageenan and its enzymatic hydrolysates: after 24 h and after 96 h of storage at − 8 °C.Explanatory notes: 50% suc+I – 50% sucrose solution with the addition of ι-carrageenan; 50% suc+B – 50% sucrose solution with the addition of the enzymatic β-galactosidase hydrolysates of ι-carrageenan; 50% suc+L – 50% sucrose solution with the addition of the enzymatic commercial lactase hydrolysates of ι-carrageenan.

was continuously purged with argon gas for 50 min before and during the measurements. The Ge crystal was previously cleaned with ultrapure organic solvents (Sigma-Aldrich). Absorption spectra at a resolution of one data point per 1 cm^{-1} (to the highest measurement accuracy) were collected in the region between 4000 and 400 cm^{-1}. The scans were then Fourier-transformed and averaged with Grams/AI 8.0 software (Thermo Fisher Scientific, USA).

3. Results and discussion

3.1. Hydrolysis results

According to the literature, the average molecular mass of ι-carrageenan is estimated at around 5.6×10^6 Da [17]. In the present study it was found that the average molecular mass of reference samples (samples of ι-carrageenan after time zero of the hydrolysis analysis) was about 1.96×10^6 Da (for the first part of examined samples) and from 3.6×10^6 to 3.8×10^6 Da (for the second part of examined samples) (Table 1). This difference between our samples and the literature may be attributed to the association and aggregation which could occur at room temperature during storage in ι-carrageenan [27]. Some reduction in molecular mass accompanies the degradation of polysaccharides such as ι-carrageenan. The molecular mass of ι-carrageenan was reduced by about 24% after acid hydrolysis in comparison to non-degraded ι-carrageenan (Table 1, Fig. 1). After enzymatic hydrolysis using β-galactosidase, molecular mass was decreased by about 16% (Table 1, Fig. 2). For commercial lactase use, only a 3% decrease in molecular weight was observed (Table 1, Fig. 3). Kamińska-Dwórznicka et al. [2]

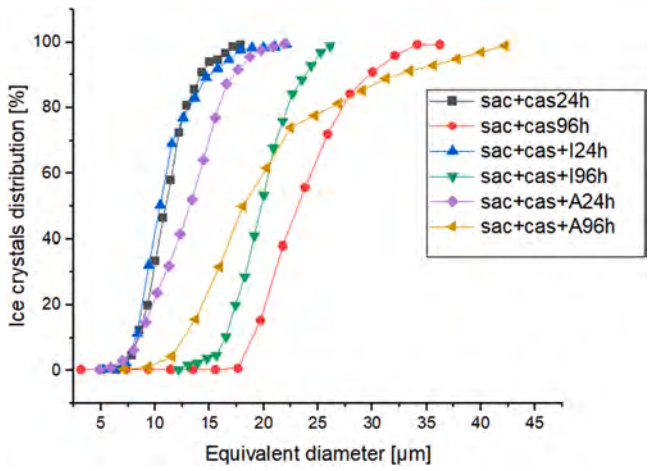


Fig. 6. Ice crystal size distribution in model sucrose solutions with the addition of sodium caseinate and ι-carrageenan and its acid hydrolysates: after 24 h and after 96 h of storage at − 8 °C. Explanatory notes: 50% suc+cas+I – 50% sucrose solution with the addition of sodium caseinate and ι-carrageenan; 50% suc+cas+A – 50% sucrose solution with the addition of sodium caseinate and acid hydrolysates of ι-carrageenan.

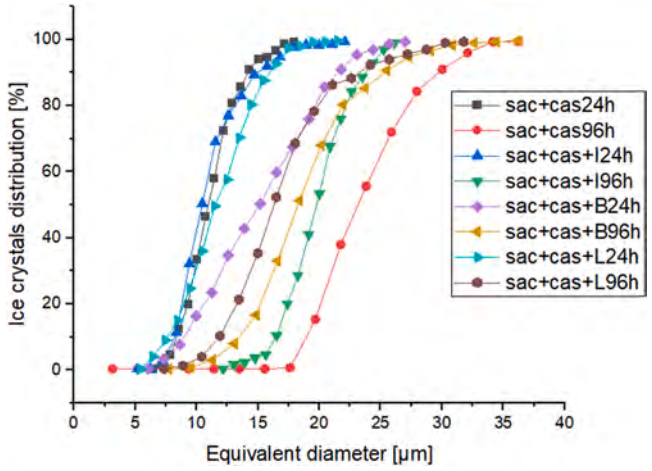


Fig. 7. Ice crystal size distribution in model sucrose solutions with the addition of sodium caseinate and ι-carrageenan and its enzymatic hydrolysates: after 24 h and after 96 h of storage at − 8 °C. Explanatory notes: 50% suc+cas+I – 50% sucrose solution with the addition of sodium caseinate and ι-carrageenan; 50% suc+cas+B – 50% sucrose solution with the addition of sodium caseinate and the enzymatic β-galactosidase hydrolysates of ι-carrageenan; 50% suc+cas+L – 50% sucrose solution with the addition of sodium caseinate and the enzymatic commercial lactase hydrolysates of ι-carrageenan.

reported that after hydrolysis with β-galactosidase, the molecular mass of κ-carrageenan was decreased by about 16%. Owing to this result, the enzymatic hydrolysis with commercial lactase use is not the best way to degrade the molecular mass of such polysaccharides as carrageenan.

Moreover, the enzyme β-galactosidase was used instead of carrageenase due to the fact of its availability. In the present research, it was found that the enzyme will influence the internal β(1−4) linkages as carrageenase does. Moreover, this enzyme was also used in research by Kamińska-Dwórznicka et al. [2] and it brought satisfactory results. Additionally, as mentioned in the methodology section, the lactase enzyme was used as a cheaper equivalent to β-galactosidase. Since these are both in the same enzyme group, an attempt to understand the mechanism of degrading ι-carrageenan was made. The chromatographical analysis indicated that the extension and type of hydrolysis generated a shift in the time of discharge of the molecules. First, the highest molecular mass was noted for samples after enzymatic hydrolysis (Table 1). After 72 h of hydrolysis by β-galactosidase, the oligosaccharides reached the lowest mass compared to the shorter hydrolysis time. Nonetheless, after 24 h of enzymatic hydrolysis by commercial lactase, the molecular mass reached 3.5×10^6 Da while 3.6×10^6 was observed for the standard sample. In a study by Henares et al. [13] the hydrolytic activity of carrageenase from *Pseudoalteromonas carrageenovora* was examined using ι-carrageenan as the substrate. NMR spectroscopy indicated that the beta-1,4-linkage of the D-galactose-4-sulfate unit was hydrolysed however, it is specific for carrageenase use and we do not know exactly what kind of linkages in ι-carrageenan were hydrolysed by examined enzymes.

The tendency of decreasing molecular mass in the course of time was also observed in the acid hydrolysis of ι-carrageenan (Fig. 1). Concerning acid hydrolysis of ι-carrageenan in the study by Briones and Sato [28], it was confirmed that obtained oligosaccharides after hydrolysis contained the basic sugar unit 3,6-anhydrogalctose and also sulphated galactose. Based on ESI MS spectra analysis, the ions for sodium salt forms were linked to both sulphated oligosaccharides. Additionally, a high level of purity of both obtained hydrolysates was noticed. Moreover, in the study by Karlsson and Singh [17], the molecular mass of ι-carrageenan, after 120 min of acid hydrolysis at 55 °C, was significantly depolymerized compared to the initial average molecular mass before hydrolysis. The reduction of molecular mass could be caused by a decrease of the free sulphate groups in carrageenan. The standard sample with the highest molecular mass (1.94×10^6 Da) left the column first, and it was gradually followed by other samples with lower mass. A similar effect was noted in acid hydrolysis of κ-carrageenan, described by Kamińska-Dwórznicka et al. [26]. Also, the samples after 3 h of hydrolysis by hydrochloric acid had the lowest molecular mass. The obtained results show the differences in the mechanism of degradation of carrageenan chains depending on the type of hydrolysis. Additionally, based on the statistical analysis, only the molecular mass of the sample after hours of acid hydrolysis was relevant. In comparison to enzymatic hydrolysis, only for the designated molecular mass of samples after 48 and 72 h were significant differences noted. In the hydrolysates gained after commercial lactase use, no statistically significant differences were observed (Table 1). Based on the conclusion formulated by Kamińska-Dwórznicka et al. [2] and Kamińska-Dwórznicka et al. [20] in the matter of molecular mass of κ-carrageenan after hydrolysis, only samples with the lowest average molecular mass were used for further analysis.

3.2. *Recrystallisation process*

The sucrose solutions were prepared as a model of non-milk ice cream whereas the sucrose solutions with the addition of sodium caseinate were treated as a model of milk ice cream. The hydrolysates after the longest time of hydrolysis and the highest molecular mass reduction were chosen for further analysis (Table 1).

After 24 h of storage, the average recorded diameter of ice crystals in the sucrose solution without any addition (50% suc) was 11.87 µm. Also, it was the smallest size out of all samples examined at the beginning. Nonetheless, after 96 h, it changed significantly, because the diameter (X_{50}) increased to almost 28 µm (Table 2; Fig. 4; Fig. 5). The application of non-degraded ι-carrageenan (50% suc+I) did not trigger relevant consequences, because it had no vital effect on ice recrystallisation inhibition. After 24 h of storage ice crystals were larger than in samples without additives, 14.34 µm. Additionally, during storage the growth of ice crystals was visible and it reached the same level as was noted for the reference sample (27.57 µm). Similar results for the ι-carrageenan addition were previously reported by Kiran-Yildirim and Gaukel [29] – the mean ice crystal diameter for sucrose solutions with ι-carrageenan addition was 31.095 µm after 96 h of storage.

When analysing the value of ice crystal diameter at 50% of the cumulative distribution of the sample (X_{50} diameter), the addition of

Table 2
Comparison of ice crystal size distribution after different storage times.

Time of storage and solution variant		**Average diameter D_A in the class with the highest frequency [μm] ± SD**	Minimal size of ice crystals [μm]	Maximal size of ice crystals [μm]
50% suc	24 h	**11.87 ± 2.63**	6.70	20.46
	96 h	**27.54 ± 2.85**	22.46	36.75
50% suc+I	24 h	**14.34 ± 3.81**	6.42	24.14
	96 h	**27.57 ± 4.37**	20.41	45.92
50% suc+A	24 h	**13.01 ± 2.03**	9.23	17.28
	96 h	**20.50 ± 4.11**	11.29	30.14
50% suc+B	24 h	**15.55 ± 6.86**	4.43	34.23
	96 h	**20.78 ± 6.03**	7.48	37.30
50% suc+L	24 h	**17.27 ± 3.27**	7.25	26.09
	96 h	**21.67 ± 8.84**	6.62	47.86

Explanatory notes: 50% suc+I – 50% sucrose solution with the addition of ι-carrageenan; 50% suc+A – 50% sucrose solution with the addition of the acid hydrolysates of ι-carrageenan; 50% suc+B – 50% sucrose solution with the addition of the enzymatic β-galactosidase hydrolysates of ι-carrageenan; 50% suc+L – 50% sucrose solution with the addition of the enzymatic commercial lactase hydrolysates of ι-carrageenan.

Table 3
Comparison of ice crystal size distribution after different storage times.

Time of storage and solution variant		**Average diameter D_A in the class with highest frequency [μm] ± SD**	Minimal size of ice crystals [μm]	Maximal size of ice crystals [μm]
50% suc +cas	24 h	**11.16 ± 2.21**	6.88	17.74
	96 h	**23.76 ± 4.01**	13.03	35.54
50% suc+cas+I	24 h	**11.07 ± 2.71**	6.61	19.19
	96 h	**19.82 ± 2.73**	13.67	26.66
50% suc+cas+A	24 h	**11.38 ± 2.73**	5.65	22.27
	96 h	**20.35 ± 6.38**	8.13	37.10
50% suc+cas+B	24 h	**14.00 ± 2.97**	7.88	21.90
	96 h	**18.77 ± 4.61**	7.71	37.94
50% suc+cas+L	24 h	**11.76 ± 3.13**	5.42	22.52
	96 h	**16.92 ± 4.56**	7.34	33.31

Explanatory notes: 50% suc +cas+I – 50% sucrose solution with the addition of sodium caseinate and ι-carrageenan; 50% suc+cas+A – 50% sucrose solution with the addition of sodium caseinate and acid hydrolysates of ι-carrageenan; 50% suc+cas+B – 50% sucrose solution with the addition of sodium caseinate and the enzymatic β-galactosidase hydrolysates of ι-carrageenan; 50% suc+cas+L – 50% sucrose solution with the addition of sodium caseinate and the enzymatic commercial lactase hydrolysates of ι-carrageenan.

hydrolysates of ι-carrageenan affected the recrystallisation process (Fig. 4, Fig. 5; Table 2). The most propitious effect of IRI (Ice Recrystallization Inhibition) in sucrose solutions was observed for the addition of oligosaccharides gained after acid (50% suc+A) and enzymatic hydrolysis with β-galactosidase (50% suc+B) use. For sucrose solution with the addition of acid hydrolysates, X_{50} was lower than 20 μm (Fig. 4). The average diameter of ice crystals after 96 h was exactly 20.5 μm, and it is well below the detection threshold defined for ice crystals in ice cream [2,7].

Another point of view was presented by Leiter et al. [14], who found that increasing the sodium ion concentration (after acid hydrolysis) leads to decreasing the IRI activity of κ- carrageenan, and it was assumed that the influence of ions on this parameter is related to the gelation properties of κ- carrageenan. However, Thrimawithana et al. [11] mentioned that ions such as sodium ions are only able to bond ionically to the sulphate groups, in comparison to calcium ions and potassium ones, which have a significant impact on carrageenans. Based on this study we also think that other ions such as sodium present in the sample after hydrolysis did not affect the IRI activity of hydrolysed carrageenans.

The addition of enzymatic hydrolysates (50% suc+B) (after β-galactosidase treatments) also successfully restricted ice crystal growth compared to non-degraded ι-carrageenan and the sample without any addition (Table 2, Fig. 5). At the end of storage, the average diameter of ice crystals also did not exceed 21 μm. Nonetheless, the addition of the enzymatic hydrolysates (50% suc+L) (after commercial lactase use) also brought the same effect. The X_{50} parameter was around 19.5 μm (Fig. 5). According to Kamińska-Dwórznicka et al. [2,20], the application of acid and enzymatic hydrolysates of κ-carrageenan was more effective than a pure form of carrageenan addition. Nevertheless, not only does molecular mass reduction influence this process but also the type of oligosaccharides obtained [2,20]. Presumably, the loss of sulphate groups in polysaccharides generates a tendency to create a more flexible conformation and less extended due to their lower electrostatic intermolecular repulsion [17].

In sucrose solution with the addition of sodium caseinate, there was a more noticeable result in crystal size reduction, in comparison to pure sucrose solution (50% suc+cas). After 96 h of storage parameter, X_{50} was at the level of 23 μm (Fig. 6), while for pure sucrose it was more than 27 μm (Fig. 4). It was known that ι-carrageenan with calcium salt exhibits unusual thixotropic behaviour and produces a softer, more resilient gel than with potassium salts [30]. Moreover, based on the research by Thrimawithana et al. [31] ι-carrageenan with the addition of KCl has the ability to create a system with fewer cross-links than with the addition of $CaCl_2$. Additionally, with calcium chloride, it could form regular intra-helical cross-links. In our study for the addition of non-degraded ι-carrageenan (50% suc+cas+I) initially, the average diameter of ice crystals was 11.07 μm and after 96 h it had increased only to 19.82 μm, while for pure sucrose with ι-carrageenan addition it was also around 27 μm after 96 h of storage (Tables 2 and 3).

Acid hydrolysate addition brings a similar effect, as was observed for the sucrose sample without sodium caseinate addition. After 24 h the average diameter of ice crystals with the addition of acid hydrolysates (50% suc+cas+A) was estimated at 11.38 μm and after 96 h of storage, it was increased to 20.35 μm (Table 3, Fig. 6). A similar effect was noted for the sample with the enzymatic hydrolysates (β-galactosidase use) (50% suc+cas+B) addition, the average X_{50} parameter after 96 h was approximately 18 μm (Fig. 7). The most effective addition to preventing the recrystallization process proved to be the addition of hydrolysates obtained after commercial lactase (50% suc+cas+L) use. After 24 h the

average diameter was comparable to other variants and it was less than 11.76 μm. Nonetheless, at the end of storage (after 96 h), the addition of hydrolysates obtained by commercial lactase hydrolysis caused inhibition of the growth in size of the crystals and the average diameter was 16.92 μm (Table 3). Presumably, this significant effect may be related to the hypothesis, that the structure and mechanism of hydrolysis had more influence on IRI than the molecular mass of hydrolysates. Because during the hydrolysis by lactase, the molecular mass did not significantly change (Table 1) however the lucrative results were obtained for this sample. Even if the two types of lactases (commercial and pure β-galactosidase) degraded carrageenan to a different level when comparing only molecular mass they still influenced the same type of galactosides bond and create the same type of oligosaccharides. It was already hypothesised by Kiran-Yildirim et al. [1] that the structural features of carrageenans such as the amount and position of sulfate and other functional groups may be crucial for IRI activity. Moreover, based

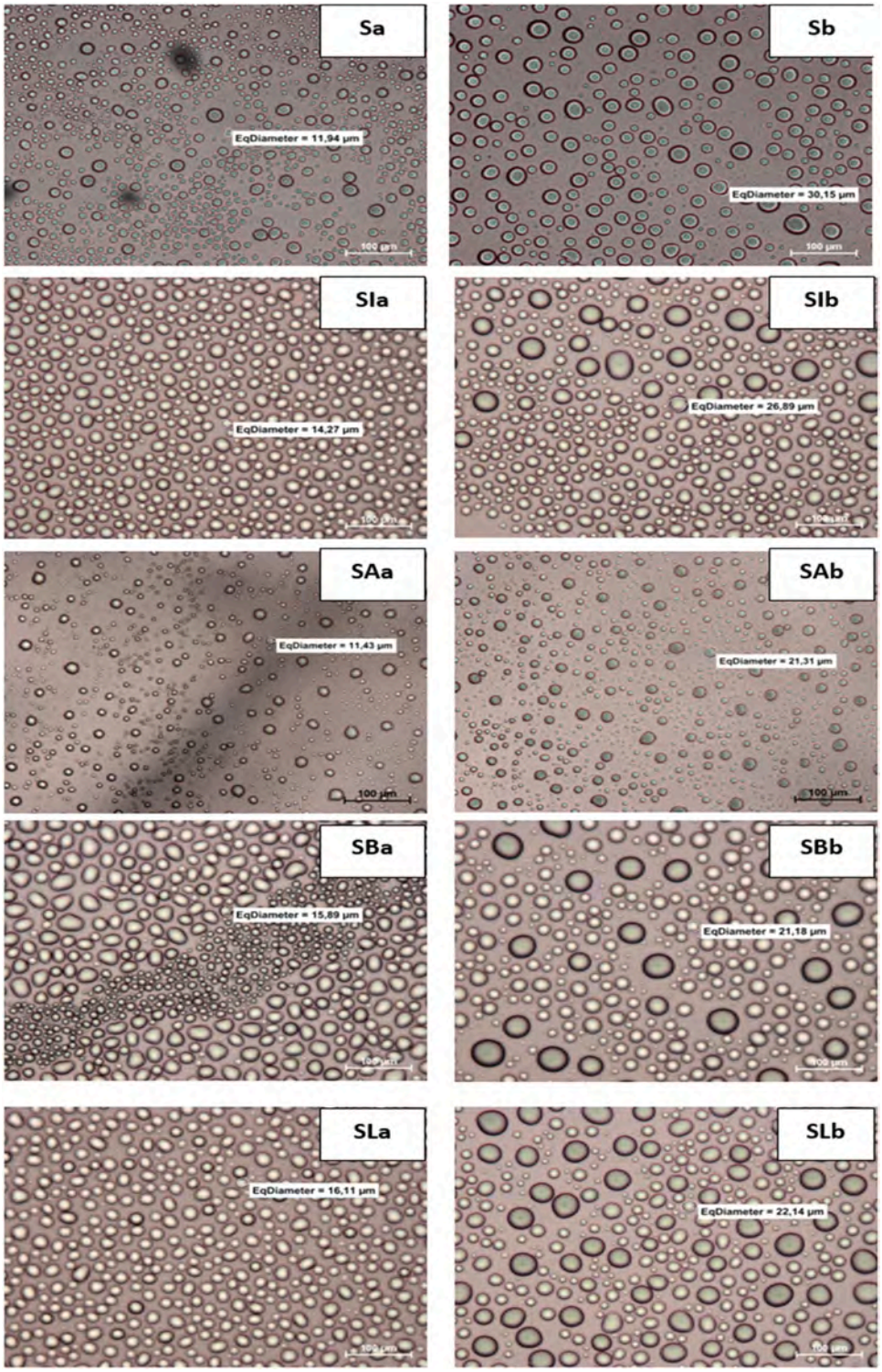


Fig. 8. Microscopic images of ice crystals in model sucrose solutions (S) with the addition of ι-carrageenan (SI) and its acid (SA) β-galactosidase (SB) and commercial lactase (SL) hydrolysates: after 24 (a) and 96 h (b) of storage at − 8 °C.

on the results presented by Kamińska-Dwórznicka et al. [2] the size of ice crystals in frozen model sucrose solution with the addition of enzymatic hydrolysates of κ-carrageenan was less than 6 μm. In comparison to the present results, the average diameter of ice crystals was larger, and after 96 h it was approximately 19 μm. Such variance was caused by the different forms of carrageenan and distinct method of crystallization. Moreover, the addition of sodium caseinate to sucrose was used as a model of milk ice cream, and also it might be a reason for these differences. Nonetheless, in the present study, the size of ice crystals was less than 20 μm. This size of ice crystals in ice cream is highly accepted for eligible texture and composition. Additionally, the hydrolysates of ι-carrageenan had a better influence than iota itself.

In research conducted by Wu [32] the commercial enzyme α-amylase was used for hydrolysis of κ-carrageenan. The aim of this research was to optimise the use of this enzyme and use the carrageenan-derived oligosaccharides for process production. In the conclusion of these results,

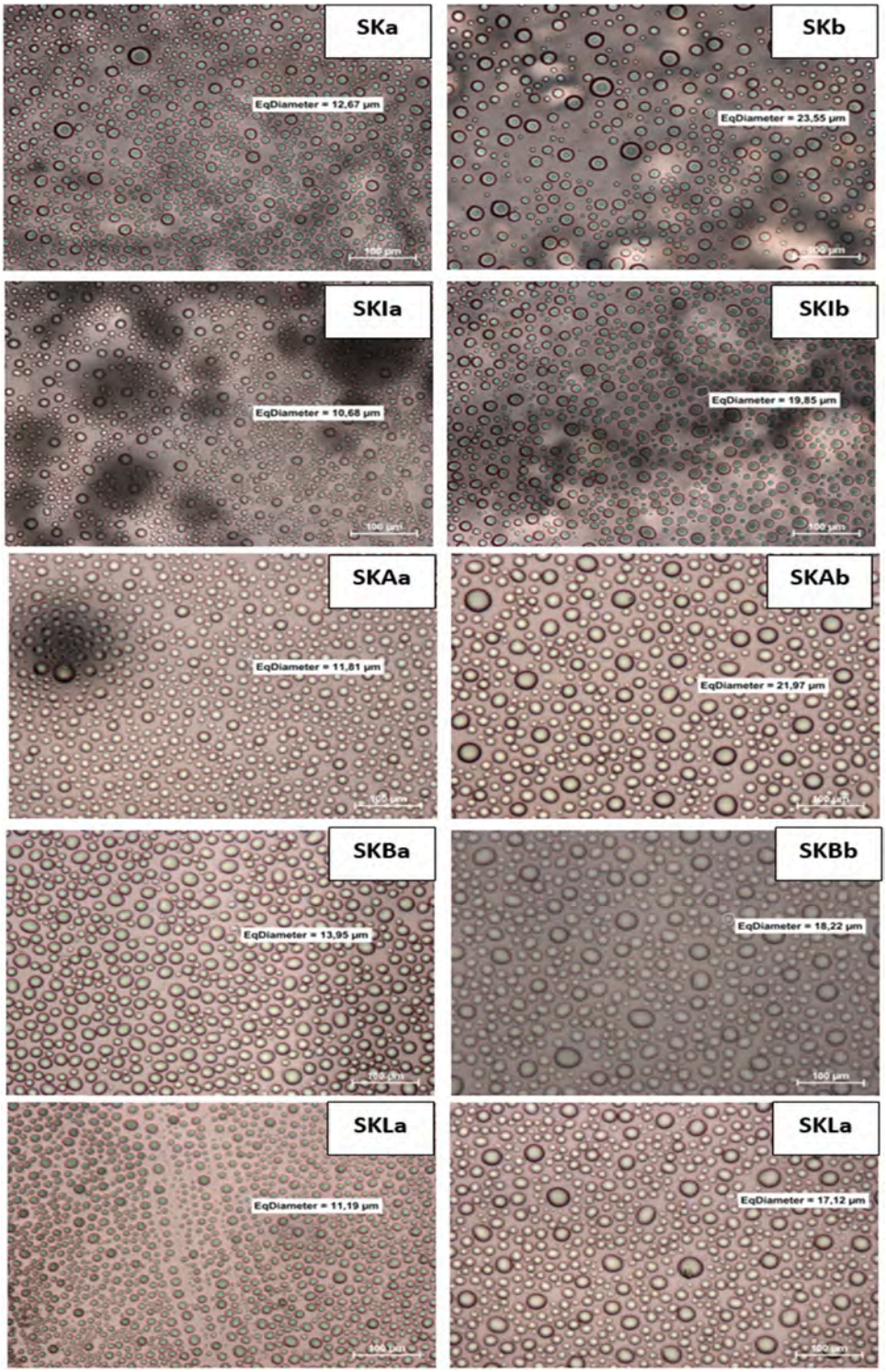


Fig. 9. Microscopic images of ice crystals in model sucrose solutions with the addition of sodium caseinate (SK) and with the addition of ι-carrageenan (SKI) and its acid (SKA) β-galactosidase (SKB) and lactase (SKL) hydrolysates: after 24 (a) and 96 h (b) of storage at − 8 °C.

the obtained oligosaccharide product had a high level of purity and the yield was 96.5%. It indicates that using commercial and cheaper enzymes may bring positive results with respect to carrageenans [32].

Crystal structure observed from the images also brings information about the progress of the recrystallisation of ice. In sucrose solution and sucrose solutions with the addition of sodium caseinate, the shape of ice crystals was regular and round (Fig. 8; Fig. 9). Accordingly, at the time of storage of samples, some changes may suggest that coalescence between adjacent ice crystals occurred. Presumably, the benefit of milk proteins from sodium caseinate addition relied on the fact that it creates with water molecules a structure that filled the empty spaces, consequently forming the regular appearance of ice structure (Fig. 9). The different interactions may explain such a phenomenon with the surface of crystals and stabilisers during storage. According to the results conducted by Kiran-Yildirim et al. [1], the shape modifications of ice crystals should increase with increasing the IRI activity of ι-carrageenan. However, in comparison to the present research, such changes were not observed.

3.3. FTIR analysis

The spectra of the samples selected for the study are presented in Fig. 10. In order to simplify the interpretation of the obtained results, all the characteristic bands and their corresponding vibrational modes and functional groups [33–38] are presented in Table 4. The analysis of the obtained spectra, reveals very intense vibration bands associated with stretching groups -OH with a maximum at about 3350 cm^{-1}, these results correspond to the previous research [33–37]. The next area of vibrations with a maximum at 2919, 2886 cm^{-1} are the bands characteristic of the C-H stretching vibrations in the ι-carrageenan structure. The bands with a maximum at 1643 cm^{-1} are deformation vibrations of the -OH groups. The area of the bands with a maximum at 1418 cm^{-1} are deformation vibrations corresponding to the -O-CH group. The very intense band with a maximum at about 1213 cm^{-1} corresponds to the vibration stretching of the group O═S═O in the ι-carrageenan structure, which was also examined by Moniha et al. [33]. They showed the differences in spectra examined for pure ι-carrageenan and with the addition of ammonium nitrate [33]. It was also investigated [35,36]. Furthermore, it is worth mentioning the vibrations with a maximum at 845 and 700 cm^{-1} corresponding to the stretching vibrations of the O-SO_3 groups in the general structure of the ι-carrageenan. However, from the point of view of the applied process, the most interesting areas are those including the bands, at 1214, 1020, 914 cm^{-1}, and the area of vibration of the -OH stretching groups mentioned at the beginning of the description, i.e., the band with a maximum of about 3350 cm^{-1}. The band with a maximum at 1214 cm^{-1} is the stretching vibrations characteristic of the configuration ν(O═S═O). On the other hand, a very intense band with a maximum at 1020 cm^{-1} indicates stretching vibrations characteristic for the C-O groups in the carrageenan molecules. The last region of vibrations located at a about 914 cm^{-1} are stretching vibrations characteristic of the C-O-C configuration in the ι-carrageenan structure. The band with the maximum at 914 cm^{-1} may also be partly derived from the O-SO_3 moiety.

As we can see, the bands of the -OH group is slightly shifted towards shorter wavenumbers in samples L24 and B72, i.e. after enzymatic hydrolysis in relation to the pure sample of PI ι-carrageenan. This may indicate a decrease in the vibration intensity of the -OH groups, and thus a decrease in their mobility. In the case of sample A3, the shift is also

Table 4
Location of the maxima of the FTIR absorption bands, with assignment of particular vibrations to the respective samples: PI, A3, L24 and B72 with Fig. 10, registered within the spectral range of 500–3740 cm^{-1} [33–38].

FTIR	Type and origin of vibrations
Positioning of band [cm^{-1}]	
3350	ν_s(-OH)
2919	ν(C-H)
2886	
2846	
1643	δ(-OH)
1625	
1462	δ_w(-O-CH)
1418	δ_w(-OH) in C-OH group and ν_w(C-H)
1372	
1319	ν(C–H)
1247	ν_s(O═S═O)
1213	
1160	ν(C-O)
1094	
1066	
1020	
998	
965	
914	ν(C-O-C) and ν(O-SO_3)
866	ν(O-SO_3)
845	
801	δ(C–H)
770	
717	ν(O-SO_3)
700	
670	
602	δ(C–H)
573	

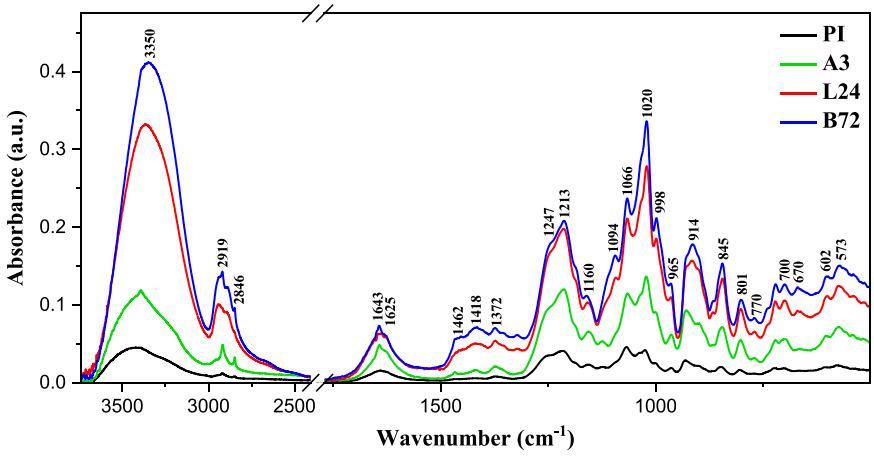


Fig. 10. FTIR infrared spectra in the spectral range: 500–3740 cm^{-1} for the tested samples, respectively: PI, A3, L24 and B72. Spectra were measured at room temperature. Explanatory notes: PI – pure ι-carrageenan; A3 – the acid hydrolysate of ι-carrageenan; B72 – the enzymatic β-galactosidase hydrolysates of ι-carrageenan; L24 – the enzymatic commercial lactase hydrolysates of ι-carrageenan.

visible but much smaller. It is very interesting to observe the reduction of the relative intensity of the bands from a maximum at 1213 and 914 cm^{-1}. They come from the vibrations of groups which include sulfur atoms. This fact clearly proves the possibility of reducing the number of sulfate groups, which may result in greater flexibility of the polymer structure of the sample thus prooving of our main hypothesis. Clear changes are also observed at a maximum of 1020 and 1063 cm^{-1}, i.e., for C-O stretching vibrations, especially in the case of L24 and B72 enzymatic hydrolysis. This confirms that the applied processes also have a noticeable effect on the main carrageenan ring. It is also worth emphasizing the changes in the intensity of the bands below 980 cm^{-1}. This result proves that the discussed processes have a different impact on the bonds between the main structural units of carrageenan. Finally, one should also pay attention to the L24 and B72 spectra. They have a very similar course, which is a clear proof that in an industrial application one can use a cheaper and more accessible equivalent. The application of infrared spectroscopy will be the subject of further research related to this topic. The fundamental aim is to quickly and easily monitor the changes taking place in selected samples by observing a molecular response.

4. Conclusion

The present study showed that new substances obtained by hydrolysis might bring a positive IRI (Ice Recrystallisation Inhibition) effect. The best method for reducing the molecular mass of ι-carrageenan was acid hydrolysis, with about 24% of molecular mass reduction.

Furthermore, all examined hydrolysates inhibited the recrystallisation process in model sucrose solutions. The average diameter of ice crystal did not exceed 22 μm after 96 h. Moreover, in the sucrose solution with the addition of sodium caseinate, the effect was even more visible and the most efficient was the addition of the oligosaccharides after commercial lactose hydrolysis. At the end of storage, the size of obtained crystals was less than 17 μm. The effectiveness of ι-carrageenan hydrolysates in ice recrystallisation inhibition may suggest that structural composition, as well as the position of functional groups, should be a subject for further studies. The analysis of the spectra obtained via FTIR spectroscopy confirms the above conclusions. The intensity and shape of the bands from the areas with the maximum at 3350, 1214, 1020, and 914 cm^{-1} provide clear evidence for the above hypotheses, also from a molecular approach. The most informative areas are the stretching vibrations characteristic of groups such as -OH, O = S = O, C-O, and C-O-C in the main core of the ι-carrageenan structure.

CRediT authorship contribution statement

Anna Kamińska-Dwórznicka: Conceptualization. Anna Kot: Software, Investigation and analysis of particular methods: Hydrolysis, Writing – original draft preparation. Anna Kot, Anna Kamińska-Dwórznicka, Ewa Jakubczyk: Validation. **Andrzej Antczak**: SEC analysis. **Arkadiusz Matwijczuk**: FTIR analysis. **Anna Kamińska-Dwórznicka, Ewa Jakubczyk**: Freezing. **Anna Kot, Anna Kamińska-Dwórznicka**: Microscopy structure analysis. **Anna Kamińska-Dwórznicka, Anna Kot**: Writing – review & editing. **Anna Kamińska-Dwórznicka, Ewa Jakubczyk**: Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] B. Kiran-Yildirim, J. Hale, D. Wefers, V. Gaukel, Ice recrystallisation inhibition of commercial κ-, ι-, and λ-carrageenans, J. Food Eng. 290 (2021), 110269. ⟨https://www.x-mol.com/paperRedirect/1289640905495158784⟩.

[2] A. Kamińska-Dwórznicka, P. Skrzypczak, E. Gondek, Modification of kappa carrageenan by β-galactosidase as a new method to inhibit recrystallization of ice, Food Hydrocoll. 61 (2016) 31–35, https://doi.org/10.1016/j.foodhyd.2016.04.046.

[3] S. Adapa, K.A. Schmidt, I.J. Jeon, T.J. Herald, R.A. Flores, Mechanisms of ice crystallisation and recrystallisation in ice cream: A review, Food Rev. Int. 16 (3) (2000) 259–271, https://doi.org/10.1081/FRI-100100289.

[4] K. Cook, R. Hartel, Mechanisms of Ice Crystallization in Ice Cream Production, Compr. Rev. Food Sci. Food Saf. 9 (2) (2010) 213–222, https://doi.org/10.1111/j.1541-4337.2009.00101.x.

[5] V. Gaukel, A. Leiter, W.E.L. Spiess, Synergism of different fish antifreeze proteins and hydrocolloids on recrystallisation inhibition of ice in sucrose solutions, J. Food Eng. 141 (2014) 44–50, https://doi.org/10.1016/j.jfoodeng.2014.05.016.

[6] A. Regand, H.D. Goff, Structure and ice recrystallisation in frozen stabilised ice cream model systems, Food Hydrocoll. 17 (2003) 95–102, https://doi.org/10.1016/S0268-005X(02)00042-5.

[7] F.T. Ndoye, G. Alvarez, Characterisation of ice recrystallisation in ice cream during storage using the focused beam reflectance measurement, J. Food Eng. 148 (2015) 24–34, https://doi.org/10.1016/j.jfoodeng.2014.09.014.

[8] Y.D. Livney, D.P. Donhowe, R.W. Hartel, Influence of temperature on crystallization of lactose in ice-cream, Int. J. Food Sci. 30 (3) (1995) 311–320, https://doi.org/10.1111/j.1365-2621.1995.tb01380.x.

[9] Y. SWong, W.R. Hartel, Crystallization in Lactose Refining – a review, J. Food Sci. 3 (79) (2014) 257–272, https://doi.org/10.1111/1750-3841.12349.

[10] M.J. Ridout, S. Garza, G.J. Brownsey, V.J. Morris, Mixed iota-kappa carrageenan gels, Int. J. Biol. Macromol. 18 (1996) 5–8, https://doi.org/10.1016/0141-8130(95)01037-8.

[11] T. Thrimawithana, S. Young, D. Dunstan, R. Alany, Texture and rheological characterisation of kappa and iota carrageenan in the presence of counter ions, Carbohydr. Polym. 82 (1) (2010) 69–77, https://doi.org/10.1016/j.carbpol.2010.04.024.

[12] P. Chauhan, A. Saxena, Bacterial carrageenans: an overview of production and biotechnological applications, 3 Biotech 6 (2) (2016), https://doi.org/10.1007/s13205-016-0461-3.

[13] B.M. Henares, E.P. Enriquez, F.M. Dayrit, N.R.L. Rojas, Iota-carrageenan hydrolysis by Pseudoalteromonas carrageenovora IFO12985, Philipp. J. Sci. 139 (2) (2010) 131–138.

[14] A. Leiter, A. Ludwig, V. Gaukel, Influence of heating temperature, pH and ions on recrystallization inhibition activity of κ-carrageenan in sucrose solution, J. Food Eng. 195 (2017) 14–20, https://doi.org/10.1016/j.jfoodeng.2016.09.016.

[15] M. Baharamparvar, M.Mazaheri Tehrani, Application and functions of stabilizers in ice cream, Food Rev. Int. 27 (4) (2011) 389–407, https://doi.org/10.1080/87559129.2011.563399.

[16] W.R. Blakemore, A.R. Harpell, Carrageenan, in: A. Agents Imeson (Ed.), Food Stabilisers, Thickeners and Gelling, Blackwell Publishing Ltd, UK, 2010, pp. 73–94.

[17] A. Karlsson, S.K. Singh, Acid hydrolysis of sulphated polysaccharides. Desulphation and the effect on molecular mass, Carbohydr. Polym. 38 (1999) 7–15, https://doi.org/10.1016/S0144-8617(98)00085-X.

[18] V.L. Campo, D.F. Kawano, D.B. da Silva Jr., I. Carvalho, Carrageenans: Biological properties, chemical modifications and structural analysis– A review, Carbohydr. Polym. 77 (2009) 167–180, https://doi.org/10.1016/j.carbpol.2009.01.020.

[19] Z. Yao, H. Wu, S. Zhang, Y. Du, Enzymatic preparation of κ-carrageenan oligosaccharides and their anti-angiogenic activity, Carbohydr. Polym. 101 (2014) 359–367, https://doi.org/10.1016/j.carbpol.2013.09.055.

[20] A. Kamińska-Dwórznicka, M. Matusiak, K. Samborska, D. Witrowa-Rajchert, E. Gondek, E. Jakubczyk, The influence of kappa carrageenan and its hydrolysates on the recrystallization process in sorbet (Part B), J. Food Eng. 167 (2015) 162–165, https://doi.org/10.1016/j.jfoodeng.2015.06.014.

[21] A. Kamińska, V. Gaukel, Monitoring the growth of crystals in ice cream, Food, Sci., Technol., Qual. 62 (1) (2009) 57–64.

[22] J.G. Parsons, S.T. Dybing, D.S. Coder, K.R. Spurgeon, S.W. Seas, Acceptability of ice cream made with processed wheys and sodium caseinate, J. Dairy Sci. 68 (1985) 2880–2885, https://doi.org/10.3168/jds.S0022-0302(85)81181-4.

[23] M. Tang, Y. Lei, Y. Wang, D. Li, L. Wang, Rheological and structural properties of sodium caseinate as influenced by locust bean gum and κ-carrageenan, Food Hydrocoll. 122 (2021), 106251, https://doi.org/10.1016/j.foodhyd.2020.106251.

[24] V. Arsdel, M.J. Copley, D.K. Tressler, The freezing preservation of foods. Westport USA, AVI Publ. Comp. (1968).

[25] S.G. Min, Untersuchungen zur Rekristallisation, von Eis in gefrorenen Lebensmitteln, Diss. Univ. Hohenh. (1994).

[26] A. Kamińska-Dwórznicka, A. Antczak, K. Samborska, A. Lenart, Acid hydrolysis of kappa-carrageenan as a way of gaining new substances for freezing process modification and protection from excessive recrystallisation of ice, Int. J. Food Sci. Technol. 50 (8) (2015) 1799–1806, https://doi.org/10.1111/ijfs.12820.

[27] C. Viebke, P.A. Williams, Determination of molecular mass distribution of κ-carrageenan and xanthan using asymmetrical flow field-flow fraction, Food Hydrocoll. 14 (2000) 265–270, https://doi.org/10.1016/S0268-005X(99)00066-1.

[28] A.V. Briones, T. Sato, Structural studies on ι-carrageenan derived oligosaccharides and its application, Adv. Chem. Eng. 4 (2014) 17–22, https://doi.org/10.4236/aces.2014.41003.

[29] B. Kiran-Yildirim, V. Gaukel, Ice Crystal Growth in Sucrose Solutions Containing Kappa- and Iota-Carrageenans, Chem. Eng. Technol. 43 (6) (2020) 1040–1047, https://doi.org/10.1002/ceat.201900644.
[30] M. Garcia Tasende, J.A. Manriquez-Hernandez, Carrageenan properties and applications: a review, in: L. Pereira (Ed.), , Carrageenans: Sources and Extraction Methods, Molecular Structure, Bioactive Properties and Health Effects, Nova Science Publishers, New York, 2016, pp. 17–50.
[31] T. Thrimawithana, S. Young, R. Alany, Effect of cations on the microstructure and in-vitro drug release of κ- and ι-carrageenan liquid and semi-solid aqueous dispersions, J. Pharm. Pharm. 63 (1) (2010) 11–18, https://doi.org/10.1111/j.2042-7158.2010.01171.x.
[32] S. Wu, Degradation of κ-carrageenan by hydrolysis with commercial α-amylase, Carbohydr. Polym. 89 (2012) 394–396.
[33] V. Moniha, M. Alagar, S. Selvasekarapandian, B. Sundaresan, G. Boopathi, Conductive bio-polymer electrolyte iota-carrageenan with ammonium nitrate for application in electrochemical devices, J. Non Cryst. Solids 481 (2018) 424–434, https://doi.org/10.1016/j.jnoncrysol.2017.11.027.
[34] M.G. Tecson, D.H. Camacho, V.D. Ebajo Jr, L.V. Abad, Structural investigation of degradation products of irradiated Kappa-carrageenan, Radiat. Phys. Chem. 194 (2022), 110015, https://doi.org/10.1016/j.radphyschem.2022.110015.
[35] V. Moniha, M. Alagar, S. Selvasekarapandian, B. Sundaresan, R. Hemalatha, Development and characterization of bio-polymer electrolyte iota-carrageenan with ammonium salt for, Electrochem. Appl., Mater. Today.: Proc. 8 (2019) 449–455, https://doi.org/10.1016/j.matpr.2019.02.135.
[36] N.A.A. Ghani, R. Othaman, A. Ahmad, F.H. Anuar, N.H. Hassan, Impact of purification on iota carrageenan as solid polymer electrolyte, Arab. J. Chem. 12 (3) (2019) 370–376, https://doi.org/10.1016/j.arabjc.2018.06.008.
[37] D. Kluczyk, A. Matwijczuk, A. Górecki, M.M. Karpinska, M. Szymanek, A. Niewiadomy, M. Gagos, Molecular organization of dipalmitoylphosphatidylcholine bilayers containing bioactive compounds 4-(5-heptyl-1, 3, 4-thiadiazol-2-yl) benzene-1, 3-diol and 4-(5-methyl-1, 3, 4-thiadiazol-2-yl) benzene-1, 3-diols, J. Phys. Chem. 120 (47) (2016) 12047–12063, https://doi.org/10.1021/acs.jpcb.6b09371.
[38] K. Samborska, A. Wiktor, A. Jedlińska, A. Matwijczuk, W. Jamróz, K. Skwarczyńska-Maj, D. Kiełczewski, M. Tułodziecki, Ł. Błażowski, D. Witrowa-Rajchert, Development and characterization of physical properties of honey-rich powder, Food Bioprod. Process. 115 (2019) 78–86, https://doi.org/10.1016/j.fbp.2019.03.004.

Anna Kot

Anna Kamińska-Dwórznicka

Vegan ice cream: A review

DOI 10.15199/65.2022.12.5

SUMMARY:
There are a plethora of reasons why people are more interested in a vegan diet and at the same in a vegan frozen dessert. Environmental, ethical, and healthy motives or just even curiosity contribute to developing the need for such products. The main purpose of this study was to develop and understand of the phenomenon of vegan ice cream. Another key point in this paper was to assemble basic information about vegan ice cream, its ingredients, experimental production and the newest research in the scientific world. All the mentioned examples show that there are many great alternatives to milk ingredients and a wide variety of compounds. In addition, the combination of distinctive compounds has contributed to a comprehensive range of possibilities in vegan ice cream production.

STRESZCZENIE:
Istnieje wiele powodów, dla których wzrasta zainteresowanie dietą wegańską, a tym samym wegańskimi mrożonymi deserami. Motywy ekologiczne, etyczne, zdrowotne, a nawet ciekawość przyczyniają się do rozwoju zapotrzebowania na taki produkt. Głównym celem tego artykułu było wyjaśnienie popularności lodów wegańskich. Kolejnym kluczowym punktem było zebranie podstawowych informacji o lodach wegańskich, ich składnikach, eksperymentalnej produkcji oraz najnowszych badaniach w świecie nauki. Wszystkie wymienione przykłady pokazują, że istnieje wiele prosperujących alternatyw dla składników mleka krowiego. Natomiast szeroka gama tych surowców oraz ich kombinacji zapewnia ogromne możliwości w produkcji wegańskich lodów spożywczych.

TYTUŁ:
Lody wegańskie: przegląd

KEY WORDS:
vegan ice cream, vegan, ice cream, ice cream ingredients

SŁOWA KLUCZOWE:
lody wegańskie, weganizm, lody, składniki do lodów

According to the physical definition of ice cream, it is a multiphase system in which air, fat and ice crystals are dispersed in a vicious, concentrated and unfrozen solution [19, 28]. In 2021, the global ice cream market was valued at USD 79.0 billion and it is expected to broaden at an annual growth rate (CAGR) of 4.2% from 2022 to 2030. The fuel for this growth is connected with the rising demand for innovative flavours, types of ice cream or health consciousness [43].

One of the pivotal aims of food technologists in the ice cream industry is to produce ice cream with an ice crystal size and distribution that results in the smoothness of the product and inhibits the recrystallisation problem [17]. For instance, ice crystals between 10 to 20 μm give a favourable texture while crystals larger than 50 μm cause the undeniable quality of products such as coarse or grainy [18, 23, 36]. The sort of nutrients and the energy value of ice cream depend upon the used ingredients. According to that ice cream may be a source of food energy and become an excellent and desirable food for growing children or adults who need to maintain weight [20]. Despite this, ice cream is still underestimated and it is treated as just only frozen dessert. However, by drawing on the research by Spence et al. (2019), the new potential of ice cream was highlighted. Due to the dynamic contrast experienced by eating ice cream and the residual sensitivity to cold, this frozen dessert makes an excellent medium for the transmission of nutrients/energy to the elderly who are in care/hospital or are malnourished. Based on that perceiving ice cream as a childish or comfort food is not exactly correct. Owing to the fact that the popularity of ice cream would like to seems to be an effective vehicle for the delivery of the new meaning [37].

VEGAN ICE CREAM FOR WHOM?

Nowadays, climate change is perceived as one of the incredibly significant phenomena caused by human activity. Unfortunately, the food system is responsible for around 30% of total GHG emissions globally. As it was noticed in the paper by Konstantas et al. (2019), there are not many studies which show the influence of the impact ingredients of ice cream on global warming. For example in one of them, it was estimated that the GWP (global warming potential) of ice cream is at 4 kg CO_2 eq./kg. [24]. According to the United Nations, adjusting our diet to a plant-based diet is essential to counteract climate change. On grounds that the exclusion of a meat-based diet can contribute to the promotion of more sustainable use of natural resources [7, 29]. Moreover, besides the lucrative and promising conviction of influence on the environment of a plant-based diet, people start to adopt and successfully adhere to vegan or vegetarian diets. Additionally, such an overview of diet is also connected with expanding awareness of ecological, ethical and healthy benefits for people [29, 38, 39]. Another issue which is taken into consideration while we buy food is obviously our health restrictions. Lactose intolerance which occurs in the absence or deficiency of the enzyme lactase forces people to remove dairy products from their lives. Based on that there are a consecutive group of people who would try to find a product which is deprived of animal ingredients [34, 40]. Another limiting factor in consuming milk ice cream and developing vegan alternatives is an allergy to milk protein. Cow's milk allergy is one of the highest spread allergies in early life (among infants and chil-

dren). It was estimated that in developing countries it is ranging from 0.5% to 3% at the age of 1 year [16, 40]. In a view of all that has been mentioned reasons, to face the indispensable increasing demand for vegan products, food producers start to create vegan-labelled ice creams which are on the rise nowadays. As a consequence, we will be able to indulge ourselves in eating our favourite frozen dessert without any guilt and health ailments.

DEFINITION OF VEGAN ICE CREAM

Under the circumstances of developing new formulations of products or processes, it is required to measure and describe the properties of ice cream but also the ice cream mixes and the all ingredients which are used in the process production [10, 14]. Furthermore, it is necessary to clarify the definition of vegan ice cream. The term vegan ice cream refers to a frozen and aerated mixture which is made based on plant protein, vegetable fat and additions, without any animal-based ingredients. Moreover, what should be highlighted here, is the example of sorbet. This sort of ice cream is also made without animal milk or fat, fruit-based, however sometimes animal-based stabiliser such as gelatin is used for sorbet production. Consequently, this sort of ice cream can not be always classified as vegan ice cream. In addition, thankfully by creating vegan ice cream a plethora of people is able to enjoy such frozen dessert without sacrificing their principles or taking the risk of health ailments. Taking into consideration, the pivotal role of cow's milk and milk products in creating the structure of ice cream and the sensory profile, the tremendous challenge is to replace it in vegan ice cream production. According to this, scientists and food technologists try to use distinctive ingredients from more known to even unpopular in the industry, to obtain satisfying results and acceptance from consumers. To systematize the current knowledge, present recipes and innovative operations in vegan ice cream production were collected in ***table 1***.

INGREDIENTS FOR vegan ice cream production

The group of ingredients in ice cream are immensely variable, starting from basic as protein to different sorts of stabilisers or emulsifiers. According to the classification made by Clarke (2004), there exist three groups of ingredients for ice cream products: the major which represents substantial quantities such as protein, sugar or fat; minor ingredients which shows in small quantities such as stabilizers, emulsifiers and last but not least, components as chocolate, fruit pieces or nuts. Obviously, the choice of ingredients depends on the type of product, cost, availability of raw materials and additionally the scale of production [14].

In traditional milk ice cream, the basic product for production is usually cow's milk, liquid and also in powder. For vegan ice cream production, a wide variety of plant proteins were used or tested to replace such ingredients. Nowadays, the tremendous popularity among vegan recipes has pea protein. It is relatively a new sort of plant protein and due to its low cost, availability and health benefits, it becomes more popular. As a result, it is widely studied by industrial and academic researchers. Pea protein stands out with its emulsifying or gelling properties. Moreover, in comparison to soy protein, it has better digestibility and fewer allergic responses. Additionally, as a sustainable source with a lower carbon footprint, it may be a good alternative for animal protein [30, 31, 33]. Initial work on using pea protein and almond drinks in ice cream was undertaken by Kot et al. (2020). This research indicates that preparing ice cream based on such a recipe contributed to its successful acceptance among panellists and the promising physical qualities of vegan ice cream [25]. As a known and also frequently use type of vegetable protein, there is soy protein. Contemporary, soy protein is the largest commercially available plant protein. Moreover, soy protein is used according to its properties such as water-holding, binding or emulsifying. In addition, soybean protein might affect the steroids and also influence the metabolism of cholesterol. Albeit it must not be forgotten that soy allergy is also a common food allergy, especially in childhood [5, 33]. What is more in the research by Lomolino et al. (2020), the addition of potato protein was tested in vegan ice cream. It was reported that potato protein with a combination of stabilisers, provides the initial quality of ice cream even if ice cream did not resist thermal stress [32].

The next ingredient in the recipe for ice cream is a group of stabilisers. The most common in the ice cream industry are locust bean gum, guar gum, xanthan gum or carrageenan. They are mostly polysaccharides that are able to interact with water, improve the viscosity and texture of ice cream and additionally provide a sense of creaminess or lubricity [4]. Moreover, using stabilisers in ice cream is strictly connected with the inhibition of the recrystallisation process. Furthermore, it is known that stabilisers show the synergism effect. According to that, the group of plant gums are successfully used in the production of vegan ice cream. For instance, in the research by Kot et al. (2020), the combination of xanthan gum and locust bean gum in pea protein vegan ice cream was used. It was noted that this combination contributed to the lowest size of ice crystals, less than 20 μm and prolong the melting time (***table 1***).

To replace liquid cow's milk which is usually a crucial ingredient in milk ice cream, plant milk or even just water was tested in vegan ice cream production. Plant-based milk may be defined as water-soluble extract based on their oil seeds, cereals, seeds or/and legumes. As a result of the similarity of the nutritional value or functional properties, the milk analogues allow them to be valuable substitutes for milk. It must not be forgotten that the nutritional value of vegetable drinks depends on the quality, the type of raw material and the process of production. Moreover, the variety and accessibility of plant milk have grown significantly worldwide due to the fact that it should be available to consumers [6, 35]. Currently, in vegan ice cream production the popularity as a cow's replacement ingredients gain soy, coconut, almond or even walnut drink. For instance, soy is a valuable source of protein and fat. Seeds of soy consist of 35-45% protein and 20% of fat. In addition, soy drinks may form a stable network which is similar to gel structure. Moreover, the lecithin of soybean extract is able to act as an emulsifier and as a result, protect against freezing damage [1, 5, 40]. In the paper by Bisla et. al (2012), the ice cream was prepared based on a soy drink with the addition of watermelon seed drink and guava pulp. The result of the sensory analysis (such as appearance, flavour, mouth feel or overall acceptability) showed that the most acceptable was ice cream with 50% of soy and 50% of watermelon drink and 50 g guava pulp. Moreover, based on the nutritional analysis it was shown a high value of protein (11.2 g/100 g) and fat (7.26 g/100 g). In addition, such produced ice cream is characterised by excellent content of iron (1.56 mg/100 g) and vitamin C (89.92 mg/100 g) in comparison to the control sample of ice cream based on traditional milk [13]. Coconut drink is another popular ingredient in vegan ice cream. It contains 80% of water, 3.6 g fat and 0.3 g protein.

Moreover, it is rich in minerals, vitamins, proteins, lipids and antioxidants and it is easily digested. Additionally, one of the main components of coconut drink is coconut oil which for instance characterises a significant portion of lauric acid. Thus, coconut drinks may be a good candidate to increase the calories in a product due to their high-fat intake [1, 2, 40]. Nowadays, there was a trend in which coconut drinks or coconut oil were extremely popular even in vegan ice cream. In the study by Beegum et al. (2021), the effect of coconut drink, tender coconut pulp, tender coconut water and coconut sugar were investigated on the attributes of ice cream. Given the results obtained the coconut additions contributed to the acceptance of appearance, flavour and taste among panellists. Moreover, increasing the coconut drink and pump provided a pivotal amount of polyphenolics and antioxidants. On the other hand, the lower overrun and total solids were achieved by adding a plant-based protein [11] (***table 1***). Almond drink is also used as a milk substitute which has a high number of phytochemicals, vitamin E, monounsaturated and polyunsaturated fatty acids, potassium and arginine. Additionally, the almond drink is able to provide a thicker texture and creamier taste to the product [1, 26, 40]. In the research by Bekiroglu et al. (2022), vegan ice cream was prepared based on a fresh and dried walnut drink. This sort of milk analogue stands out with a rich nutritional value. Additionally, the nut in raw and processed form may be applied as a way against the formation of carcinogenic substances from dietary materials [12]. Overall, in the mentioned study the addition of walnut drink improved the rheological properties but on the other hand, it detrimentally influenced the brightness of ice cream. Moreover, referring to the physicochemical properties, walnut drink increased the fat content due to the high-fat content of walnut. Consequently, it can be concluded that both dry and fresh walnut drink in vegan ice cream is beneficial for this frozen dessert [12]. Other authors, Atalar et al. (2021) provided an analysis of using hazelnut drink after high-pressure homogenisation as a replacement for cow milk, to prepare ice cream. Results evidenced that the addition of hazelnut drink improved the textural, melting and rheological properties. Furthermore, high viscosity, consistency index and high material stiffness were observed. In addition, based on the higher capacity of TPC (total phenolic content) and antioxidants as a result of hazelnut drink, provide the new product with health-promoting properties [9].

The next curious issue was taken into consideration in the research by Aboulfazli et al. (2016), in which the ice cream based on soy or coconut drink was riched with probiotics. It is known that milk products have the potential to incorporate probiotics culture to create new functional products. As a result of this research both used vegetable drinks, soy and coconut, which gave a richer medium of amino acids and sugar content than cow's milk in ice cream. Therefore, there is a possibility to produce ice cream based on the milk analogue with the probiotic culture which may be used as a new functional food [2]. Furthermore, in the paper by Velotto et al. (2021), the fascinating idea was to use stevia as a sweetener and chia seeds as a thickener in the production of vegan ice cream. As a result, it was noticed that such a combination of ingredients contributed to high overrun values and desirable texture.

To follow the trend and obtain the lucrative quality of ice cream, scientists examine many recipes with a wide range of ingredients. An innovative and curious idea in vegan ice cream production has the use of inulin. There exist a few research in which this ingredient was applied to the recipe. Firstly, inulin may be beneficial for our health. Inulin has the capacity to bind water molecules and thus it plays a crucial role in stabilizing the texture [3]. To give an illustration of this, in the research by Góral et al. (2018) (***table 1***), in coconut vegan ice cream, the addition of inulin was tested. It was reported that increasing the amount of inulin, reduced the time of the first drop during melting.

Recently developments in the field of vegan ice cream have led to interest also in the taste and flavour that is significant in ice cream. As evidenced in the study by Diniz et al. (2022), to enrich the choice of vegan ice cream, peanut and cocoa-flavoured were made based on the water-soluble plant extracts of baru nuts and cashew nuts. The peanut-flavoured ice cream had a greater proportion of oleic acid than cocoa ones. Having said that the higher melting rate and overrun were noted for the cocoa one and then for the peanut ice cream. However, it must not be forgotten about changes in the product such as consumers' acceptance or texture should be also examined. In this case, further studies are required to develop this knowledge.

Owing to the fact that not only changes in the recipe of ice cream but also changes in the technology of production are indispensable. For instance, in the study by Anwar et al. (2022), the extract of watermelon seed protein concentrates was made by using an ultrasonication process to prepare vegan ice cream. Coconut fat and coconut drink also were used in the recipe as fat replacers. Finally, it was reported that the rheological, structural and sensory quality were comparable to standard dairy ice cream formulations. In addition, in the research by Yan et al. (2022), the combination of high hydrostatic pressure treatment (HPP) and compounded phospholipid was used to modify the soybean protein isolate to produce low-fat vegetable ice cream. The results of this study showed that such a combination successfully improved the performance of soybean protein isolate (SPI). The obtained ice cream characterised a better expansion rate, melting rate and hardness than the samples without any modifications.

THE PROCESS OF PRODUCTION of ice cream

Not only do ingredients contribute to the lucrative quality of ice cream but also the process of production. The ice cream processing operation of vegan ice cream is conducted in the same way as milk ice cream. It may be divided into two distinct steps: mix manufacturing operations and the freezing process. The first step in ice cream mix manufacture is connected with creating the ice cream mix. Ice cream mix can be described as hydrocolloidal dispersion, in which proteins and polysaccharides in concentrations over the phase separation threshold, create a two-aqueous system, in compliance with thermodynamic incompatibility [19, 20, 22]. After combining and blending all ingredients in an appropriate order, pasteurisation is conducted. The main aim of this operation is to safeguard the healthy condition of the product. The next crucial step is the homogenisation process. The essential purpose of this step is to obtain a stable and uniform suspension by reducing the size of fat globules to less than 2 μm. Moreover, homogenisation is required to accomplish a stable emulsion [20]. The last step before the freezing process is the maturation of the ice cream mix. This step is significant due to the fact that during this time beneficial changes occur as the fat crystallization, hydration of stabilisers and membrane rearrangement to obtain a better quality product and smoother texture. After finishing the ice cream mix manufacture, the freezing process is managed. In the ice cream freezer, the ice cream mix is converted into ice cream by simultaneously aerating, freezing and beating. After the freezing

Table 1. Summary of recent studies on the production and quality of vegan ice cream

Tabela 1. Podsumowanie ostatnich badań dotyczących produkcji i jakości lodów wegańskich

Ingredients for vegan ice cream	Processing conditions	Effect	Reference
Sterilized coconut drink, cane sugar, elderberry syrup, sunflower lecithin, inulin, LBG	weighting components, blending and aeration, freezing (–6°C), hardening (–30°C for 24 h)	■ decreasing in the cryoscopic temperature and melting time as a result of increasing the amount of inulin and LBG ■ the higher amount of inulin and LBG were added the higher the overrun was obtained ■ samples with LBG had higher hardness than ice cream with inulin ■ the addition of stabilisers contributed to the brightening of ice cream while the addition of inulin led to darkening. ■ according to the sensory evaluation ice cream with LBG at 0.8 g/100 g and 4 g/100 g inulin obtained the highest score	Góral et al. (2018)
Almond drink, almond syrup, inulin, pea protein, emulsifier E471, LBG and xanthan gum	weighting compounds, mixing, pasteurisation at 95°C for 1.5 min, cooling to obtain 25°C, freezing, hardening at –18°C for 24 h	■ the size of ice crystals did not exceed 21 µm ■ the addition of stabilisers contributed to the highest score in the organoleptic evaluation ■ the stabilisers did significantly influence the physical parameters of ice cream as overrun or density ■ the same stabilisers and technology may be used in milk and also in the vegan ice cream production	Kot et al. (2020)
Vegan recipe: salep, sucrose, hazelnut drink (was obtained by cold-pressed hazelnut cake by the removal of oil, then mixed with the distilled water and homogenised by using high-pressure homogenisation at 100 MPa)	weighting components, heated and homogenised (10 min; 16,000 rpm, 70°C), pasteurisation (80° for 10 min), cooling to 4°C and ageing at 4°C for 24 h, freezing at 0°C for 10 min (the final temperature of ice cream was at –7° ± 1°C), stored at –18°C for 24 h	■ by increasing the amount of hazelnut drink the water-holding properties, flow behaviours, and emulsifying capacities were improved ■ a higher amount of hazelnut drink reduced the melting rate and also retarded the first dropping time values ■ the 75% of hazelnut drink in the recipe for vegan ice cream contributed to improving the acceptability of the product according to the sensory parameters ■ the hazelnut drink increased the TPC (total phenolic content) and antioxidant capacity of the final product	Atalar et al. (2021)
Vegan recipe: Coconut drink, sucrose, coconut sugar, tender coconut water, tender coconut pulp, water, stabilisers & emulsifier (CMC, xanthan gum, guar gum, GMS)	all ingredients were mixed and pasteurized at 75°C for 15 min, homogenised at 2000/500 psi, ageing at 5°C for an hour, freezing, hardening at –28°C	■ vegan ice cream obtained lower overrun ■ the addition of coconut products yelled in increased total phenolics and salts ■ vegan ice cream was preferred over dairy ice cream during the sensory evaluation, in order to flavour and taste	Beegum et al. (2021)
Vegan recipe: fat coconut powder, emulsifier blend (containing: mono- and diglycerides of fatty acids, guar gum, locust bean gum and sodium alginate), glucose syrup, maltodextrin, dextrose monohydrate, 100% pure stevia powder, 100% chia seeds powder	the weighting of components, heating liquid ingredients to 50°C, pasteurisation at 85°C for 1 min, cooling to 4°C, stirring for about 10 h, then continuously stirring for 7-8 min at –8°C, storing at –18°C	■ the replacement of sugar and common emulsifier contributed to the highest overrun ■ the chia seeds and stevia gain improvement in the textural parameters as consistency index *K* and flow behaviour index *n* ■ the sensory evaluation proved that stevia and chia seeds gained the highest score	Velotto et al. (2021)
WSPC - watermelon seed protein concentrate (extraction with the ultrasonication process at 25 kHz, 80 W for 10 min), coconut fat, sugar, stabiliser, emulsifier, coconut drink	mixing all ingredients for 8-10 min at 48-50°C, pasteurisation for 20 min at 75°C, homogenisation at 2000 psi for 2 min, cooling at 15°C, ageing at 4°C for overnight, freezing at –5°C, storing at –25°C	■ functional properties of WSPC were improved with US-assisted extraction ■ by increasing the amount of WSPC the melting resistance, viscosity and textural hardness were increased ■ based on the sensory acceptability, the most satisfactory sample was the one with 10% of WSPC	Anwar et al. (2022)
Vegan recipe: monodigliserid, salep, dried walnut milk/fresh walnut drink (the walnut drink was made and then homogenised at 25 MPa and pasteurised at 92°C through 15 min)	weighting components, heating drink to obtain 40°C, pasteurising all ingredients at 72°C for 15 min, cooling to 4°C, ageing for 24 h at 4°C, freezing, and storing at –18°C for 8 weeks	■ the use of walnut drink improved the rheological properties of ice cream (the higher consistency coefficient (*K*) and lower *n* value) ■ walnut drink contributed to the decreasing the brightness ■ the overrun of ice cream with walnut drink was higher than with the cow milk ■ based on the sensory evaluation ice cream with walnut drink received a similar score as ice cream with cow milk	Bekiroglu et al. (2022)
Mixed water-soluble plant extract of baru nuts and cashew nuts, peanut paste, cocoa powder, sugarcane syrup, xanthan gum, water, emulsifying agent/emustab, stabilising agent	the weighting of the ingredients, homogenisation for 10 min, agitation and cooling at –30°C for 40 min, storing at –18°C	■ the cocoa-flavoured vegan ice cream was characterised by a higher melting rate and overrun than the peanut-flavoured one ■ the peanut-flavoured ice cream was characterised by 9 different types of fatty acid	Diniz et al. (2022)
Vegetable recipe: HHP-modified soy protein isolate and compounded phospholipids, cream, cane sugar, emulsifier (sucrose ester, monoglyceride), stabilisers (carrageenan, locust bean gum)	the well-mixed slurry was refrigerated at 4°C for 1 hour, then freeze	■ the HPP treatment significantly changed the secondary structure of the protein ■ the ice cream with the addition of modified SPI exhibited better results in expansion rate, melting rate and hardness than unmodified samples ■ the addition of phospholipids contributed to the increase in the surface charge and the particle size ■ sensory evaluation showed good acceptability, close to the milk ice cream	Yan et al. (2022)

operation, ice cream is hardened at the typical temperature of –18°C [14, 19].

As was mentioned in the begging, ice cream is a multiphase system and one of these phases is the emulsion. During the initial step of ice cream production, the emulsion is created. The quality of the ice cream mix before and after the maturation process has a tremendous effect on the final product. Due to the fact that during the pasteurisation and homogenisation process, the structure of emulsion is created which may consequently influence the structure of ice crystals in ice cream. Moreover, the changes which can occur while the maturation step as the destabilisation of fat or increase/decrease particles might contribute to the size of ice crystals and the quality of ice cream as texture, creaminess or meltdown behaviour [22].

Taking all these points into consideration, it is vital to highlight the physical properties of ice cream emulsion which are connected with the stability, particle size distribution or rheological properties. Nowadays, there are studies in which scientists pay attention to the physical properties of vegan ice cream mixes. For instance, in the research by Kot et al. (2021) the influence of used stabilisers and the homogenisation conditions were examined on the stability properties of vegan ice cream mixes. It was reported that homogenisation contributed to the destabilisation of ice cream mixes. Moreover, the addition of a combination of iota carrageenan, locust bean gum and xanthan gum, had a beneficial influence not only on the stability but also on the reduction of the size of particles. Furthermore, the same authors in other research [27], used ultrasound homogenisation to produce vegan ice cream mixes. In this paper, it has occurred that ultrasound homogenisation provided better stability of ice cream mixes in comparison to traditional homogenisation. In addition, the combination of stabilisers (iota carrageenan or its hydrolysates with locust bean gum and xanthan gum) and ultrasound contributed to the reduction of the size of particles.

CONCLUSION:

The general concept of production of experimental vegan ice production in the mentioned research is incredibly scintillating. Those results appeared to confirm that this frozen dessert may be paralleled to the traditional milk ice cream. The diversity among ingredients or combinations of them provides successful results for current and also for next research. Consequently, there exists tremendous hope for all who suffer from milk allergies, lactose intolerance or vegans, to relish the taste of this delicious frozen dessert. Obviously, further studies on this current topic are therefore required to develop knowledge about the properties of vegan ice cream and also the dependencies between ingredients. ■

Mgr inż. A. Kot (ORCID 0000-0003-3850-1743), dr hab. A. Kamińska-Dwórznicka, prof. SGGW (ORCID 0000-0002-2835-2265) – Department of Food Engineering and Process Management, Institute of Food Sciences, Warsaw University of Life Sciences (WULS-SGGW), Warsaw;
e-mail: anna_kot1@sggw.edu.pl

LITERATURE:

[1] Abdullah N., N. Wahab, N. Saruan, H. Matias-Peralta, N. Xavier, N. Muhammad, B.A. Talip, M.F.A. Bakar. 2018. „Effect of replacing coconut milk with almond milk in spicy coconut gravy on its sensorial, nutritional and physical properties". *Materials Today: Proceedings* 5 (10) : 21919-21925. DOI: 10.1016/j.matpr.2018.07.051.
[2] Aboulfazli F., A.B. Shori, A.S. Baba. 2016. „Effects of the replacement of cow milk with vegetable milk on probiotics and nutritional profile of fermented ice cream". *LWT – Food Science and Technology* 70 (7) : 261-270. DOI: 10.1016/j.lwt.2016.02.056.
[3] Akbari M., M.H. Eskandari, M. Niaskosari, A. Bedeltavana. 2016. „The effect of inulin on the physicochemical properties and sensory attributes of low-fat ice cream". *International Dairy Journal* 57 : 52-55. DOI: 10.1016/j.idairyj.2016.02.040.
[4] Akbari M., M.H. Eskandari, Z. Davoudi. 2019. „Application and functions of fat replacers in low-fat ice cream: A review". *Trends in Food Science & Technology* 86 : 34-40. DOI: 10.1016/j.tifs.2019.02.036.
[5] Akesowan A. 2009. „Influence of soy protein isolate on physical and sensory properties of ice cream". *Thai Journal of Agricultural Science* 42 (1) : 1-6.
[6] Alozie Y.E., U.S. Udofia. 2015. „Nutritional and sensory properties of almond (*Prunus amygdalu* var. Dulcis) seed milk". *World Journal of Dairy & Food Sciences* 10 (2) : 117-121. DOI: 10.5829/idosi.wjdfs.2015.10.2.9622.
[7] Alvaro C. 2017. „Ethical veganism, virtue, and greatness of the soul". *Journal of Agricultural and Environmental Ethics* 30 (6) : 765-781. DOI: 10.1007/s10806-017-9698-z.
[8] Anwar S., M. Baig, Q. Syed, R. Shukat, M. Arshad, H. Asghar, M. Arshad. 2022. „Dairy ingredients replaced with vegan alternatives: valorisation of ice cream". *International Journal Of Food Science & Amp; Technology* 57 (9) : 5820-5826. DOI: 10.1111/ijfs.15895.
[9] Atalar I., A. Kutr, O. Gul, F. Yazici. 2021. „Improved physicochemical, rheological and bioactive properties of ice cream: Enriched with high pressure homogenized hazelnut milk". *International Journal of Gastronomy and Food Science* 24 : 100358. DOI: 10.1016/j.ijgfs.2021.100358.
[10] Bahram-Parvar M. 2015. „A review of modern instrumental techniques for measurements of ice cream characteristics". *Food Chemistry* 188 : 625-631. DOI: 10.1016/j.foodchem.2015.05.017.
[11] Beegum P., J. Nair, M. Manikantan, R. Pandiselvam, S. Shill, S. Neenu, K. Hebbar. 2021. „Effect of coconut milk, tender coconut and coconut sugar on the physico-chemical and sensory attributes in ice cream". *Journal Of Food Science And Technology* 59 (7) : 2605-2616. DOI: 10.1007/s13197-021-05279-y.
[12] Bekiroglu H., H. Goktas, D. Karaibrahim, F. Bozkurt, O. Sagdic. 2022. „Determination of rheological, melting and sensorial properties and volatile compounds of vegan ice cream produced with fresh and dried walnut". *International Journal of Gastronomy and Food Science* 28 : 100521. DOI: 10.1016/j.ijgfs.2022.100521.
[13] Bisla G., P. Archana, S. Verma, S. Sharma. 2012. „Development of ice creams from Soybean milk & Watermelon seeds milk and Evaluation of their acceptability and Nourishing potential". *Advances in Applied Science Research* 3 (1) : 371-376.
[14] Clarke C. 2004 *The science of ice cream*. Cambridge, UK : The Royal Society of Chemistry.
[15] Diniz L., E. Jesus, C. Francisco, L. Tormen, L. Bertan. 2022. „Mixed water-soluble nut-based plant extracts to produce vegan ice creams". *Research, Society And Development* 11 (7) : e39011729892. DOI: 10.33448/rsd-v11i7.29892.
[16] Flom J. D., S.H. Sicherer. 2019. „Epidemiology of cow's milk allergy". *Nutrients* 11 : 1051. DOI: 10.3390/nu11051051.
[17] Flores A.A., H.D. Goff. 1999. „Ice crystals size distribution in dynamically frozen model solutions and ice cream as affected by stabilizers". *Journal of Dairy Science* 82 (7) : 1399-1407. DOI: 10.3168/jds.S0022-0302(99)75366-X.
[18] Gaukel V., A. Leiter, W.E.L. Spiess. 2014. „Synergism of different fish antifreeze proteins and hydrocolloids on recrystallization inhibition of ice in sucrose solutions". *Journal of Food Engineering* 141 (3) : 44-50. DOI: 10.1016/j.jfoodeng.2014.05.016.
[19] Goff H.D. 1997. „Colloidal aspects of ice cream – a review". *Journal of Dairy Science* 7 (6-7) : 363-373. DOI: 10.1016/S0958-6946(97)00040-X.
[20] Goff H.D., R.W. Hartell. 2013. *Ice Cream. Seventh Edition*. Springer Science+Business Media New York.
[21] Góral M., K. Kozłowicz, U. Pankiewicz, D. Góral, F. Kluza, A. Wójtowicz. 2018. „Impact of stabilizers on the freezing process, and physicochemical and organoleptic properties of coconut milk-based ice cream". *LWT-Food Science and Technology* 92 : 516-522. DOI: 10.1016/j.lwt.2018.03.010.
[22] Innocente N., M. Biasutti, E. Venir, M. Spaziani, G. Marchesini. 2009. „Effect of high-pressure homogenization on droplet size distribution and rheological properties of ice cream mixes". *Journal of Dairy Sciences* 92 (5) : 1864-1875. DOI: 10.3168/jds.2008-1797.
[23] Kamińska-Dwórznicka A., E. Gondek, S. Łaba, E. Jakubczyk, K. Samborska. 2019. „Characteristics of instrumental methods to describe and assess the recrystallization process in ice cream systems". *Foods* 8 (4) : 117. DOI: 10.3390/foods8040117.
[24] Konstantas A., L. Stamford, A. Azapagic. 2019. „Environmental of ice cream". *Journal of Cleaner Production* 209 : 259-272. DOI: 10.1016/j.jclepro.2018.10.237.
[25] Kot A., A. Kamińska-Dwórznicka, A. Barańska. 2020. „Study of the properties of vegan ice cream based on almond drink". *Zeszyty Problemowe Postępów Nauk Rolniczych* 600 : 21-30. DOI: 10.22630/ZPPNR.2020.600.3.
[26] Kot A., A. Kamińska-Dwórznicka, S. Galus, E. Jakubczyk. 2021. „Effects of different ingredients and stabilisers on properties of mixed based on almond drink for vegan ice cream production". *Sustainability* 13 (21) : 12113. DOI: 10.3390/su132112113.
[27] Kot A., A. Kamińska-Dwórznicka, E. Jakubczyk. 2022. „Study on the influence of ultrasound homogenisation on the physical properties of vegan ice cream mixes". *Applied Sciences* 12 : 8492.
[28] Koxhlot M.N.R., B. Eisenmann, J. Hinrichs. 2001. „Effect of the fat globule sizes on the meltdown of ice cream". *Journal of Dairy Science* 84 (1) : 31-37. DOI: 10.3168/jds.S0022-0302(01)74448-7.
[29] Krizanova J., D.L. Rosenfeld, A.J. Tomiyama, J. Guardiola. 2021. „Pro-environmental behaviour predicts adherence to plant-based diets". *Appetite* 163 (4) : 105243. DOI: 10.1016/j.appet.2021.105243.
[30] Liang H.-N., C. Tang. 2014. „Pea protein exhibits a novel Pickering stabilization for oil-in-water emulsions at pH 3.0". *LWT – Food Science and Technology* 58 (2) : 463-469. DOI: 10.1016/j.lwt.2014.03.023.
[31] Lu Z.X., J.F. He, Y.C. Zhang, D.J. Bing. 2020. „Composition, physicochemical properties of pea protein and its application in functional foods". *Critical Reviews in Food Science and Nutrition* 60 : 15, 2593-2605. DOI: 10.1080/10408398.2019.1651248.
[32] Lomolino G., S. Zannoni, A. Zabara, M. Da Lio, A. De Iseppi. 2020. „Ice recrystallisation and melting in ice cream with different proteins levels and subjected to thermal fluctuation". *International Dairy Journal* 100 : 104557. DOI: 10.1016/j.idairyj.2019.104557.
[33] O'Sullivan J., B. Murray, C. Flynn, I. Norton. 2016. „The effect of ultrasound treatment on the structural, physical and emulsifying of animal and vegetable protein". *Food Hydrocolloids* 53 : 141-154. DOI: 10.1016/j.foodhyd.2015.02.009.
[34] Panghal A., S. Janghu, K. Virkar, Y. Gat, V. Kumar, N. Chhikara. 2018. „Potential non-dairy probiotic products – A healthy approach". *Food Bioscience* 21 : 80-89. DOI: 10.1016/j.fbio.2017.12.003.
[35] Rincon L., R. Braz Assunção Botelho, E.R. de Alencar. 2020. „Development of novel plant-based milk based on chickpea and coconut". *LWT – Food Science and Technology* 128 : 109479. DOI: 10.1016/j.lwt.2020.109479.
[36] Smith A.K., H.D. Goff, Y. Kakuda. 2000. „Microstructure and rheological properties of whipped cream as affected by heat treatment and addition of stabilizer". *International Dairy Journal* 10 (4) : 295-301. DOI: 10.1016/S0958-6946(00)00043-1
[37] Spence Ch., J. Navarraa, J. Youssef. 2019. „Using ice-cream as an effective vehicle for energy/nutrient delivery in the elderly". *International Journal of Gastronomy and Food Science* 16 : 100140. DOI: 10.1016/j.ijgfs.2019.100140.
[38] Stremmel G., O. Elshiewy, Y. Boztug, F. Carneiro-Otto. 2022. „Vegan labeling for what is already vegan: Product perceptions and consumption intentions". *Appetite* 175 : 106048. DOI: 10.1016/j.appet.2022.106048.
[39] Tan N.P., T.S. Conner, H. Sun, S. Loughnan, L.D. Smillie. 2021. „Who gives a veg? Relations between personality and Vegetarianism/Veganism". *Appetite* 163 : 105195. DOI: 10.1016/j.appet.2021.105195.
[40] Vanga S.K., V. Raghavan. 2018. „How well do plant based alternatives fare nutritionally compared to cow's milk?". *Journal of Food Science and Technology* 55 (1) : 10-20. DOI: 10.1007/s13197-017-2915-y.
[41] Velotto S., L. Parafati, A. Ariano, R. Palmeri, F. Pesce, D. Planeta, V. Alfeo, A. Todaro. 2021. „Use of stevia and chia seeds for the formulation of traditional and vegan artisanal ice cream". *International Journal of Gastronomy and Food Science* 26 : 100441. DOI: 10.1016/j.ijgfs.2021.100441.
[42] Yan G., Y. Cui, D. Lia, Y. Ding, J. Han, S. Wang, Q. Yang, H. Zheng. 2022. „The characteristics of soybean protein isolate obtained by synergistic modification of high hydrostatic pressure and phospholipids as a promising replacement of milk in ice cream". *LWT– Food Science and Technology* 160 : 113223. DOI: 10.1016/j.lwt.2022.113223.
[43] [nternet 1 : https://www.grandviewresearch.com/industry-analysis/ice-cream-market?fbclid=IwAR1Twrel9IFlQ0PqP_0qA0MIky5y1Ai6FDUlLCYy5MwFHYARKwYHT4jGrOk [access 25.08.2022].

Zeszyty Problemowe Postępów Nauk Rolniczych
nr 600, 2020, 21–30
DOI 10.22630/ZPPNR.2020.600.3

STUDY OF THE PROPERTIES OF VEGAN ICE CREAM BASED ON ALMOND DRINK

Anna Kot✉, Anna Kamińska-Dwórznicka, Alicja Barańska
WULS-SGGW, Institute of Food Sciences

Summary. The objective of this study was to create a formula of vegan ice cream based on almond drink with the same unitary operations applied for the milk ice cream. Additionally, the influence of selected stabilizers on the physical, organoleptic characteristics of ice cream and crystal structure was studied. A mixture of two stabilizers locust bean gum (LBG) and xanthan gum was applied. The obtained results showed that the addition of stabilizers had no significant effect on the physical parameters of ice cream. However, the addition prevented recrystallization and the ice crystal equivalent diameter did not exceed 21 μm. The organoleptic evaluation the ice cream with stabilizers obtained the highest score. In conclusion, this paper showed that the same parameters and additives could be used equally for milk and vegan production of ice cream.

Key words: vegan ice cream, almond drink, stabilizers

INTRODUCTION

The appropriate composition of ice cream and the interactions between ingredients contribute to obtain the positive characteristic structure of ice cream a desert appreciated by most consumers. During the final production steps, storage and commercialization of ice cream, undesirable changes might occur that have a negative impact on their structure [Kamińska-Dwórznicka et al. 2019, Lomolino et al. 2020]. As a result of the temperature fluctuation, the sizes of ice crystals change, which leads to the phenomenon of recrystallization, which results in product degradation [Gaukel et al. 2014]. To inhibit recrystal-

Anna Kot https://orcid.org/0000-0003-3850-1743; Anna Kamińska-Dwórznicka https://orcid.org/0000-0002-2835-2265; Alicja Barańska https://orcid.org/0000-0003-2711-6073
✉ anna_kot1@sggw.edu.pl
© Copyright by Wydawnictwo sggw

lization processes a good way is to add substances that have a preventive effect on the ice crystals growth, called stabilizers [Kamińska-Dwórznicka et al. 2013]. The main reason for using of stabilizers in the freezing process is their ability to increase the viscosity of the solution, which could lead to the limitation of water molecules migration during storage [Soukoulis et al. 2008, Goff and Hartel 2013]. Although cow's milk is an important source of nutrients, many people suffer from cow's milk allergy and lactose intolerance. Additionally, the consumers choose plant's products, instead of milk ones because of other factors such as the presence of cholesterol, health and environment consideration [Kundu et al. 2018, Vanga and Raghavan 2018]. The solution to this problematic issue can be the consumption of almond drink instead of cow's milk [Vanga and Raghavan 2018]. Almond has a relatively high number of phytochemicals (including phenolic acids, phytosterols), polyphenolic compounds (flavonoids and pro-anthocyanidins), vitamin E, monounsaturated and polyunsaturated fatty acids, arginine and potassium. Due to this beneficial nutrient profile, almond has antioxidant and anti-inflammatory properties and it can reduce cardiovascular diseases risk [O'Neil et al. 2016, Yüksel et al. 2017].

This study aimed to prepare the initial formula for vegan ice cream. Besides this, the recipe was prepared accordingly with the milk ice cream production. Moreover, the addition of two stabilizers was compared with the sample with any additions, on the physical and sensory properties of obtained ice cream.

MATERIAL AND METHODS

Materials

The following ingredients were used for preparing the best options of vegan ice cream production: roasted almond original (Alpro), almond syrup (Monin), saccharose (Diamant Cukrownia Glinojeck), glucose (Biomus), inulin (Orafti BENEO), pea protein (Natural YS S85F, Roguette), emulsifier E471 (Fooding Shanghai), milk powder without lactose (Mlekovita) – eliminate from the final formula, LBG (Fooding Shanghai), xanthan gum (Fooding Shanghai).

Ice cream production

The development of the final formula required preliminary testing, during which seven other recipes were tested. Ultimately, from the seventh tried one was finally the basic for the ice cream production (Table 1). All ingredients were weighed separately with a determined formula. Then dry components were mixed with the liquid ones, using Bosch MaxoMixx 750W blender. The Vorwerk thermomixer was used for pasteurization process of the ice cream mix at 95°C per 1.5 min. Afterwards, the mix was cooled to 25°C, and frozen through 40 min using the ice cream maker Nemox Gelato Pro 1700 (Nemox, Italy). Then the samples were placed into a plastic boxes and hardened at –18°C for 24 h. In the meantime, a control sample of ice cream without any stabilizers (WOS)

was prepared. All the physical measurements for control sample and for the sample with the stabilizers addition (WS) were carried out twice. Only organoleptic analysis was carried out three times out.

Table 1. Final formula of vegan ice cream

Tabela 1. Finalna receptura lodów wegańskich

Ingredients Składniki	Weight Udział wagowy [g]	Percentage Udział procentowy [%]
Almond drink Napój migdałowy	587.36	73.42
Almond syrup Syrop migdałowy	64.00	8.00
Inulin Inulina	96.00	12.00
Pea protein Białko grochu	48.00	6.00
Emulsifier E471 Emulgator E471	3.20	0.40
Locust bean gum (LBG) Mączka chleba świętojańskiego (LBG)	0.80	0.10
Xanthan gum Guma ksantanowa	0.64	0.08

Methods

pH

Ice cream pH was measured after pasteurization and cooling 25°C, using the pH--meter Elektroda Elmetron EPP3t with temperature sensor Pt-1000B, according to the device's instruction.

Density

Density was carried out using the glass pycnometer at ambient temperature, according to the method presented by Dłużewska et al. [2003]. The pycnometer was weighed on an analytical weight with an accuracy of 0.1 g. Then it was filled with ice cream mix and capped tightly and weighed on an analytical weight with an accuracy of 0.1 g.

Melting test

In order to study the ice cream melting behaviour, the method described by Dłużewska et al. [2003] was used. For this purpose, a cooled metal cylinder (of known volume) was filled with ice cream and kept at –18°C for 24 h. Then the cylinder was placed on a glass funnel so that it wasn't touching this funnel. The melting time was measured from the moment the first drop appeared in the narrowed part of the funnel.

Determination of the overrun

The overrun of the final product was determined according to the method described by Ismail et al. [2013]. A known volume of ice cream mix and ice cream after freezing was weight.

Microstructural analysis of ice crystals

Ice crystals were analyzed based on the images taken after 24 h of storage at –18°C. The first step of this determination was sampling. The small amount of ice cream was put on cool slide using a spatula and covered by a cool slip glass, at the storage temperature. The images were taken using the Nikon Alpha Phot-2 microscope with the cooling system Linkam Scientific PE 94 and camera Nikon DS.-Fi1. The obtained images were analyzed using NIS Elements D software. The 200 to 300 ice crystal were marked for a particular sample, and then the area, equivalent diameter and standard deviation were calculated using the NIS Elements D Imaging software (ver. 3.00, Nikon). The frequency distribution of crystals size was figured using Microsoft Excel 2011 macro data analysis. A method developed by Regand and Goff [2003] was applied to report the relative frequency of any class range was figured as the number of the crystals in that range (range frequency) divided by the total number of crystals.

Organoleptic evaluation

The organoleptic analysis was carried out with a group of 10 people. The analysis was conducted using the 5-point scale, for 1 – the less desirable features and 5 – the most desirable features. All the samples were prepared 24 h before and stored at –18°C. Then the ice cream samples were kept at room temperature for at least 10 min (for softening), portioned and placed into plastic cups. The group of testing panellists evaluated the following categories: taste, colour, smell, consistency and melting time.

Statistical analysis

All obtained results were processed using a one-way analysis of variance with the Tukey test at the significance level of $\alpha = 0.05$, program Statistica 13.1.

RESULTS AND DISCUSSION

The first parameter which was measured in prepared samples was the pH. In the research conducted by Fiol et al. [2017], the pH for milk ice cream with added lactose and sodium caseinate as the main ingredients, measure from 7.7 to 7.8 [Fiol et al. 2017]. These results indicate that both with (WS) or without stabilizers (WOS) ice cream had similar pH (Table 2). However, the statistical analysis indicates no significant differences between WS and WOS. According to Makinde and Adebile [2018] research, the pH of almond drink is the range from 6.53 to 6.93. Considering the composition (Table 1), the almond drink was more than 70% of ice cream and could have contributed most to the pH of the ice cream mix. Additionally, the pea protein added to both samples might have contributed in increasing of the pH. Stabilizers addition shouldn't affect the pH of ice cream.

The density of ice cream matrix varied in composition and it might be in the range from 1.0544 to 1.1232 g·ml^{-1} [Goff and Hartel 2013]. The density increase of the ice cream mix is not a desirable factor, because it could disturb the appropriate overrun of such product [Florowska et al. 2013]. In our study there were no significant differences between the examined samples. Additionally, the density had no influence on the other physical factors like overrun or melting time (Table 2). The overrun was described as the percentage of the ice cream's expansion which is achieved from the amount of air bubbles incorporated during freezing process [Tomer and Kumar 2013]. Measured overrun values of prepared ice cream were among 26.56 and 32.02% for WS and WOS respectively. The ice cream without stabilizers (WOS) was the one characterized by a higher value of this parameter. According to the statistics, the differences was not significant ($p > 0.05$). Elsabie and Aboel Einen [2017] proved that the ice frozen product based on plant milk had a lower overrun (40.2–44.2%) different from those based on cow's milk (52.83%). This was affected by the lower fat amount in frozen dessert based on plant products. So, also, in our case, the addition of LBG and xanthan, contributes to the decrease of this parameter (Table 2). Moreover, it was reported that according to the study Lomolino et al. [2020], ice cream based on the vegan recipes had a lower overrun (24 and 26%) than the milk ice cream (84 and 83%). Such low overrun is connected with the lack of milk proteins. These ingredients are responsible for forming foam due to their amphiphilic character. The addition of stabilizers may increase the melting resistance because of their abilities – such as micro-viscosity enhancement or water-holding [BahramParvar et al. 2013]. In the prepared ice cream, the melting time for the WS sample with the complex of stabilizers was better than for the WOS sample. It was almost one hour – 57.08 min (Table 2). Basing on the statistical analysis, the samples were significantly different. Also, the results presented by Dłużewska et al. [2003] confirm that the addition of locust bean gum and xanthan gum as stabilizers to ice cream caused elongation in the melting time. It was probably caused by the stabilizers addition that could trap the water molecules and then in consequence extend the melting time. Based on the knowledge of the research from Akin et al. [2007], the addition of inulin had an influence on the melting time. The

Table 2. Results of physical analysis

Tabela 2. Wyniki pomiaru właściwości fizycznych

Physical properties Właściwości fizyczne	WOS	ws
pH	$7.42^{A} \pm 0.1250$	$7.34^{A} \pm 0.1250$
Density – Gęstość [g·cm^{-3}]	$1.089^{A} \pm 0.0005$	$1.089^{A} \pm 0.0010$
Overrun – Puszystość [%]	$32.02^{A} \pm 1.2350$	$26.56^{A} \pm 1.3100$
Melting time – Czas topnienia [min : s]	$42 : 45^{A} \pm 0 : 37$	$57 : 08^{B} \pm 1 : 44$

WOS – ice cream sample without the addition of stabilizers (control sample); WS – ice cream sample with the addition of stabilizers (locust bean gum, xanthan gum). Values are reported as means ±standard deviation. Various letters indicate significant ($p < 0.05$) differences in samples.

WOS – lody bez dodatku stabilizatorów (próbka kontrolna); WS – lody z dodatkiem stabilizatorów (mączki chleba świętojańskiego, gumy ksantanowej). Wyniki pomiarów przedstawione są jako średnia z ±odchyleniem standardowym. Różne litery oznaczają znaczące ($p < 0,05$) różnice w próbkach.

researchers used inulin as prebiotic ingredient in probiotic milk cream. Moreover, they showed that inulin prolonged the melting time of ice cream. Such substances due to their affinity to water molecules, could prevent free movement of water [Akin et al. 2007]. Moreover, in a study El-Nagar et al. [2002] the addition of inulin retarded the melting time of yog-ice cream based on stirred yoghurt and milk ice cream. The increasing of inulin addition in samples, reduced the rate of meltdown [El-Nagar et al. 2002].

The results of the sensory analysis depended by the people's subjective perception and by the conditions in which the experiment was conducted. The average result for all indicators in the control sample (WOS) was between 3.87 and 4.40 but for the ice cream sample (WS) was from 4.07 to 4.77 (Fig. 1). During the sensory analysis of WOS sample and WS one, the statistical differences between them, were recognized only for the melting time ($p > 0.05$). But for the other examined factors such flavor, colour and taste, the differences were not significant.

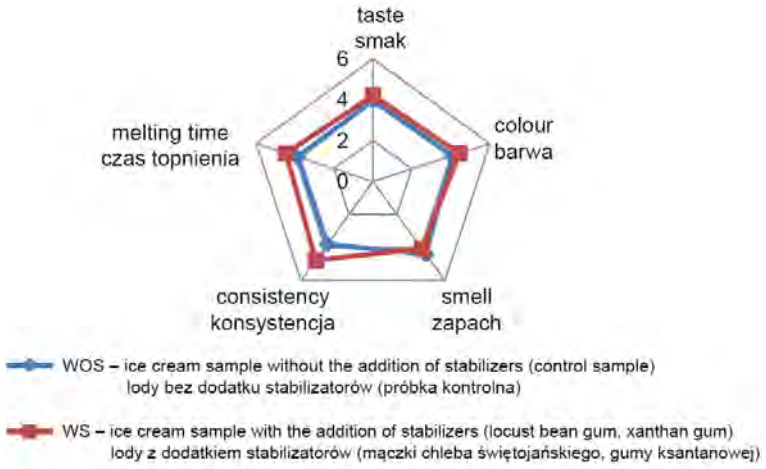


Fig. 1. Results of organoleptic evaluation

Rys. 1. Wyniki analizy organoleptycznej

There was no noticeable difference in taste for ice cream with (WS) and without stabilizers addition (WOS), however, the characteristic taste of almonds was recognized in both formulae. That phenomenon could be caused by the preference for the sweet taste. Góral et al. [2018] showed that ice cream based on coconut milk with locust beam gum is also sweeter and more acceptable by panellists. The most important impact on the prepared ice cream samples, was given by the almond drink and the pea protein. The flavour sensation was considered as acceptable and smooth. But, for the sample with locust beam gum and xanthan gum addition, this indicator was recognized as more aromatic. The colour of both samples was similar to creamy, with shades of beige and ecru. On the other hand, the WOS sample had more intensive colour than the one with the stabilizers addition (WS). Relating to the structure of ice cream, during the consumption, the WOS sample was considerably lower in consistency with higher ice crystals noticeable (Fig. 1). Based on the obtained results, the sample with stabilizers presented a longer

melting time than the sample without stabilizers. The addition of stabilizers in ice cream improve this parameter.

The ice crystals are a crucial factor which determines the quality of frozen desserts. Small size of it (10–20 μm) is required to achieve the acceptable texture of ice cream. Large ice crystals with more than 20 μm confer a coarse or grainy structure of a product [Kamińska-Dwórznicka et al. 2015]. In our study, the presented images showed the structure of ice crystals after 24 h of storage at –18°C. On the first image of the WOS sample (without stabilizers) (Fig. 2a), the coalescence phenomenon was visible. The close ice crystals were linked together and made one major one. The crystals in this sample had greater size and the diameter was between 28.72 and 89.21 μm (Table 3). The shape of crystals was diverse, round and angular included. The empty spaces around them were noticed. On the images from sample with stabilizers, the shape of the crystals was regular with round edges (Fig. 2b). The ice crystals were smaller and tightly distributed. Consequently, it can be difficult to see an empty space in such structures. The obtained results showed, that its diameter had the range from 8.87 to 23.48 μm and average diameter was 17.21 ±1.79 μm so it was less than 25 μm.

a b

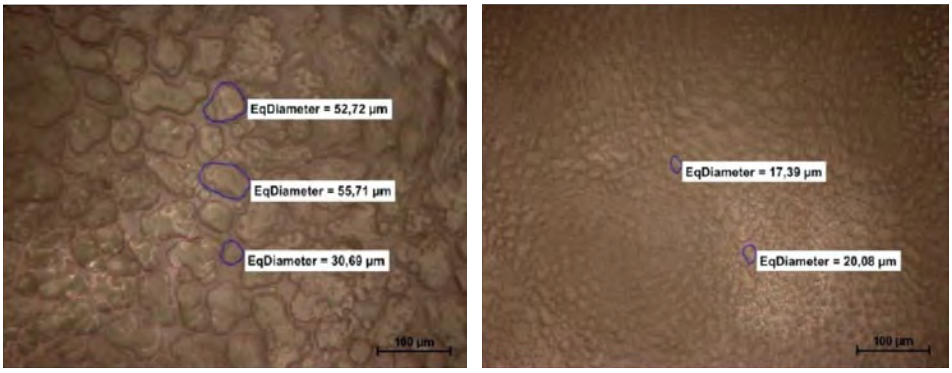


Fig. 2. Images of ice crystals in the samples after 24 h of storage at –18°C: a – WOS control sample – ice cream without the addition of stabilizers; b – WS sample – ice cream with the addition of stabilizers (locust bean gum, xanthan gum)

Rys. 2. Zdjęcia kryształów lodu w próbkach po 24 h przechowywania w –18°C: a – próbka kontrola WOS – lody bez dodatku stabilizatorów; b – próbka WS – lody z dodatkiem stabilizatorów (mączki chleba świętojańskiego, gumy ksantanowej)

Concluding on this, the recrystallization process was limited by the use of stabilizers in the composition of ice cream. Referring to organoleptic analysis, panellists did not feel sandiness in this sample and perceived a smooth and consistent texture. Lomolino et al. [2020] showed that ice cream with milk protein and stabilizers had more uniform and smaller crystals than ice cream without milk protein. Also, ice cream based on vegan protein (potato protein), had a heterogenous growth of ice crystals. In addition, the size of ice crystals was more than 20 μm [Lomolino et al. 2020]. Moreover, according to the study of Sofjan and Hartel [2004] on milk ice cream with used stabilizers: guar gum, xanthan

Table 3. Comparison of ice crystals size distribution after 24 h of storage at –18°C

Tabela 3. Porównanie rozmiarów kryształów lodu po 24 h przechowywania w –18°C

Ice cream mix variant Wariant mieszanki lodowej	Ice crystals size Średnica kryształów lodu [μm]		Average diameter (D_A) in the class with the highest frequency ±standard deviation (S_D) Uśredniona wartość – średnica (D_A) w klasie z największą częstotliwością ±odchylenie standardowe (S_D) [μm]
	min	max	
WOS	28.72	89.21	51.69 ±1.53
ws	8.87	23.48	17.21 ±1.79

WOS – ice cream sample without the addition of stabilizers (control sample); WS – ice cream sample with the addition of stabilizers (locust bean gum, xanthan gum).

WOS – lody bez dodatku stabilizatorów (próbka kontrolna); WS – lody z dodatkiem stabilizatorów (mączki chleba świętojańskiego, gumy ksantanowej).

gum and carrageenan, the ice crystals size decreased when overrun increased. That means that the ice cream with lower overrun had the highest recrystallization rate. This relation can be caused by heat transfer rates upon aeration [Sofjan and Hartel 2004]. In our study, the ice cream had a lower overrun repeatedly.

CONCLUSIONS

The production of vegan ice cream based on almond drink may be conducted with the same technology as traditional milk ice cream. The physical parameters of the examined ice cream had the same or similar values as milk based ice cream. Using the LGB and xanthan gum as stabilizers in the ice cream mix lower the overrun and prolonged the melting time. Moreover, the addition of stabilizers improved the microstructure of ice cream which was also marked during the sensory analysis.

REFERENCES

Akın M.B., Akın M.S., Kırmacı Z., 2007. Effects of inulin and sugar levels on the viability of yoghurt and probiotic bacteria and the physical and sensory characteristics in probiotic ice-cream. Food Chem. 104, 93–99.

BahramParvar M., Tehrani M.M., Razavi S.M.A., 2013. Effects of a novel stabilizer blend and presence of κ-carrageenan on some properties of vanilla ice cream during storage. Food Biosci. 3, 10–18.

Dłużewska E., Gazda B., Leszczyński K., 2003. Wpływ wybranych hydrokoloidów polisacharydowych na jakość koncentratów lodów owocowych. Acta Sci. Pol. Technol. Aliment. 2(1), 97–107.

El-Nagar G., Clowes G., Tudorică C.M., Kuri V., Brennan C.S., 2002. Rheological quality and stability of yog-ice cream with added inulin. Int. J. Dairy Technol. 55(2), 89–93.

Elsabie W.B., Aboel Einen K.A.M., 2017. New Healthy Imitated Ice Dairy Products. J. Food Dairy Sci. 8(6), 253–256.

Fiol C., Prado D., Romero C., Laburu N., Mora M., Alava J.I., 2017. Introduction of a new family of ice creams. Int. J. Gastron. Food Sci. 7, 5–10.

Florowska A., Wójcik E., Florowski T., Dłużewska E., 2013. Wpływ dodatku preparatów błonnikowych na wybrane wyróżniki jakości lodów. ZPPNR 574, 11–18.

Gaukel V., Leiter A., Spieß W.E.L., 2014. Synergism of different fish antifreeze proteins and hydrocolloids on recrystallisation inhibition of ice sucrose solutions. J. Food Eng. 141, 44–50.

Goff H.D., Hartel R.W., 2013. Ice Cream. 7th ed. Springer, New York.

Góral M., Kozłowicz K., Pankiewicz U., Góral D., Kluza F., Wójtowicz A., 2018. Impact of stabilizers on the freezing process, and physicochemical and organoleptic properties of coconut milk-based ice cream. LWT-Food Sci. Technol. 92, 516–522.

Ismail E.A., Al-Saleh A.A., Metwalli A.A.M., 2013. Effect of inulin supplementation on rheological properties of low-fat ice cream. Life Sci. J. 10(3), 1742–1746.

Kamińska-Dwórznicka A., Antczak A., Samborska K., Pomarańska-Łazuka W., 2013. Wpływ kappa karagenu i jego hydrolizatów na proces zamrażania modelowych roztworów sacharozy. ZPNNR 575, 63–70.

Kamińska-Dwórznicka A., Gondek E., Łaba S., Jakubczyk E., Samborska K., 2019. Characteristic of instrumental methods to describe and assess the recrystallization process in ice cream systems. Foods 8(4), 117. https://doi.org/10.3390/foods8040117

Kamińska-Dwórznicka A., Matusiak M., Samborska K., Witrowa-Rajchert D., Gondek E., Jakubczyk E., Antczak A., 2015. The influence of kappa carrageenan and its hydrolysates on the recrystallization process in sorbet. J. Food Eng. 167(Part B), 162–165.

Kundu P., Dhankhar J., Sharma A., 2018. Development of non diary milk alternative using soymilk and almond milk. Food Nutr. J. 6(1), 203–210.

Lomolino G., Zannoni S., Zabara A., Da Lio M., De Iseppi A., 2020. Ice recrystallisation and melting in ice cream with different proteins levels and subjected to thermal fluctuation. Int. Dairy J. 100, 104557. https://doi.org/10.1016/j.idairyj.2019.104557

Makinde F.M., Adebile T.V., 2018. Influence of processing treatments on quality of vegetable milk from almond (*Terminalia catappa*) kernels. Act. Sci. Nutr. Health 2(6), 37–42.

O'Neil C.E., Nicklas T.A., Flugoni III V.L., 2016. Almond Consumption is associated with better nutrient intake, nutrient adequacy, and diet quality in adult: National health and nutrition examination survey 2001–2010. Food Nutr. Sci. 7, 504–515.

Regand A., Goff H.D., 2003. Structure and ice recrystallization in frozen stabilized ice cream model systems. Food Hydrocoll. 17, 95–102.

Sofjan R.P., Hartel R.W., 2004. Effects of overrun on structural and physical characteristics of ice cream. Int. Dairy J. 14, 255–262.

Soukoulis Ch., Chandrinos I., Tzia C., 2008. Study of the functionality of selected hydrocolloids and their blends with κ-carrageenan on storage quality of vanilla ice cream. LWT-Food Sci. Technol. 41, 1816–1827.

Tomer V., Kumar A., 2013. Development of High Protein Ice-Cream Using Milk Protein Concentrate. IOSR-JESTFTJ 6(5), 71–74.

Vanga S.K., Raghavan V., 2018. How well do plant based alternatives fare nutritionally compared to cow's milk? J. Food Sci. Technol. 55(1), 10–20.

Yüksel A., Şat İ., Yüksel M., Çakmakçı S., 2017. A comparative study on the physicochemical characteristics, organic acid profiles, mineral compositions and sensory properties of ice creams produced with different types of nuts. Carpath. J. Food Sci. Technol. 9(3), 137–151.

BADANIE WŁAŚCIWOŚCI LODÓW WEGAŃSKICH NA BAZIE NAPOJU MIGDAŁOWEGO

Streszczenie. Celem przedstawionych badań było opracowanie receptury lodów wegańskich na bazie napoju migdałowego, na podstawie procesu produkcyjnego tradycyjnych lodów mlecznych. W prezentowanych badaniach zbadano wpływ wybranych stabilizatorów na fizyczne i organoleptyczne właściwości lodów oraz strukturę kryształów po 24 h przechowywania. W tym celu przygotowano próbkę kontrolną oraz próbkę badawczą z mieszanką dwóch rodzajów stabilizatorów, tj. mączki chleba świętojańskiego (LBG) oraz gumy ksantanowej. Uzyskane wyniki wykazały, że dodatek mieszanki stabilizatorów nie wpłynął znacząco na właściwości fizyczne lodów. Dodatek stabilizatorów pozwolił zaś na ukształtowanie korzystniejszej struktury kryształów, a średnica kryształu lodu nie przekroczyła 21 μm. W ocenie organoleptycznej lody ze stabilizatorami uzyskały najwyższy wynik. W pracy wykazano, że te same parametry oraz dodatki używane w produkcji lodów mlecznych mogą być stosowanie przy wytwarzaniu lodów wegańskich.

Słowa kluczowe: lody wegańskie, napój migdałowy, stabilizatory

sustainability

MDPI

Article

Effects of Different Ingredients and Stabilisers on Properties of Mixes Based on Almond Drink for Vegan Ice Cream Production

Anna Kot *, Anna Kamińska-Dwórznicka, Sabina Galus and Ewa Jakubczyk

Department of Food Engineering and Process Management, Insitute of Food Sciences, Warsaw University of Life Sciences (WULS-SGGW), Nowoursynowska 159C, 02-776 Warsaw, Poland; anna_kaminska1@sggw.edu.pl (A.K.-D.); sabina_galus@sggw.edu.pl (S.G.); ewa_jakubczyk@sggw.edu.pl (E.J.)
* Correspondence: anna_kot1@sggw.edu.pl

Abstract: A plant-based diet is beneficial not only to human health but also for environmental sustainability. As consumers, we play a vital role in balancing hedonic consumption with long-term sustainable behaviours, such as reduced animal products consumption. As a result of the changeable trend in the food industry, there are considerably more requirements for food manufacturers. This study aimed to determine the influence of different ingredients and selected stabilisers (iota carrageenan and its acid and enzymatic hydrolysates) on the physicochemical properties of ice-cream mixes. The effect of maturation during 24 h on the selected properties was also observed. The particle size distribution, stability, density, viscosity and morphology after preparation and after 24 h of maturation at the temperature of 4 °C were tested. Finally, it was found that the addition of stabilisers and the homogenisation process caused a decrease in the particle size diameter and they contributed to the obtained higher value of viscosity in comparison to samples without stabilisers. Moreover, the use of stabilisers and the homogenisation process negatively affected the stability of the ice-cream mix due to the fact that the stability rate (TSI) was about 6.0. The data provided by this paper are valuable for intensifying the potential application of vegan ice cream. Additionally, this product may be useful to reduce agricultural waste and for fundamental product development as an alternative beneficial food product in the close future.

Keywords: vegan ice cream; almond drink; iota carrageenan

check for updates

Citation: Kot, A.; Kamińska-Dwórznicka, A.; Galus, S.; Jakubczyk, E. Effects of Different Ingredients and Stabilisers on Properties of Mixes Based on Almond Drink for Vegan Ice Cream Production. *Sustainability* **2021**, *13*, 12113. https://doi.org/10.3390/su132112113

Academic Editor: Dario Donno

Received: 30 September 2021
Accepted: 28 October 2021
Published: 2 November 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Food is essential for life, but it also causes an extensive impact on the environment. Having been increasingly industrialised and globalised, the food industry has faced a plethora of challenges. For instance, feeding the increasing population and simultaneously reducing environmental impacts, such as greenhouse gas or climate change. It was estimated that the food sector contributes to 26% of global greenhouse gas emissions. Therefore, finding sustainable alternatives is crucial to alleviate the associated environmental footprint [1–3]. The intake of animal products in our diet is the most significant factor which determines the footprint of worldwide food consumption, and the most effective approach to reducing "dietary emissions" is to decrease the consumption of animal products [2]. In this sense, a lifestyle based on pro-environmental behaviours enables consumers to adhere to a plant-based diet. Consequently, in the close future, such a global move toward changing our eating habits will be extremely beneficial for the environment [4]. Not only is a shift to a plant-based diet necessary to counteract the detrimental climate change but also it has been shown to deliver health benefits. For instance, shifting away from a meat-based diet would lead to lowering both cholesterol levels and blood pressure or balancing blood sugar, even reducing the risk of developing certain cancers. Additionally, the reduced intake of animal proteins can be driven by consumer dietary restrictions such as, for example, a cow milk allergy or lactose intolerance. Factors such as allergies for animal products or ethical

choices (vegan, vegetarian or flexitarian), have tempted people and the food industry to scrutinise the idea of a plant-based diet and look for some alternatives in daily life [4–6].

It is widely acknowledged that ice cream is made and also eaten in almost every country. By means of their remarkable organoleptic properties, it stands out with tremendous popularity among society [7]. In ice cream-making, the first step is to blend the liquid and solid ingredients in the appropriate order, obtaining a liquid called ice-cream mix. In turn, the physicochemical and organoleptic properties of ice cream are determined by the relevant quality of the ice-cream mix; it is absolutely vital to select the best ingredients and focus on the properties of the ice-cream mix [7,8]. Ice-cream mix can be defined as hydrocolloidal dispersion, in which proteins and polysaccharides in concentrations over the phase separation threshold, create a two-aqueous system, according to the thermodynamic incompatibility [8–10].

Out of the numerous plant drinks, almond drink was typed to present research to preparing ice-cream mix. Firstly, the almond drink has become one of the most popular plant-based alternatives. Nonetheless, the various health benefits that are involved in the consumption of almonds are also the key factors that help boost the demands of consumers for an almond drink. It is medically proven that almond has a relatively high number of phytochemicals (including phenolic acids and phytosterols), polyphenolic compounds (flavonoids and pro-anthocyanidins), vitamin E, monounsaturated and polyunsaturated fatty acids, potassium and arginine. Because of this beneficial nutrient profile, almond has antioxidant and anti-inflammatory properties and it can reduce cardiovascular disease risk. Moreover, almond drink has advantages in a balanced diet, including preparing the low-calorie meals and unique taste [11,12].

Moreover, in the presented study, inulin was used as an ingredient to prepare a vegan ice-cream mix. Inulin can be used in ice cream production as a prebiotic, sugar or fat replacer and texture modifier. Inulin intake could be beneficial for human health. It can increase mineral absorption and promotes the growth of the micro-flora digestive tract. As an ingredient, it can be used to prepare low-caloric food for diabetics to control their blood sugar levels [13,14]. As a substitution for milk in powder, pea protein is used. It is emerging as an alternative to conventional products which are derived from animal proteins, one which is known as a nutritional ingredient in the food industry owing to its emulsifying and gelation properties or its hypoallergenic attributes. The protein fractions which were found in this pea protein are albumins, globulins and other minor fractions, such as prolamins and glutelins [15,16].

Stabilisers are a group of ingredients that are able to increase viscosity and stability during temperature fluctuations, improve texture or reduce the rate of meltdown and slow down moisture migration out of ice cream during storage. Moreover, they may control the incorporation of air in the factory freezer and help produce a stable foam [17]. The most common stabilisers in the ice cream industry are carrageenans. These are naturally occurring carbohydrate polymers. Chemically, they are made up of D-galactose and 3,6-anhydro-D-galactose units which are bonded together with α-1,3 and β-1,4 linkages in attendance of sulphate groups. Depending on the number and position of sulphate esters, three forms of carrageenan were mentioned: kappa, iota and lambda [18–21]. Iota carrageenan contains two sulphate groups per unit. Moreover, it has the ability to form a soft elastic gel in the presence of calcium ions [18,19,22]. In this paper, the acid and enzymatic hydrolysates of iota carrageenan were used as an alternative stabiliser instead of iota carrageenan. According to the satisfactory results of hydrolysis of kappa-carrageenan concerning the size of ice crystals of ice cream, described by Kamińska-Dwórznicka et al. [23,24] we decided to check the effectiveness of the hydrolysates of iota carrageenan in creating structure in the initial step of production of ice cream—ice-cream mix preparation.

In considering the significance of the influence of different ingredients and stabilisers in preparing ice-cream mix, in this research, the vegan ice-cream mix with the different sorts of stabilisers was scrutinised. Moreover, the physical properties analysis was con-

ducted before and after the homogenisation process and 24 h after the maturation of the ice-cream mix.

2. Materials and Methods

2.1. Hydrolysates Preparation

Iota carrageenan powder samples were obtained from Sigma-Aldrich. The enzymes for enzymatic hydrolysis were: β-galactosidase (1000 U/mg, from *Escherichia coli*), soluble in glycerol and TRIS buffer (pH 7.4), which was obtained from Sigma-Aldrich, St. Louis, MI, USA and commercial lactase from Serowar s.c. Szczecin. For acid hydrolysis, 0.1 M hydrochloric acid and 0.1 M sodium hydroxide obtained from Chempur were used.

For enzymatic hydrolysis iota carrageenan was first dissolved in distilled water to obtain a 0.4 mg/mL solution and heated up to 40 °C. This hydrolysis was carried out using two different enzymes. The first one was β-galactosidase. It was conducted for 72 h at 37 °C and then the sample was neutralised at 48 °C for 5 min, cooled to stop any further reactions (Sigma-Aldrich—Product Information; [24]). After repeating the solution of iota carrageenan, the second enzyme was used—commercial lactase (1 mL/1 L solution; 1 drop/50 mL solution). It was conducted for 24 h, at 5 °C. then the sample was neutralised at 48 °C for 5 min, cooled in order to stop any further reactions (Serowar—Product Information). For both treatments samples were stored frozen at −18 °C and thawed just before analysis.

For acid hydrolysis (using 0.1 M hydrochloric acid) iota carrageenan was first dissolved in distilled water to obtain a 10 mg/mL solution and heated up to 40 °C. Samples after 3 h of hydrolysis were taken, then neutralised and cooled. Finally, samples were stored frozen at −18 °C and thawed before analysis [23].

2.2. Ice Crem Mixes

2.2.1. Materials for Use for Different Ice-Cream Mix Recipes

Vegan ice-cream mixes were prepared using: the roasted almond original (Enerbio, Rossmann, Hanover, Germany), almond syrup (Monin, Bourges, France), inulin (Orafti BENEO, Tienen, Belgium), pea protein (Nutralys S85 plus-, Roquette, Lestrem, France), emulsifier E471 (Fooding Shanghai, Shanghai, China), LBG-Locust Bean Gum (Fooding Shanghai, Shanghai, China), xanthan gum (Fooding Shanghai, Shanghai, China), iota carrageenan (Fluka, Sigma-Aldrich, St. Louis, MI, USA) or new obtained stabilisers: the hydrolysates of ι-carrageenan after acid hydrolysis, after enzymatic hydrolysis by β-galactosidase and after enzymatic hydrolysis by lactase. The characteristics of samples are described in Table 1.

2.2.2. The Ice-Cream Mix Production

All ingredients were weighed separately with a fixed recipe. Then dry components were mixed with the liquid ones, using Bosch MaxoMixx 750 W blender (Bosch, Gerlingen, Germany). For the pasteurisation process in the ice-cream mix, Vorwerk thermomixer was used at the temperature of 85 °C per 1.5 min. Afterwards, it was cooled to a temperature of 25 °C. Then the homogenisation was made using the homogeniser IKA T 25 digital ULTRA-TURRAX 20 rpm (IKA®-Werke GmbH & Co. KG, Staufen, Germany) through 2.5 min.

Table 1. The characteristic of samples.

Sample	Characteristics
CWH	control sample without the stabilisers and without the homogenisation treatment
CH	control sample without the mix of stabilisers and with the homogenisation treatment
IWH	sample with the addition of the stabilisers (ι-carrageenan, locust bean gum, xanthan gum) and without the homogenisation treatment
IH	sample with the addition of the stabilisers (ι-carrageenan, locust bean gum, xanthan gum) and with the homogenisation treatment
AWH	sample with the addition of the stabilisers (the acid hydrolysates of ι-carrageenan, locust bean gum, xanthan gum) and without the homogenisation treatment
AH	sample with the addition of the stabilisers (the acid hydrolysates of ι-carrageenan, locust bean gum, xanthan gum) and with the homogenisation treatment
BWH	sample with the addition of the stabilisers (the enzymatic β-galactosidase hydrolysates of ι-carrageenan, locust bean gum, xanthan gum) and without the homogenisation treatment
BH	sample with the addition of the stabilisers (the enzymatic β-galactosidase hydrolysates of ι-carrageenan, locust bean gum, xanthan gum) and with the homogenisation treatment
LWH	sample with the addition of the stabilisers (the enzymatic commercial lactase hydrolysates of ι-carrageenan, locust bean gum, xanthan gum) without the homogenisation treatment
LH	sample with the addition of the stabilisers (the enzymatic commercial lactase hydrolysates of ι-carrageenan, locust bean gum, xanthan gum) and with the homogenisation treatment

2.3. Ice-Cream Mix Properties Analysis

2.3.1. Density

Density is carried out using a glass pycnometer at ambient temperature. The pycnometer was weighed on an analytical weight with an accuracy of 0.1 g. Then it was filled with ice-cream mix and capped tightly and weighed on an analytical weight with an accuracy of 0.1 g. The mass of ice-cream mix was calculated from the mass difference, using the formula:

$$d = \frac{m}{v} \quad (1)$$

where, d—density of ice cream (g/cm^3), m—the weight of ice cream (g), v—the volume of ice cream (cm^3), according to the method [25]. The density of the ice-cream mix was conducted before and after the ageing stage (24 h).

2.3.2. Particle Size Distribution

A laser diffraction instrument Cilas 1190 (Cilas, Orléans, France) was used to determine the particle size of the ice-cream mix. The emulsions were suspended in water at the obscuration of 10%. The results were presented as particle size distribution diagrams and

expressed as the median diameter D_{50}. This analysis of the ice-cream mix was conducted before and after the ice cream maturation (24 h).

2.3.3. Emulsion Stability Evaluation

Measurements of stability were conducted using a Turbiscan Lab Expert (Formulation SA, Toulouse, France). The date of backscattered (BS) light recorded during measurements was processed using Turbisoft 2.0.0.33 software, which allowed us to compare the stability based on the so-called Turbiscan Stability Index (TSI). This analysis of the ice-cream mix was conducted before and after the ice cream maturation (24 h).

2.3.4. Rheological Properties

Rheological measurements of different ice-cream mix samples and after ageing (24 h) were conducted using a Haake Mars 40 rheometer (Thermo Scientific Inc., Karlsruhe, Germany) equipped with a TM-PE-C temperature module controller. The measurements were carried out with a CCB/CC25 DIN/Ti concentric cylinder geometry (gap size 5.3 mm) within the shear rate of 0–100 s^{-1} at a constant temperature of 25 °C. All measurements were performed in triplicate for each kind of ice-cream mix. The data obtained from RheoWin v.4.86. Job Manager (Thermo Scientific, Karlsruhe, Germany) were plotted with apparent viscosity (η_{app}) as a function of shear rate ($\dot{\gamma}$) in the semi-logarithmic scale (the viscosity curves). The flow behaviour was also analysed and obtained data were fitted to the following models:

Bingham:

$$\eta_{app} = \eta_p + \tau_0 \left(\dot{\gamma}\right)^{-1} \quad (2)$$

Ostwald de Waele:

$$\eta_{app} = K\dot{\gamma}^{n-1} \quad (3)$$

Herschel–Bulkley:

$$\eta_{app} = \tau_0 \left(\dot{\gamma}\right)^{-1} + K\dot{\gamma}^{n-1} \quad (4)$$

where, η_{app}—the apparent viscosity (Pa s), $\dot{\gamma}$—the shear rate (s^{-1}), η_p—the plastic viscosity (Pa s), τ_0—yield stress (Pa), K—the consistency index (Pa s^n), n—flow behaviour index (dimensionless).

The adequacy of fitted models was estimated using the regression analysis which delivered the values of a correlation coefficient (R) and a chi-square (χ^2).

2.3.5. Microstructural Analysis of Ice-Cream Mix

Ice crystal mix was analysed based on the images taken before and after 24 h of maturation at 4 °C. The first step of this determination was sampling. A small amount of ice-cream mix was put on the slide using a spatula and covered by a slip glass. The images were taken using the Olympus BX 43F microscope and camera Olympus CAM-SC 50 (Olympus, Tokyo, Japan). This analysis of the ice-cream mix was conducted before and after the ice cream maturation (24 h).

2.3.6. Statistical Analysis

The analysis of variance (ANOVA) was performed using STATISTICA 13 software. The significance of the test was set on $\alpha = 0.05$. Data are expressed as a mean with standard deviation (±SD) and the differences between groups were evaluated using the Tukey HSD test.

3. Results and Discussion

3.1. The Density of Ice-Cream Mix

Table 2 show the influence of stabilisers and the homogenisation process on the density of ice-cream mixes. Overall, the density of the ice-cream mixes varies with composition and it can be changed by the variable amount of ingredients [26,27]. Additionally, the

increasing density of the ice-cream mix is not a desirable factor, due to the fact that it could have a detrimental influence on the overrun of ice cream [28].

Table 2. The density of ice-cream mixes.

Sample	Density [g/cm^3]	Density after 24 h of Maturation [g/cm^3]
CWH	1.00 ± 0.03 ^{a,b}	1.00 ± 0.03 ^{a}
CH	1.00 ± 0.03 ^{a,b}	1.00 ± 0.03 ^{a}
IWH	1.09 ± 0.11 ^{b}	1.09 ± 0.11 ^{a}
IH	0.98 ± 0.06 ^{a,b}	0.98 ± 0.06 ^{a}
AWH	1.00 ± 0.03 ^{a,b}	0.98 ± 0.03 ^{a}
AH	0.94 ± 0.03 ^{a}	0.98 ± 0.02 ^{a}
BWH	1.00 ± 0.03 ^{a,b}	1.01 ± 0.02 ^{a}
BH	0.94 ± 0.05 ^{a}	0.99 ± 0.03 ^{a}
LWH	0.99 ± 0.03 ^{a,b}	1.01 ± 0.04 ^{a}
LH	0.92 ± 0.03 ^{a}	0.97 ± 0.03 ^{a}

a–b The differences between mean values with the same letter in rows are statistically insignificant ($p < 0.05$).

In the presented study the density before the maturation ranged from 0.92 to 1.09 g/cm^3 (Tables 1 and 2). The statistical appraisal showed that significant differences in density were observed between samples with different stabilisers before the maturation. The lowest value of density was noticed for the sample LH (ice-cream mix with the addition of enzymatic (lactase treatment) hydrolysates of iota carrageenan). However, the highest value of density was obtained by sample with the addition of iota carrageenan without homogenisation treatment IWH. The difference between samples indicates that the stabilisers and the homogenisation influence density of ice-cream mixes. After the maturation, the density of ice-cream mix varied from 0.97 to 1.09 g/cm^3 but there were no statistically significant differences in this parameter among analysed samples. The results indicate that the maturation stage contributes to obtaining the stability of ice-cream mixes (Table 1). According to the study of Warren and Hartel [29], the density of commercial ice-cream mix was reported from 1070 to 1160 kg/m^3. These values are similar to the density which was found in our study. Additionally, in a study conducted by Alves et al. [30], the density of non-dairy ice-cream mixes was observed at approximately the same level as in our study, between 1096.51 to 1122.85 g/L.

3.2. The Stability of Ice-Cream Mix

Ice-cream mix is a multiphase product and when ice cream contains fat it can be treated as an oil-in-water emulsion. In this sense, describing the physical properties of ice cream bases, it is necessary to investigate such factors as stability and particle size distribution. Additionally, the stability of the emulsion refers to resisting the undesirable changes during the storage time (for example sedimentation or coalescence) [31]. In the presented study, the stability of ice-cream mixes was scrutinised using the turbidimetric method. This method contributed to collecting information about the profile of emulsion and ongoing changes which are not visible to the human eye. The destabilisation of emulsion often occurs due to particle size increase (coalescence), particle aggregation (flocculation) or particle migration (creaming and sedimentation) [32]. The results of the stability of ice-cream mixes were expressed as the TSI factor values (Turbiscan Stability Index) in Table 3 and as the Back Scattering (Figure 1a,b).

Table 3. The stability of ice-cream mixes.

Sample	TSI
CWH	2.1
CH	3.0
IWH	2.3
IH	4.1
AWH	2.1
AH	6.4
BWH	3.6
BH	6.5
LWH	1.7
LH	5.2

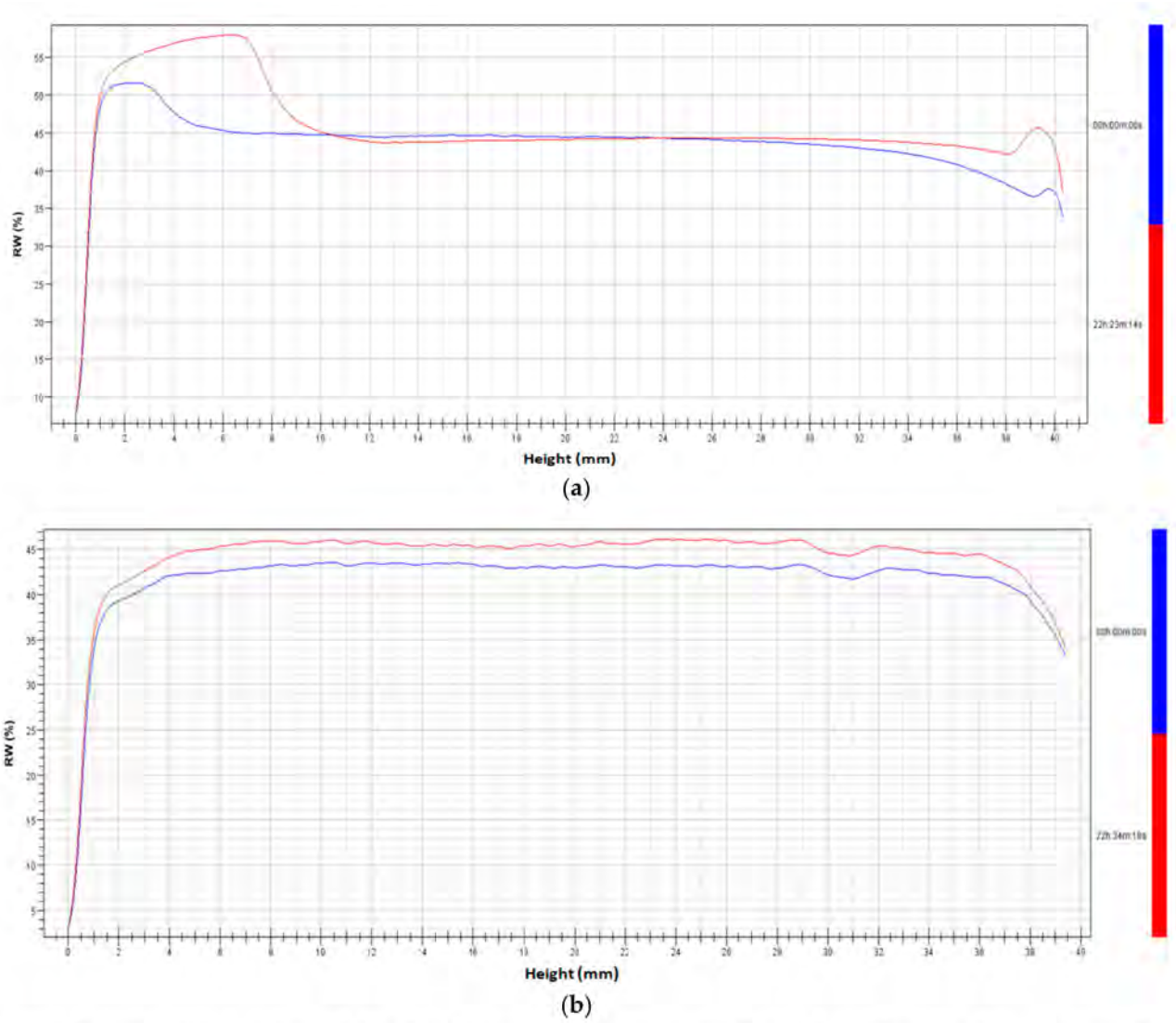


Figure 1. Variation of backscatter with time for chosen samples during maturation under refrigeration ((**a**)—sedimentation and creaming; (**b**)—coalescence).

Based on the presented results it was noticed that the homogenisation treatment had a significant influence on the stability of ice-cream mixes. TSI value of samples without homogenisation treatment achieved the lowest value and at the same, the highest stability. In those groups of samples without homogenisation treatment, the ice-cream mix with the addition of enzymatic (lactase treatment) hydrolysates of iota carrageenan (LWH) had the lowest TSI 1.7 (Table 3). The highest TSI value (the lowest stability) was observed for the sample with the addition of enzymatic (β-galactosidase treatment) hydrolysates of iota carrageenan BWH (3.6). The sample IWH and AWH had the TSI value close to the control sample (CWH), around two.

In comparison to samples after homogenisation treatment, deterioration was observed. The TSI value was recorded from 3 to 6.5 (Tables 1 and 3). The lowest value of TSI was recorded for the control sample (CH– at 3 value) while in the sample with the addition of stabilisers, the highest destabilisation has occurred. The sample with mentioned stabilisers, BH, AH and LH, had the highest destabilisation; TSI was noticed in order at 6.5, 6.4 and 5.2 values. Moreover, for sample AH (with the addition of acid hydrolysates of iota carrageenan) the highest increase in TSI value was recorded compared to the same sample (with the same stabilisers-AWH) but without the homogenisation treatment.

As a result of BS% (Back Scattering), the ice-cream mixes (with the addition of stabilisers and no matter of homogenisation treatment) were prone to coalescence during the 24 h of maturation (Figure 1a,b). This destabilisation was caused by decreasing the size of particles. Additionally, this dependence was confirmed by the particle size distribution in the presented research (Table 4). On the other hand in the control sample (CWH and CH), despite homogenisation treatment, a different sort of destabilisation was noticed. Based on the results of Back Scattering, it was pointed out that the creaming occurred on the top of the test vial, while sedimentation occurred at the bottom of the vial (Figure 1a). This process was explained as the moving of particles in ice-cream mixes. Presumably, the lack of stabilisers in this control recipe of ice-cream mix contributed to the additional space between particles and a different relationship could be observed. Nonetheless, in a study by Voronin et al. [17] the disability of milk ice-cream mixes was noticed after the HPJ technology. Most samples were similarly stable to creaming and, additionally, rapid separation was seen. The authors hypothesised that the separation in samples was due to the presence of particles with air that was less dense than the serum phase. Moreover, the ability of samples in direction to the creaming was occurred by partially coalesced fat [17]. Then in research conducted by Cheng et al. [33] destabilisation was also observed in ice-cream mix model solutions. In this research, the interaction of polysaccharides with milk protein was studied as a result of the destabilisation of the emulsion system. It was indicated that the stability of the ice cream model emulsion depends on the types and concentrations of polysaccharides. For instance, carboxymethylcellulose may effectively be used to delay phase separation than guar gum [33]. In our study, the differences between the stabilisation of emulsion in using stabilisers were noticed. However, the homogenisation process significantly contributed to the decreasing stability of ice-cream mixes. Therefore, in all probability, the air bubbles may be the reason for such phenomena, as the researchers noticed in the study by Voronin et al. [17]. Additionally, Keeney [34] established that a certain amount of fat destabilisation is necessary to obtain good properties of the texture of ice cream. Moreover, the study conducted by Berger and White [35] and Berger et al. [36] proved that such destabilisation influences the properties of ice cream concerning creamy mouthfeel or meltdown behaviour [37].

Table 4. Median D_{50} of ice-cream mixes.

Sample	D_{50}	D_{50} after 24 h of Maturation
CWH	15.65 ± 0.62 ^{c}	17.23 ± 0.49 ^{d}
CH	20.54 ± 0.45 ^{d}	11,35 ± 0.48 ^{b}
IWH	30.40 ± 0.44 ^{e}	28.50 ± 0.43 ^{e}
IH	13.77 ± 0.19 ^{b}	11.51 ± 0.94 ^{b}
AWH	38.36 ± 0.49 ^{f}	11.41 ± 0.97 ^{b}
AH	13.06 ± 0.53 ^{a,b}	6.33 ± 0.12 ^{a}
BWH	42.15 ± 0.19 ^{g}	11.29 ± 0.53 ^{b}
BH	13.42 ± 0.40 ^{b}	6.88 ± 0.33 ^{a}
LWH	38.31 ± 0.66 ^{f}	13.20 ± 0.45 ^{c}
LH	11.91 ± 0.16 ^{a}	6.37 ± 0.48 ^{a}

a–g The differences between mean values with the same letter in rows are statistically insignificant ($p < 0.05$).

3.3. The Particle Sizes of Ice-Cream Mixes

The particle size of the dispersed phase is influential on the stability of the emulsion, according to Stoke's law. As a result, the higher stability is connected with the smaller size of particles, along with being homogenously distributed [31]. Additionally, the size of particles would have the beneficial or detrimental influence of creating the ice crystal structure in the final product.

The mean diameter was conducted before and after 24 h of maturation. The mean diameter D (4, 3) before 24 h of maturation ranged from 11.91 to 42.15 µm (Tables 1 and 4). The lowest value of mean diameter (11.91 µm) was observed for the sample LH (with the addition of the enzymatic (lactase) hydrolysates of *ι*-carrageenan) after the homogenisation. For ice-cream mixes with the addition of *ι*-carrageenan (IH) and its other two hydrolysates (BH and AH) with the homogenisation treatment, the mean diameter was around 13 µm. To summarise, the samples with the addition of the hydrolysates of *ι*-carrageenan and after the homogenisation treatment had the lowest value of particle size. Innocente et al. [9], in their research on the impact of the pressure (mechanical) and high-pressure homogenisation process on the particle size distribution in ice-cream mixes for the production of milk ice cream, showed that mechanical homogenisation reduces the mean volume diameters of the particles of the dispersed phase compared to the non-homogenised mixture. Additionally, in a study by Biasutti et al. [38] on the effects of high-pressure homogenisation of ice-cream mixes, the sample which was treated by conventional homogenisation had the lowest value of particle size compared to the sample without homogenisation.

After the maturation, the value of D (4, 3) was observed from 6.33 to 28.50 µm (Tables 1 and 4). The lowest value of mean diameter was observed for the samples AH, LH and BH (with the addition of hydrolysates of *ι*-carrageenan after the homogenisation treatment), the mean diameter was around 6 µm. In comparison to this value before maturation time, it was almost half of the reduction of particle size. The highest value of mean diameter was noticed for the sample with the *ι*-carrageenan and without the homogenisation treatment (IWH). Additionally, there was a visible increase in particle size in the control sample CWH. The statistical analysis resulted in significant differences between samples before and after the maturation time. Based on that, used stabilisers—but also the homogenisation treatment—influenced particle size in ice-cream mixes. Moreover, looking at the level of reduction of D (4, 3), the samples with the stabilisers (the hydrolysates of iota carrageenan addition) and without the homogenisation treatment significantly decreased the size of particles. For instance, the sample with the enzymatic (β-galactosidase) hydrolysates of *ι*-carrageenan reduced the mean diameter by almost 31 µm (Tables 1 and 4). Similar results were observed for other hydrolysates of *ι*-carrageenan. However, for the sample with the addition of iota carrageenan, there was a non-significant reduction. Based

on the literature, in the study by Kamińska-Dwórznicka et al. [23,24] on the influence of kappa-carrageenan and its hydrolysates on the recrystallisation process in ice model sucrose solution, better results of prohibition of excessive growth of ice crystals had the hydrolysates of carrageenan than pure carrageenan were proven. We can suppose that the addition of kappa-carrageenan was also active in the emulsion stage, which resulted in better ice crystal structure creation.

Considering the size of the particle, the particle size distribution of the ice-cream mix before and after the maturation was also analysed (Figure 2a–d). Before the maturation, all samples without homogenisation treatment had a double or trimodal particle size distribution (Figure 2a). However, after 24 h of maturation, in all mentioned ice-cream mixes the trimodal of particle size distribution was observed based on three characteristic peaks in the figure (Figure 2b). Only sample CWH (without the stabilisers) and AWH (with the addition of acid hydrolysates of iota carrageenan) did not change the sort of distribution of particles. Moreover, for samples with the homogenisation treatment more unification was noticed. Before the maturation, all samples had the double modal of size distribution (Figure 2c). Additionally, after the 24 h of maturation for all ice-cream mixes the same fourfold particle size of the distribution was also observed. The four peaks are pointed out in Figure 2d. The changes of the model of size distribution were confirmed by the value of D_{50} (Tables 1 and 4), in which a significant reduction of particle size was observed. Innocente et al. [9] showed in their research that homogenised mixtures were characterised by a monomodal (single) particle size distribution, while the non-homogenised mixture was characterised by bimodal distribution. The homogenisation process made the particles uniform in size, therefore there is only one peak in the particle size distribution graphs of homogenised blends. Additionally, in a study by Voronin et al. [17], monomodal distribution was observed for the control sample of ice-cream mix and samples which were treated by HPJ. However, it was noticed that the sample with the addition of emulsifier had a bimodal distribution. Additionally in research conducted by Warren and Hartel [39] on the influence of emulsifiers on ice-cream mix and ice cream structure, bimodal and also trimodal distribution of particle size was observed, which may reflect enhanced partial coalescence of fat droplets in ice-cream mixes. Therefore, based on our results of RW%, the coalescence was also observed for ice-cream mixes. It may be concluded that this destabilisation of ice-cream mixes contributed to increasing diversity in the distribution of particle size.

3.4. The Rheological Properties

The rheological properties refer to the flow behaviour and mouthfeel of ice cream. They are crucial in mechanical treatment of ice-cream mix such as stirring and flowing through pipelines and other technological equipment during continuous process [40]. The rheological behaviour of fluids can be described by different models using Newtonian, Bingham Plastic, Casson, Ostwald de Waele and Herschel–Bulkley equations. Rheological models present the mathematical description of the relation between the shear rate and shear stress or shear rate and apparent viscosity. The accuracy of fitting the rheological models to properties of ice-cream mix samples was carried out using a non-linear regression method. The model that best fit the rheological data was conducted by comparison of coefficients R and Chi-square. The best model should be described by the highest correlation coefficient (R) and the lowest values of Chi-square parameter (χ^2). Tables 5 and 6 show that R and χ^2 parameters of the Herschel–Bulkley model fulfilled this requirement. The values of the correlation coefficient for this model ranged from 0.9984 to 1 and the chi-square parameter varied from 19.4 to 21,495.1, which can be considered to be satisfying for analysed ice-cream mix samples. Thus, the Herschel–Bulkley model was selected as adequate to characterise the rheological properties of all samples. This model was also applied to describe the flow behaviour of ice-cream mixes with different compositions [9,33,40–42].

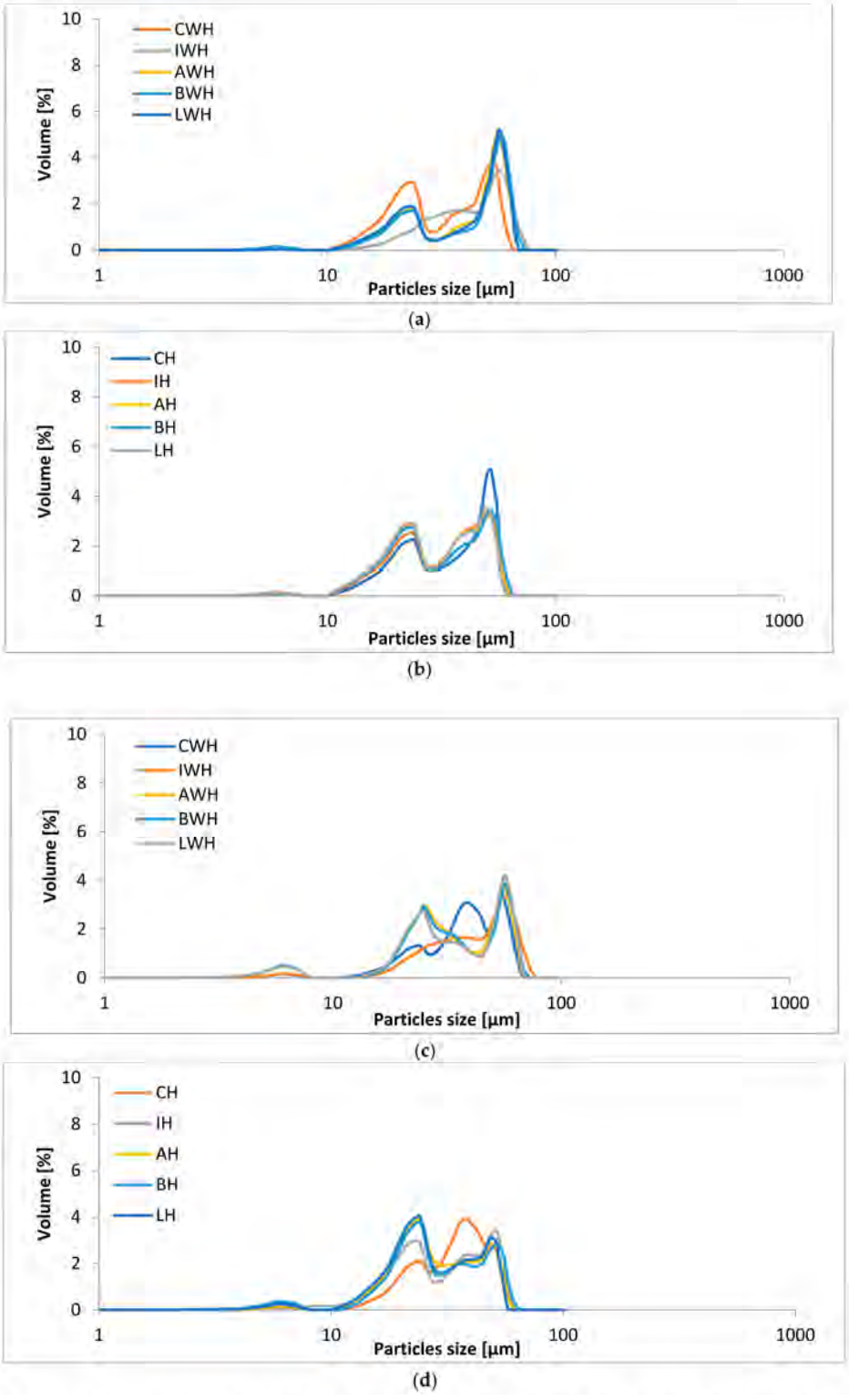


Figure 2. Particles size distribution: (**a**) before 24 h of maturation of ice-cream mix; (**b**) after 24 h of maturation of ice-cream mix; (**c**) before 24 h of maturation of ice-cream mix; (**d**) after 24 h of maturation of ice-cream mix.

Table 5. The goodness of fit of Bingham, Ostwald de Waele and Herschel–Bulkley models for ice-cream mix samples.

Sample	Coefficients	Bingham Model	Ostwald de Waele Model	Herschel–Bulkley Model
CWH	χ^2 R	1511.1 0.9998	5629.7 0.9992	26.4 0.9999
CH	χ^2 R	3794.6 0.9991	7782.7 0.9988	41.0 0.9999
IWH	χ^2 R	6,400,003.1 0.9848	653,333.3 0.9844	5252 0.9999
IH	χ^2 R	9,023,333.3 0.9840	656,333.3 0.9989	7259 0.9999
AWH	χ^2 R	897,667.6 0.9970	402,666.7 0.9987	7436.5 0.9993
AH	χ^2 R	1,393,333.3 0.9961	1,823,333.3 0.9958	8200.3 0.9998
BWH	χ^2 R	476,000.0 0.9978	497,667.0 0.9977	16,984.1 0.9984
BH	χ^2 R^2	826,333.3 0.9979	131,000.0 0.9951	5919.7 0.9998
LWH	χ^2 R	252,666.7 0.9983	281,666.7 0.9985	7536.3 0.9996
LH	χ^2 R	522,666.7 0.9973	611,100.0 0.9978	831.3 1.0000

Table 6. The goodness of fit of Bingham, Ostwald de Waele and Herschel–Bulkley models for ice-cream mix samples after 24 h.

Sample	Coefficients	Bingham Model	Ostwald De Waele Model	Herschel–Bulkley Model
CWH	χ^2 R	13,470.0 0.9988	28,005.6 0.9974	5101.1 0.9997
CH	χ^2 R	4144.7 0.9996	9231.7 0.9995	19.4 1.0000
IWH	χ^2 R	122,000.0 0.9871	798,333.3 0.9991	9784.3 0.9997
IH	χ^2 R	13,900,000.0 0.9925	2,810,000.0 0.9985	2787.7 1.0000
AWH	χ^2 R	862,000 0.9975	604,666.7 0.9982	21,495.1 0.9990
AH	χ^2 R	2,513,333.3 0.9958	611,666.7 0.9989	383.0 1.0000
BWH	χ^2 R	495,666.7 0.9980	327,000.0 0.9921	1537.1 0.9999
BH	χ^2 R	852,333.3 0.9972	327,333.3 0.9989	125.0 1.0000
LWH	χ^2 R	291,114.0 0.9987	187,333.3 0.9991	4069 0.9997
LH	χ^2 R	939,000.0 0.9968	230,666.7 0.9990	166.7 1.0000

Table 7 presents the estimated parameters of the Herschel–Bulkley model (τ_o, K, n). The values of the flow behaviour index n were lower than 1 (Tables 1 and 7) and the apparent viscosity decreased as the shear rate increased (Figure 3). It indicates that all ice-cream mix samples showed non-Newtonian shear-thinning (pseudoplastic) behaviour. The smaller values of n indicate a departure from Newtonian behaviour and characterise the higher shear-thinning attitude as well as pseudoplasticity of materials [42]. The lowest flow behaviour index was observed for the IWH (0.633–0.653) and IH (0.742–0.757) ice-cream mix which may indicate the higher stability of the product during processing. Concerning the stability of ice-cream mixes, the samples IWH and IH also had a low TSI value (at 2.3 and 4.1) which contributed to better stability properties during the maturation time. Moreover, according to other described properties, for those mentioned samples, the increase in density and additionally the decrease in particle size was observed (Tables 2 and 4). The higher shear thinning behaviour facilitates the pumping of mix and allows us to obtain a final product with desirable texture and mouthfeel properties [40,43]. Atalar et al. [44] observed that the addition of high-pressure homogenised hazelnut milk to ice-cream mix caused the decrease in n values and the higher shear thinning behaviour may lead to a reduction in energy consumption during the mixing of ice cream. The higher values of flow behaviour index (0.949–0.991) showed that ice-cream mixes without stabilisers (CWH, CH) were less pseudoplastic (Table 7). Morover, also based on the Back Scattering (Figure 1a,b) the different sorts of destabilisation were observed (creaming and sedimentation) compared to samples with the addition of stablisers. After maturation, the n values did not differ for the same type of ice-cream mix with exception of the BH sample where the low behaviour index was lower after 24 h of storage. Dogan and Kayacier [45] noticed that the flow behaviour index of the ice-cream mix decreased until 24 h of maturation and then it increased up to 42 h. The effect of homogenisation was not noticeable for many samples (Table 7). However, homogenised ice-cream mix LH and BH showed lower values of index n than samples without this treatment. The composition and type of stabiliser significantly affected the shear-thinning behaviour of mixes which can be related to different water-binding capacities of investigated stabilisers.

A significant increase in consistency K (Table 7) and apparent viscosity (Figure 3) was observed for samples after the addition of stabilisers. The ice-cream mix with iota carrageenan (IWH, IH) characterised the highest consistency index values (255.6–462.3 m Pa s^n). It was noted that the consistency index of the vast majority of samples increased after 24 h of maturation, which is in agreement with results obtained by Dogan and Kayacier [45]. The increase in viscosity can be explained by better interaction between biopolymer molecules with water, lipids and proteins after ageing. The consistency index increased for the same samples (AH and LH) after homogenisation or remained at the same level (Table 7). Yield stress τ_o also increased with the addition of stabilisers (Table 7). Samples with iota carrageenan showed the highest values of yield stress (9410.8–1508.3 m Pa s^n). Rao [46] concluded that the higher values of yield stress indicated the creation of firmer structures and more stable systems. The statistical analysis showed that yield stress did not differ after maturation but values of this parameter increased after homogenisation for most samples. The higher values of viscosity, consistency index and yield stress for IWH and IH cream mixes indicated that applied stabiliser can be a good candidate to obtain the stable product.

3.5. The Microscopic Analysis

The analysis of microscopic images of ice-cream mixes enables the assessment of the effectiveness of the two stabilisation methods used by observing the differences in particle size and their homogeneity. The process of homogenisation contributed to implementing the air bubbles into ice-cream mixes, which was visible in the picture before and after the maturation (Figure 4).

Table 7. Herschel–Bulkley model parameters of ice-cream mix at different maturation times (0 and 24 h).

Sample	Ageing Time, h	τ_o, 10^{-3} Pa	K, 10^{-3} Pa s^n	n
CWH	0	26.6 ± 0.6 ^{a}	6.8 ± 2.0 ^{a}	0.991 ± 0.005 ^{i}
	24	28.6 ± 1.9 ^{a}	9.4 ± 1.8 ^{a}	0.978 ± 0.004 ^{g,h,i}
CH	0	27.6± 0.8 ^{a}	7.8 ± 1.3 ^{a}	0.979 ± 0.010 ^{h,i}
	24	29.9 ± 1.2 ^{a}	11.8 ± 3.2 ^{a}	0.949 ± 0.036 ^{f,g,h,i}
IWH	0	1080.3 ± 49.9 ^{f}	336.0 ± 14.7 ^{h,i}	0.653 ± 0.011 ^{a}
	24	910.8 ± 113.1 ^{e,f}	462.3 ± 65.7 ^{j}	0.633 ± 0.029 ^{a}
IH	0	1410.3 ± 316.1 ^{g}	255.6 ± 78.4 ^{g,h}	0.742 ± 0.056 ^{b}
	24	1508.3 ± 119.1 ^{g}	349.2 ± 21.7 ^{i}	0.757 ± 0.011 ^{b,c}
AWH	0	444.3 ± 65.7 ^{c}	75.7 ± 9.7 ^{c,d}	0.889 ± 0.030 ^{e,f,g}
	24	509.8 ± 7.3 ^{c,d}	67.7 ± 1.8 ^{c}	0.927 ± 0.006 ^{e,f,g,h,i}
AH	0	815.8 ± 99.1 ^{d,e,f}	106.5 ± 18.0 ^{e,f}	0.855 ± 0.037 ^{d,e,f}
	24	510.2 ± 32.0 ^{c,d}	176.9 ± 7.7 ^{g}	0.777 ± 0.010 ^{b,c,d}
BWH	0	396.9 ± 13.1 ^{c}	55.5 ± 2.3 ^{b}	0.918 ± 0.008 ^{e,f,g,h}
	24	321.0 ± 42.4 ^{b,c}	56.6 ± 4.5 ^{b}	0.925 ± 0.020 ^{e,f,g,h}
BH	0	737.3 ± 105.1 ^{d,e}	56.9 ± 13.9 ^{b,c}	0.940 ± 0.051 ^{f,g,h,i}
	24	360.2 ± 34.8 ^{b,c}	97.7 ± 3.2 ^{e}	0.830 ± 0.010 ^{c,d,e}
LWH	0	315.8 ± 22.1 ^{b,c}	53.6 ± 3.6 ^{b}	0.907 ± 0.010 ^{e,f,g,h}
	24	265.0 ± 43.3 ^{b,c}	55.2 ± 6.9 ^{b}	0.913 ± 0.027 ^{e,f,g,h}
LH	0	469.2 ± 36.9 ^{c,d}	87.5 ± 7.9 ^{d,e}	0.884 ± 0.018 ^{d,e}
	24	330.8 ± 23.9 ^{b,c}	108.5 ± 1.0 ^{f}	0.806 ± 0.019 ^{b,c,d}

a–j The differences between mean values with the same letter in rows are statistically insignificant ($p < 0.05$).

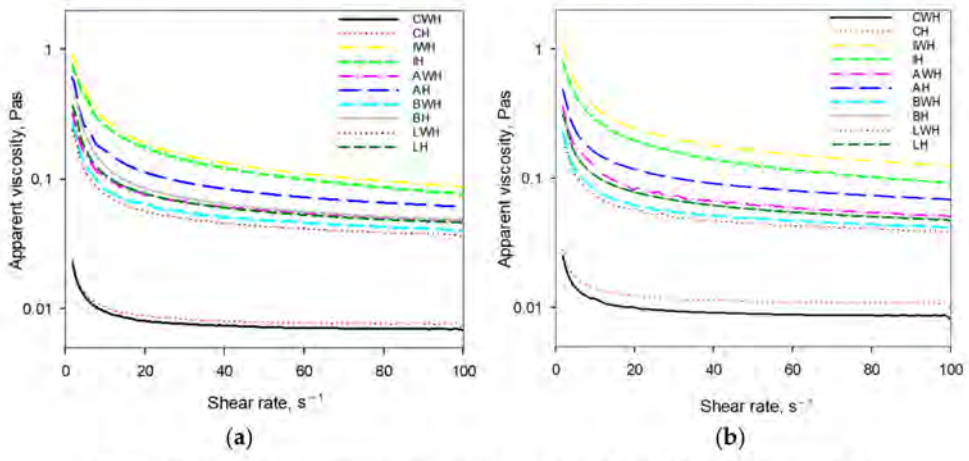


Figure 3. The viscosity curves of ice-cream mix (**a**) and after 24 h of maturation (**b**).

The reduction of particle size was also observed for all samples, which was confirmed by the particle size analysis (Table 3). The ice-cream mixes were characterised by irregular distribution of fat particles. Samples not subjected to the homogenisation methods have larger fat particles, which is also confirmed by the particle size analysis. The presented photos also show agglomerates of particles. After the 24 h maturation period, despite the decrease in particle diameter, no significant change in the degree or order in the structure of the mixtures was observed. In a study by Voronin et al. [17] in the visualisation of the microstructure of milk ice-cream mixes, the destabilised fat aggregates were also visible.

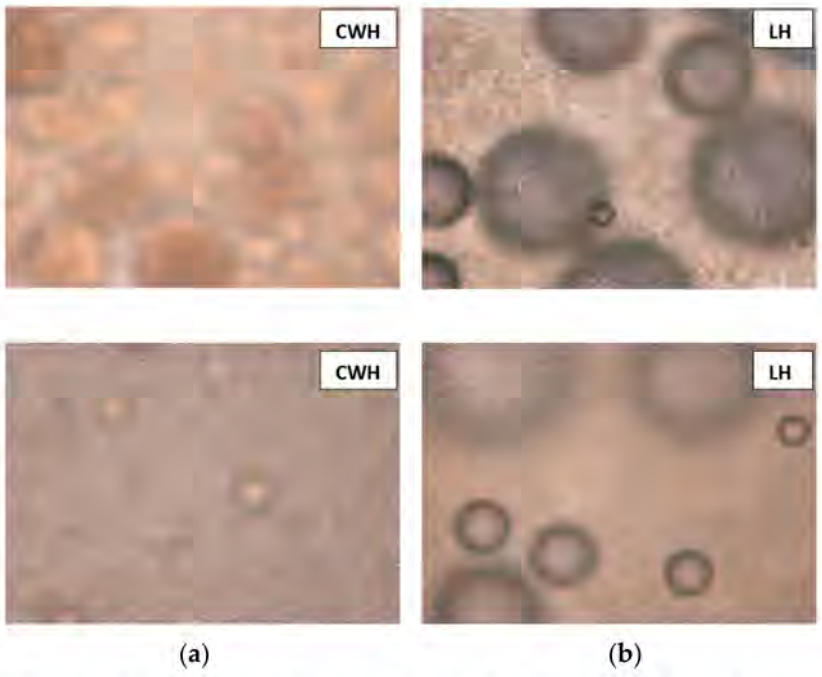


Figure 4. The chosen photo of ice-cream mix before (**a**) and after 24 h of maturation (**b**).

4. Conclusions

This presented study showed that the iota carrageenan, new stabilisers and homogenisation contributed to a significant influence on the physical properties of vegan ice cream. The homogenisation process did not affect the density of ice-cream mixes but on the other hand, this process caused the destabilisation of ice-cream mixes during the maturation time. According to the TSI (3.0 to 6.5), the higher value of this parameter was noted for the samples after homogenisation independently on the addition of stabilisers. Based on the rheological properties, the homogenisation process influenced the flow behaviour index but only for the samples after maturation time. A significant increase in consistency K and apparent viscosity was noted only for the samples with the addition of stabilisers after homogenisation. Similarly to the tendency observed for rheological properties, significant decrease in the particles sizes were noted for the samples after homogenisation and after the maturation process (D_{50} 11.51 to 6.37 μm).

The stabilisers did not influence the density of ice-cream mixes. Moreover, the used stabilisers influenced the stability of the product considerably more significant than the homogenisation process. The lowest TSI values were noted for the sample without homogenisation treatment but with the addition of stabilisers (with hydrolysates after lactase treatment LWH) at 1.7 value. Additionally, for the samples, IWH and IH (iota carrageenan addition) low TSI value was noted, which contributed to better stability properties during the maturation time. In addition, based on the rheological analysis the samples IH and IWH had higher stability according to the lowest values of the flow behaviour index. The use of stabilisers contributed to significantly decreasing particle sizes (the range of D_{50} after the maturation was from 11.29 to 28.50 μm). However, the most effective results was observed for samples with the use of hydrolysates of iota carrageenan (after acid hydrolysis-AH), which with the combination of homogenisation treatment decreased the size of particles to 6.33 μm. This particle size decrease in samples with the hydrolysates addition promises more favourable crystal structure creation in ice cream.

To sum up, the satisfying physical properties of ice-cream mixes contributed to obtaining more stable products which can be considered as the potential application of vegan ice cream. Consequently, there exists the significant possibility of decreasing the waste during production and thereby increasing the positive influence on the environment. Such an

approach will be treated as the main goal to attain and the main priority for the potential producer for whom sustainable development plays a key role.

Author Contributions: Conceptualisation, A.K. and A.K.-D.; methodology, A.K. and E.J.; software, A.K.; validation, A.K., A.K.-D., E.J. and S.G.; formal analysis, A.K. and A.K.-D.; investigation, A.K., A.K.-D. and E.J.; resources, S.G.; data curation, A.K., A.K.-D. and E.J.; writing—original draft preparation, A.K., A.K.-D. and E.J.; writing—review and editing, A.K., A.K.-D. and E.J.; visualisation, A.K.; supervision, E.J. and S.G.; project administration, A.K.-D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data generated or analysed during this study are available from the corresponding author on reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Konstantas, A.; Stamford, L.; Azapagic, A. Environmental impacts of ice cream. *J. Clean. Prod.* **2019**, *209*, 259–272. [CrossRef]
2. Sandström, V.; Valin, H.; Krisztin, T.; Havlik, P.; Herrero, M.; Kastner, T. The role of trade in the greenhouse gas footprints of EU diets. *Glob. Food Secur.* **2018**, *19*, 48–55. [CrossRef]
3. Saget, S.; Costa, M.; Santos, C.S.; Vasconcelos, M.W.; Gibbons, J.; Styles, D.; Williams, M. Substitution of beef with pea protein reduces the environmental footprint of meat balls whilst supporting health and climate stabilization goals. *J. Clean. Prod.* **2021**, *297*, 126447. [CrossRef]
4. Krizanova, J.; Rosenfeld, L.D.; Tomiyama, J.A.; Guardiola, J. Pro-environmental behaviour predicts adherence to plant-based diets. *Appetite* **2021**, *163*, 105243. [CrossRef] [PubMed]
5. Baukid, F.; Rosell, C.M.; Castellari, M. Pea protein ingredients: A mainstream ingredient to (re)formulate innovative foods and beverages. *Trends Food Sci. Technol.* **2021**, *110*, 729–742. [CrossRef]
6. Vanga, S.K.; Raghavan, V. How well do plant based alternatives fare nutritionally compared to cow's milk? *J. Food Sci. Technol.* **2018**, *55*, 10–20. [CrossRef]
7. Akdeniz, V.; Akalin Sibel, A. New approach for yoghurt and ice cream production: High intensity ultrasound. *Trends Food Sci. Technol.* **2019**, *86*, 392–398. [CrossRef]
8. Fiol, C.; Prado, D.; Romero, C.; Laburu, N.; Mora, M.; Alava, I.J. Introduction of a new family of ice creams. *Int. J. Gastron. Food Sci.* **2017**, *7*, 5–10. [CrossRef]
9. Innocente, N.; Biasutti, M.; Venir, E.; Spaziani, M.; Marchesini, G. Effect of high-pressure homogenization on droplet size distribution and rheological properties of ice cream mixes. *J. Dairy Sci.* **2009**, *92*, 1864–1875. [CrossRef]
10. Tolstoguzov, V. Some thermodynamic considerations in food formulation. *Food Hydrocoll.* **2003**, *17*, 1–23. [CrossRef]
11. Yüksel, A.; Şat, İ.; Yüksel, M.; Çakmakçı, S. A comparative study on the physicochemical characteristics, organic acid profiles, mineral compositions and sensory properties of ice creams produced with different types of nuts. *Carpathian J. Food Sci. Technol.* **2017**, *9*, 137–151.
12. O'Neli, C.E.; Nicklas, T.A.; Flugoni, V.L., III. Almond consumption is associated with better nutrient intake, nutrient adequacy, and diet quality in adult: National health and nutrition examination survey 2001–2010. *Food Sci. Nutr.* **2016**, *7*, 504–515.
13. Shoaib, M.; Shehzad, A.; Omar, M.; Rakha, A.; Raza, H.; Sharif, H.; Shakeel, A.; Ansari, A.; Niazi, S. Inulin: Properties, health benefits and food applications. *Carbohydr. Polym.* **2016**, *147*, 444–454. [CrossRef]
14. Watzl, B.; Girrbach, S.; Roller, M. Inulin, oligofructose and immunomodulation. *Br. J. Nutr.* **2005**, *93* (Suppl. 1), S49–S55. [CrossRef]
15. O'Sullivan, J.; Beevers, J.; Park, M.; Greenwood, R.; Norton, I. Comparative assessment of the effect of ultrasound treatment on protein functionality pre- and post-emulsification. *Colloids Surf. A Physicochem. Eng. Asp.* **2015**, *484*, 89–98. [CrossRef]
16. O'Sullivan, J.; Murray, B.; Flynn, C.R.; Norton, I. The effect of ultrasound on the structural, physical and emulsifying properties of animal and vegetable proteins. *Food Hydrocoll.* **2016**, *53*, 141–154. [CrossRef]
17. Voronin, G.L.; Roberts, R.; Felix, T.L.; Coupland, J.N.; Harte, F.M. Effect of high-pressure-jest processing on the physiochemical properties of low-fat ice cream mix. *J. Dairy Sci.* **2020**, *103*, 6003–6014. [CrossRef] [PubMed]
18. Chauhan, P.; Saxena, A. Bacterial carrageenans: An overview of production and biotechnological applications. *3 Biotech* **2016**, *6*, 1–18. [CrossRef] [PubMed]
19. Kiran-Yildirim, B.; Hale, J.; Wefers, D.; Gaukel, V. Ice recrystallisation inhibition of commercial κ-, ι-, and λ-carrageenans. *J. Food Eng.* **2021**, *290*, 110269. [CrossRef]
20. Weiner, L.M. Toxicological properties of carrageenan. *Agents Actions* **1991**, *32*, 46–51. [CrossRef] [PubMed]

21. Thrimawithana, T.; Young, S.; Dunstan, D.; Alany, R. Texture and rheological characterisation of kappa and iota carrageenan in the presence of counter ions. *Carbohydr. Polym.* **2010**, *82*, 69–77. [CrossRef]
22. Henares, B.M.; Enriquez, E.P.; Dayrit, F.M.; Rojas, N.R.L. Iota-carrageenan hydrolysis by Pseudoalteromonas carrageenovora IFO12985. *Philipp. J. Sci.* **2010**, *139*, 131–138.
23. Kamińska-Dwórznicka, A.; Antczak, A.; Samborska, K.; Lenart, A. Acid hydrolysis of kappa-carrageenan as a way of gaining new substances for freezing process modification and protection from excessive recrystallisation of ice. *Int. J. Food Sci. Technol.* **2015**, *50*, 1799–1806. [CrossRef]
24. Kamińska-Dwórznicka, A.; Skrzypczak, P.; Gondek, E. Modification of kappa carrageenan by β-galactosidase as a new method to inhibit recrystallization of ice. *Food Hydrocoll.* **2016**, *61*, 31–35. [CrossRef]
25. Dłużewska, E.; Gazda, B.; Leszczyński, K. Wpływ wybranych hydrokoloidów polisacharydowych na jakość koncentratów lodów owocowych. *Acta Sci. Pol. Technol. Aliment.* **2003**, *2*, 97–107.
26. Goff, H.D.; Hartel, R.W. *Ice Cream*, 7th ed.; Springer: New York, NY, USA, 2013.
27. Zambrano-Mayorga, L.; Ramirez-Navas, J.; Ochoa-Martinez, C.I. Influence of the formulation on the thermophysical properties and the quality parameters of diary ice cream. *DYNA* **2019**, *86*, 117–125. [CrossRef]
28. Florowska, A.; Wójcik, E.; Florowski, T.; Dłużewska, E. Wpływ dodatków preparatów błonnikowych na wybrane wyróżniki jakości lodów. *Zesz. Probl. Postępów Nauk Rol.* **2013**, *574*, 11–18.
29. Warren, M.M.; Hartel, R.W. Structural, compositional, and sensorial properties of United States commercial ice cream products. *J. Food Sci.* **2014**, *79*, E2005-13. [CrossRef]
30. Alves, A.B.; Santos, W.F.D., Jr.; Faria, W.C.S.; Nascimento, E.; Lanzarin, M.; Siqueria, P.B.; Siqueria, N.F.M.P.; Barros, W.M.D. Developmnet, characterization, and shelf-life of lactose-free artisan ice cream produces with different bases. *Res. Soc. Dev.* **2021**, *10*, 1–16.
31. Hu, Y.; Ting, Y.; Hu, J.; Hsieh, S. Techniques and methods to study functional characteristics of emulsion systems. *J. Food Drug Anal.* **2017**, *25*, 16–26. [CrossRef]
32. Mengual, O.; Meunier, G.; Cayre, I.; Puech, K.; Snabre, P. Characterisation of instability of concentrated dispersion by a new optical analyser: The TURBISCAN MA 1000. *Colloids Surf. A Physicochem. Eng. Asp.* **1999**, *152*, 111–123. [CrossRef]
33. Cheng, J.; Ma, Y.; Li, X.; Yan, T.; Cui, J. Effects of milk protein-polysaccharide interactions on the stability of ice cream mix model systems. *Food Hydrocoll.* **2015**, *45*, 327–336. [CrossRef]
34. Keeney, P.G. The fat stability problem in ice cream. *Ice Cream Rev.* **1958**, *8*, 42–45.
35. Berger, K.G.; White, G.W. An electron microscopical investigation of fat destabilization in ice cream. *J. Food Technol.* **1971**, *6*, 285–294. [CrossRef]
36. Berger, K.G.; Bullimore, B.K.; White, G.W.; Wright, W.B. The structure of ice cream. *Dairy Ind.* **1972**, *419–425*, 493–497.
37. Koxholt, M.; Eisenmann, B.; Hinrichs, J. Effect of the fat globule size on the meltdown of ice cream. *J. Dairy Sci.* **2001**, *84*, 31–37. [CrossRef]
38. Biasutti, M.; Venir, E.; Marino, M.; Maifreni, M.; Innocente, N. Effects of high pressure homogenisation of ice cream mix on the physical and structural properties of ice cream. *Int. Dairy J.* **2013**, *32*, 40–45. [CrossRef]
39. Warren, M.M.; Hartel, R.W. Effects of emulsifier, overrun and dasher speed on ice cream microstructure and melting properties. *J. Food Sci.* **2018**, *83*, 639–647. [CrossRef]
40. Kurt, A.; Atalar, I. Effects of quince seed on the rheological, structural and sensory characteristics of ice cream. *Food Hydrocoll.* **2018**, *82*, 186–195. [CrossRef]
41. Javidi, F.; Razavi, S.M.; Behrouzian, F.; Alghooneh, A. The influence of basil seed gum, guar gum and their blend on the rheological, physical and sensory properties of low fat ice cream. *Food Hydrocoll.* **2016**, *52*, 625–633. [CrossRef]
42. Mostafavi, F.S.; Tehrani, M.M.; Mohebbi, M. Rheological and sensory properties of fat reduced vanilla ice creams containing milk protein concentrate (MPC). *J. Food Meas. Charact.* **2017**, *11*, 567–575. [CrossRef]
43. BahramParvar, M.; Razavi, S.M.; Khodaparast, M.H. Rheological characterization and sensory evaluation of a typical soft ice cream made with selected food hydrocolloids. *Food Sci. Technol. Int.* **2010**, *16*, 79–88. [CrossRef] [PubMed]
44. Atalar, I.; Kurt, A.; Gul, O.; Yazici, F. Improved physicochemical, rheological and bioactive properties of ice cream: Enrichment with high pressure homogenized hazelnut milk. *Int. J. Gastron. Food Sci.* **2021**, *24*, 100358. [CrossRef]
45. Dogan, M.; Kayacier, A. The effect of ageing at a low temperature on the rheological properties of Kahramanmaras-type ice cream mix. *Int. J. Food Prop.* **2007**, *10*, 19–24. [CrossRef]
46. Rao, M.A. *Rheology of Fluid And Semisolid Foods: Principles and Applications*; Springer Science & Business Media: New York, NY, USA, 2007.

applied sciences

MDPI

Article

Study on the Influence of Ultrasound Homogenisation on the Physical Properties of Vegan Ice Cream Mixes

Anna Kot *, Anna Kamińska-Dwórznicka and Ewa Jakubczyk

Department of Food Engineering and Process Management, Institute of Food Sciences, Warsaw University of Life Sciences (WULS-SGGW), Nowoursynowska 159C, 02-776 Warsaw, Poland
* Correspondence: anna_kot1@sggw.edu.pl

Abstract: This study investigated the effect of ultrasound homogenisation on the physical properties of vegan ice cream mixes. Samples were prepared based on vegan recipes with different sorts of stabilisers such as iota carrageenan and iota carrageenan's acid and enzymatic hydrolysates. Ice cream mixes were compared for stability, particle size distribution, rheological properties and morphological structure. All mentioned analyses were conducted before and after 24 h of maturation at 4 °C. It was found that the ultrasound treatment decreased the size of particles and, in conjunction with the maturation stage, a significant reduction was visible (the lowest value was at 9.76 µm). The addition of the hydrolysates of iota carrageenan had a considerably better effect in reducing the size of particles than iota carrageenan. The range of TSI values was from 1.7 to 4.2. Additionally, two sorts of destabilisation occurred: sedimentation and coalescence, during the maturation of ice cream mixes, which was also visible in the images. According to the rheological properties, ice cream mixes, with the addition of stabilisers, showed non-Newtonian shear-thinning (pseudoplastic) behaviour. Moreover, the effect of ultrasound treatment on the consistency index was only pivotal for ice cream mixes with an addition of iota carrageenan and with enzymatic β-galactosidase hydrolysates of iota carrageenan.

Keywords: ultrasound; homogenisation; vegan; ice cream mix; iota carrageenan; stabilisers

check for updates

Citation: Kot, A.; Kamińska-Dwórznicka, A.; Jakubczyk, E. Study on the Influence of Ultrasound Homogenisation on the Physical Properties of Vegan Ice Cream Mixes. *Appl. Sci.* **2022**, *12*, 8492. https://doi.org/10.3390/app12178492

Academic Editors: Joanna Kapusta-Duch, Teresa Leszczyńska and Ewa Piątkowska

Received: 20 July 2022
Accepted: 22 August 2022
Published: 25 August 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

There is a myriad of elements of different nature such as sugar, fats, stabilisers, water and others, which constitute the compounds of the ice cream mix. All of them have to be correctly blended and emulsified together so there is less possibility of reducing the quality of the final product. The knowledge of the characteristics of compounds and the relationship between them is pivotal to obtaining the desirable quality of ice cream. According to that, the ice cream mix determines the sensorial characteristics, structure, resistance to melting, hardness or viscoelastic behaviour of ice cream [1–3]. Not only do ingredients contribute to the ice cream structure, but also the manufacturing process, which includes the homogenisation step.

In food engineering, homogenisation is a process that is used to reduce the size of particles and globules in the product, narrowing the size distribution or providing a stable emulsion. Consequently, there is a possibility of achieving greater stability of fat globules during the ice cream mix maturation [2,4,5]. However, as a result of the need to improve traditional food processing in recent decades, alternative methods are being implemented. The search for alternative processes has drawn attention to ultrasound [6].

In the food industry, ultrasound has been the subject of research for many years and the application of this method has now become widely used. Overall, ultrasound is defined as an acoustic wave with a frequency greater than 20 kHz, the threshold for human auditory detection. The mechanism of ultrasound is based on acoustic cavitation. It occurs because of the interaction between ultrasonic waves, liquid, and dissolved gas. Consequently, the

implosion of cavitation bubbles generates an extremely high temperature and pressure which later produces high shear energy waves and turbulence. Moreover, the ultrasound technique is an innovative and up-and-coming technology in the food industry owing to the fact that it is relatively cheap, very simple, really fast, non-toxic and energy-saving. It can also be considered a green technology on the grounds that it creates an environmentally friendly process. Additionally, the ultrasound can be used to minimize processing or increase quality and improve processing effectiveness and efficiency, providing food safety while extending the shelf life of the product [7–12]. Currently, ultrasound is commonly used for instance in the activation or deactivation of enzymes, homogenisation, emulsification, stabilisation, crystallization, or even ultrasound-assisted drying [10,13].

Ultrasound waves yield an efficient homogenising procedure compared to traditional homogenisation. Therefore, the use of ultrasound during the process of the production of ice cream may be a new promising approach [9]. In the presented study, ultrasound homogenisation was used as an alternative method to conventional homogenisation. According to the current research, it was proven that ultrasound homogenisation may produce particles with a narrow particle size distribution. Moreover, the ultrasound may improve stability and contribute to the adsorbing protein of the emulsion. Consequently, such improved stability and smaller particle size contributed to considerably better results during the freezing process due to the fact that such favourable conditions lead to creating a superior ice crystal structure and obtaining smaller ice crystals in comparison to the use of traditional homogenisation [8,14,15].

Furthermore, the ultrasound treatment would replace food additives and emulsifiers, for example in the preparation of dairy-based emulsion. The results of the study by Aslan and Dogan [16] show that ultrasound treatment could eliminate additives such as emulsifiers in the preparation of food emulsions. Not only can the findings of this study be beneficial for the economy but also for commercial aspects. Moreover, this knowledge can then be used in the ice cream mix preparation to, for instance, extend the shelf life of the ice cream. So, based on that, the power of ultrasound is a promising tool in the preparation of the ice cream mix [15,16].

The aim of this study was to develop the previous study by Kot et al. [17], conducted on vegan ice cream mixes, to compare the physical properties of ice cream mixes that were affected by different sorts of homogenisation. For this purpose, ice cream mixes with the same vegan recipe were produced but instead of traditional homogenisation, ultrasound homogenisation was used. Stability, particle size, rheological properties and morphology analysis of all the ice cream mix samples were performed.

2. Materials and Methods

2.1. The Preparation of the Hydrolysates of Iota Carrageenan

How the hydrolysis of iota carrageenan was conducted was meticulously described in the paper by Kot et al. [17]. In the mentioned research, acid and enzymatic hydrolysates of iota carrageenan were obtained. To perform acid hydrolysis, 0.1 M hydrochloric acid was used. While for enzymatic hydrolysis two sorts of enzymes were applied: β-galactosidase and its cheaper and commercial equivalent—lactase.

2.2. The Preparation of Ice Cream Mixes

2.2.1. The Materials for the Recipe for Ice Cream Mixes

The ingredients used to prepare the ice cream mixes were: 66.5% roasted almond original drink (Enerbio, Rossmann, Hanover, Germany), 16% almond syrup (Monin, Bourges, France), 12% inulin (Orafti BENEO, Tienen, Belgium), 5% pea protein (Nuturalys S85 plus, Roquette, Lestrem, France), 0.4% emulsifier E471 (Fooding Shanghai, Shanghai, China), 0.08% LBG—Locust Bean Gum (Fooding Shanghai, Shanghai, China), 0.02% xanthan gum (Fooding Shanghai, Shanghai, China), 0.01% iota carrageenan (Fluka, Sigma-Aldrich, St. Louis, MI, USA) or 0.005% newly obtained: the acid hydrolysates of iota carrageenan and enzymatic hydrolysis by β-galactosidase and enzymatic hydrolysis by commercial

lactase. The description of abbreviations of prepared ice cream mixes was characterised in Table 1.

Table 1. The description of abbreviations of samples.

Sample	Description
C	The control sample
CU	The control sample after ultrasound homogenisation
I	The sample with stabilisers (the combination of iota carrageenan, LBG and xanthan gum)
IU	The sample with stabilisers (the combination of iota carrageenan, LBG and xanthan gum) after ultrasound homogenisation
A	The sample with stabilisers (the combination of acid hydrolysates of iota carrageenan, LBG and xanthan gum)
AU	The sample with stabilisers (the combination of acid hydrolysates of iota carrageenan, LBG and xanthan gum) after ultrasound homogenisation
B	The sample with stabilisers (the combination of enzymatic β-galactosidase hydrolysates of iota carrageenan, LBG and xanthan gum)
BU	The sample with stabilisers (the combination of enzymatic β-galactosidase hydrolysates of iota carrageenan, LBG and xanthan gum) after ultrasound homogenisation
L	The sample with stabilisers (the combination of enzymatic commercial lactase hydrolysates of iota carrageenan, LBG and xanthan gum)
LU	The sample with stabilisers (the combination of enzymatic commercial lactase hydrolysates of iota carrageenan, LBG and xanthan gum) after ultrasound homogenisation

2.2.2. The Production of Ice Cream Mixes

According to the recipe, dry and liquid ingredients were weighed separately. After it, all components were mixed using a Bosch MaxoMixx 750W blender (Bosch, Gerlingen, Germany). Then, the pasteurization process was performed by using a Vorwerk thermomixer used at a temperature of 85 °C within 1.5 min and cooled to 25 °C.

2.2.3. Ultrasound Homogenisation

The next step in preparing ice cream mixes was to treat chosen samples by ultrasound by using a homogeniser Ultrasonic Liquid Processor VCX 500 (Sonics & Materials, Inc., Newtown, CT, USA) with a diameter probe (Model CV334). 250 mL of ice cream mixes for each trial were used to homogenise. The frequency of 20 kHz and exposure time of 5 min was used. Then ice cream mixes were allowed to mature for 24 h at 4 °C.

2.3. The Ice Cream Mixes' Physical Analysis

2.3.1. Stability Analysis of Ice Cream Mixes

The stability of ice cream mixes was conducted by using Turbiscan Lab Expert (Formulation SA, Toulouse, France). To record the date of backscattered (BS) light during measurements, Turbisoft 2.0.0.33 software was used, which allowed comparing stability based on the Turbiscan Stability Index (TSI). Analysis of the stability of ice cream mixes was performed before and after maturation (for 24 h; at 4 °C).

2.3.2. The Analysis of Particle Size Distribution

The Cilas 1190 (Cilas, Orléans, France)—a laser diffraction instrument was used to establish the particle size of the ice cream mixes. The emulsions of ice cream mixes were suspended in water at an obscuration of 10%. The obtained results were expressed as the median diameter of D_{50} and as diagrams of particle size distribution. The analysis was performed before and after maturation (for 24 h; at 4 °C).

2.3.3. The Morphology of the Ice Cream Mix

The morphology of ice cream mixes was determined according to the conducted before and after maturation step (for 24 h; at 4 °C). Firstly, samples of ice cream mixes were prepared using a small amount and then put on the slide using a spatula, covered with a slipped glass. Photos of the ice cream mixes were taken using the Olympus BX 43F microscope (Nikon, Shanghai, China) equipped with the Olympus CAM-SC 50 (Nikon, Tokyo, Japan).

2.3.4. The Rheological Analysis of the Ice Cream Mix

Experiments were carried out to measure the rheological properties of the ice cream mix before and after maturation, according to the methodology developed by Kot et al. [17]. Rheological tests were performed using a Haake Mars 40 rheometer (Thermo Scientific Inc., Karlsruhe, Germany) in rotational mode within a shear rate of 0–100 s^{-1}. All experiments were performed in triplicate to verify repeatability. The Herschel–Buckley model, Ostwald de Waele model and Bingham model were used to predict the flow properties of ice cream samples. A comparison of the correlation coefficient (R) and chi-square (χ2) of the analysed models showed that the Herschel–Buckley model (1) was adequate to describe the rheological properties of all ice cream samples.

$$\eta_{app} = \tau_0 (\dot{\gamma})^{-1} + K\dot{\gamma}^{n-1} \quad (1)$$

where: η_{app}—the apparent viscosity (Pa s), $\dot{\gamma}$—the shear rate (s^{-1}), τ_0—the yield stress (Pa), K—the consistency index (Pa s^n), and n—flow behaviour index (dimensionless).

2.4. Statistical Analysis

For the particle size of distribution and rheological properties, a statistical analysis was performed. The STATISTICA 13.3 software (Statsoft Polska, Kraków, Poland) was used to perform the analysis of variance (ANOVA). The significance of the test is set at $\alpha = 0.05$. The presented data are expressed as a mean with standard deviations (±SD) and also the differences between groups were evaluated using the Tukey HSD test.

3. Results

3.1. The Analysis of the Stability of Ice Cream Mixes

The results of the stability of ice cream mixes were determined as values of the TSI factor (Turbiscan Stability Index) in Figure 1 and as Back Scattering (Figure 2a,b). According to the obtained results, it was noted that the ultrasound treatment had a vital influence on the stability of ice cream mixes. The TSI value had a range from 1.7 to 4.2.

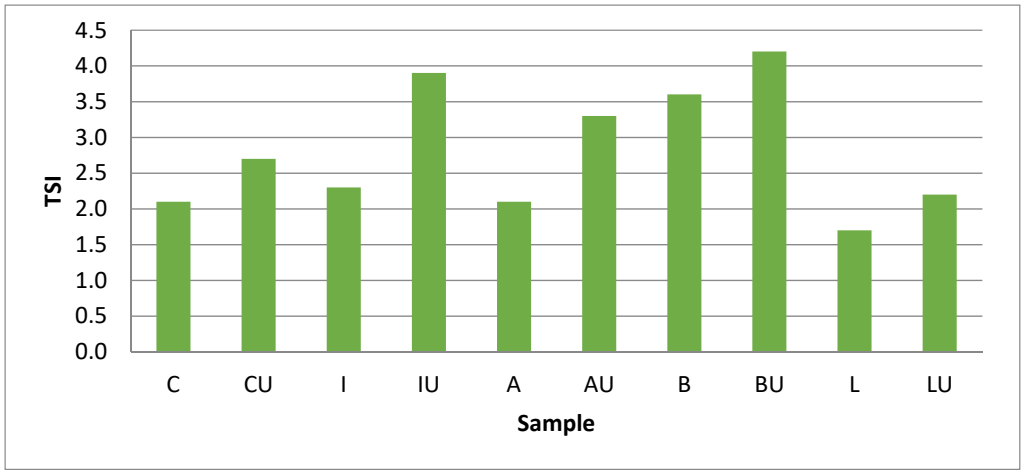
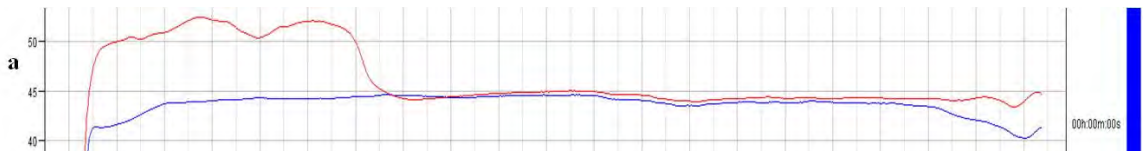


Figure 1. The turbiscan stability index (TSI) of ice cream mixes.

Figure 1. The turbiscan stability index (TSI) of ice cream mixes.



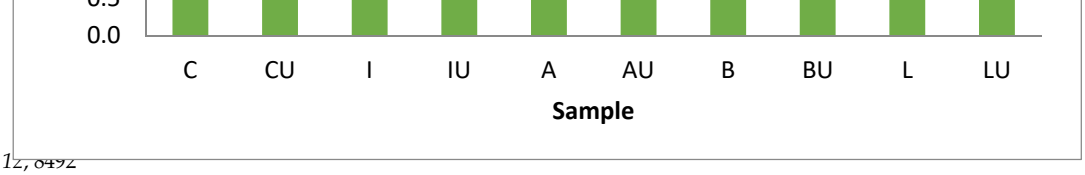


Figure 1. The turbiscan stability index (TSI) of ice cream mixes.

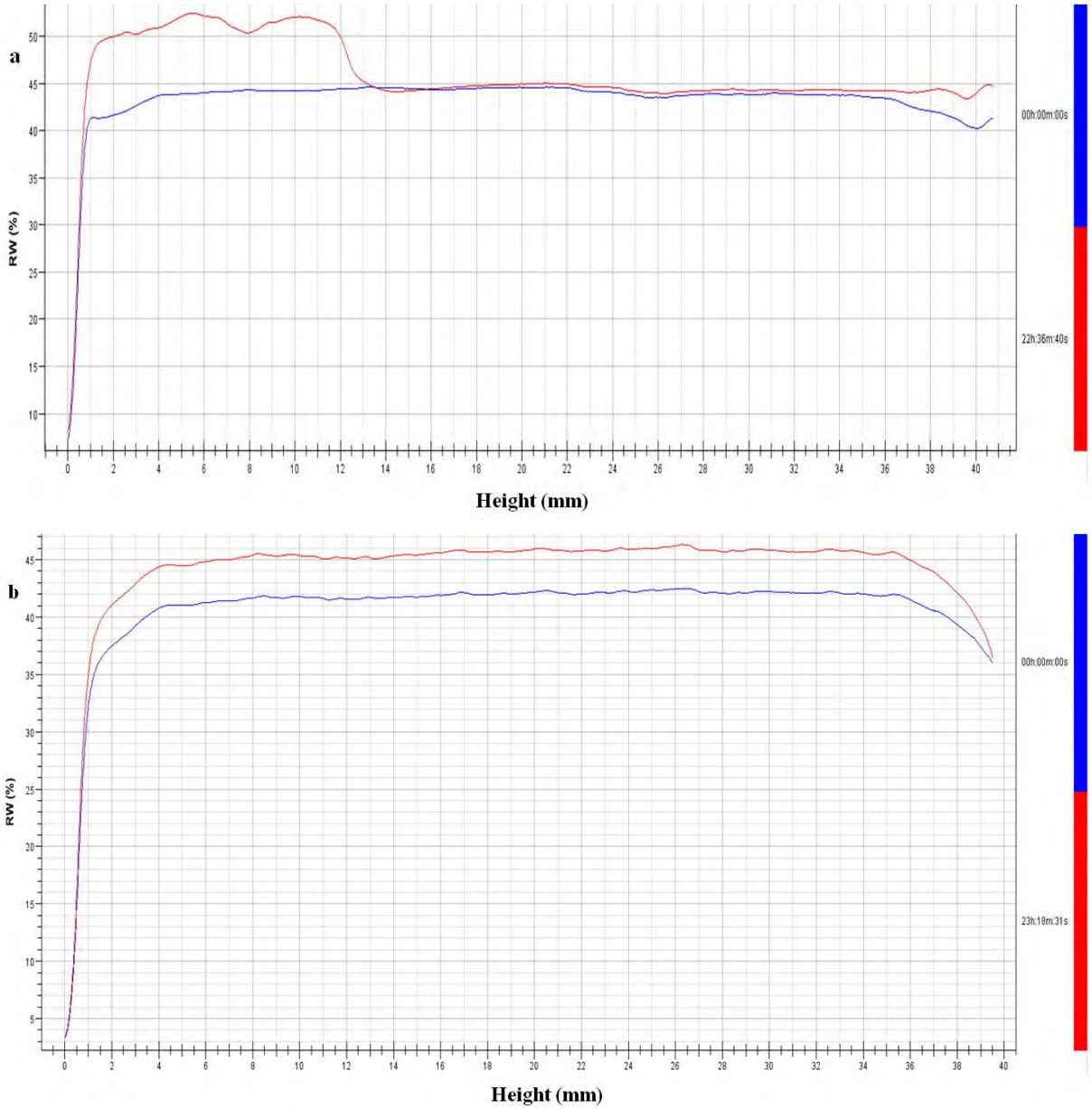


Figure 2. The variation of the backscatter at maturation time at 4 °C (**a**) sedimentation; (**b**) coalescence).

In a group of samples without ultrasound treatment, the lowest result of the TSI value (1.7) was observed in the sample with the addition of enzymatic (lactase treatment) hydrolysates of iota carrageenan (L). Additionally, samples with the addition of acid hydrolysates of iota carrageenan (A) and iota carrageenan (I), as a stabiliser, had a lower value of the TSI factor, close to the control sample (C), around 2 (Table 1 and Figure 1). Only samples with the addition of enzymatic (β-galactosidase treatment) hydrolysates of iota carrageenan (B) achieved a value of 3.6.

In the case of a group of ice cream mixes with ultrasound treatment, the same tendency was visible. The lowest TSI value (2.2) was reported for sample (LU) with the addition of enzymatic (lactase treatment) hydrolysates. In addition, diminished stability in the ice cream mixes with the addition of enzymatic (β-galactosidase treatment) hydrolysates of iota carrageenan (BU) was at 4.2 (Table 1 and Figure 1). Overall, the samples without ultrasound treatment achieved the lowest TSI value, which contributed to the highest stability of ice cream mixes. Based on that, it may be concluded that the effect of ultrasound cavitation declined the stability of ice cream mixes.

When it comes to the results of Back Scattering (BS%), two different phenomena of destabilisation occurred: coalescence and sedimentation (Figure 2a,b). Based on the presented results, it was observed that the addition of stabilisers contributed to the observed effects on the grounds that coalescence occurred in the samples with the addition of a stabiliser regardless of ultrasound treatment. Sedimentation was visible in the control sample without stabilisers also independently regardless of ultrasound treatment.

3.2. Analysis of Particle Sizes of Ice Cream Mixes

An analysis of particle size of ice cream mixes was presented as the mean diameter in Table 2 and as particle size distribution in Figure 3a,b before and after 24 h of maturation at 4 °C.

Table 2. The value of median D_{50} of ice cream mixes before and after maturation (for 24 h; at 4 °C).

Sample	D_{50} before Maturation	D_{50} after Maturation
C	15.65 ± 0.62^{a}	17.23 ± 0.49^{c}
CU	14.68 ± 0.17^{a}	9.97 ± 0.23^{a}
I	$30.40 \pm 0{,}44^{e}$	28.50 ± 0.43^{e}
IU	28.70 ± 0.16^{d}	23.73 ± 0.62^{d}
A	38.36 ± 0.49^{c}	11.41 ± 0.97^{ab}
AU	35.70 ± 0.50^{b}	11.24 ± 1.41^{a}
B	42.15 ± 0.19^{g}	11.29 ± 0.53^{ab}
BU	34.29 ± 0.14^{f}	9.76 ± 0.36^{a}
L	38.31 ± 0.66^{c}	13.20 ± 0.45^{b}
LU	36.92 ± 0.56^{b}	10.59 ± 0.09^{a}

The different superscript letters in the table represent significant differences in the means of the same parameter ($p < 0.05$). Values represent means ± standard deviations.

Before the maturation process (directly after preparing the ice cream mixes), the mean diameter of D_{50} ranged from 14.68 to 42.15 µm (Tables 1 and 2). The lowest value was noted for the control sample, CU, with the ultrasound treatment. For all ice cream mixes with the addition of stabilisers, the mean diameter was significantly higher (from 28.70 to 42.15 µm) than the previously mentioned CU sample. Nonetheless, it was observed that the ultrasound treatment reduced the size of particles in all samples no matter the addition of stabilisers. For instance, sample B (with enzymatic (β-galactosidase) hydrolysates of iota carrageenan)) without ultrasound homogenisation, had a D_{50} at 42.15 µm (it was the highest result), while the same sample but with ultrasound treatment achieved a level of 34.29 µm. According to that, the decrease in the size of the particle by acoustic cavitation was beneficial. The statistical analysis resulted in significant differences between samples before maturation time, so based on that it may be said that the ultrasound and use of stabilisers influenced the particle size of ice cream mix particles (Tables 1 and 2).

Taking into consideration the maturation time of ice cream mixes, which is crucial in ice cream production, the same analysis was conducted after 24 h. A noticeable reduction in D_{50} was noted in comparison to the results before maturation. The range of particle size was from 9.76 to 28.50 µm. Additionally, the same tendency of lower value, as before maturation, was visible for samples with ultrasound treatment. The samples with the addition of hydrolysates of iota carrageenan with ultrasound treatment had the lowest value of D_{50} (Tables 1 and 2). For instance, the sample BU (with the enzymatic (β-galactosidase) hydrolysates of iota carrageenan)) achieved 9.76 µm, sample LU (with enzymatic (lactase) hydrolysates of iota carrageenan)) at 10.59 µm and AU (with acid hydrolysates of iota carrageenan) at 11.24 µm (Tables 1 and 2). Moreover, according to statistical appraisal, the three mentioned samples with the control sample, CU, are in the same homologous group. It may be concluded that ultrasound treatment was a more crucial contribution to reducing the size of particles than the use of stabilisers. Only the sample with the addition of iota carrageenan with and without ultrasound treatment (I and IU) had the highest value of particle size at 28.5 µm and 23.73 µm.

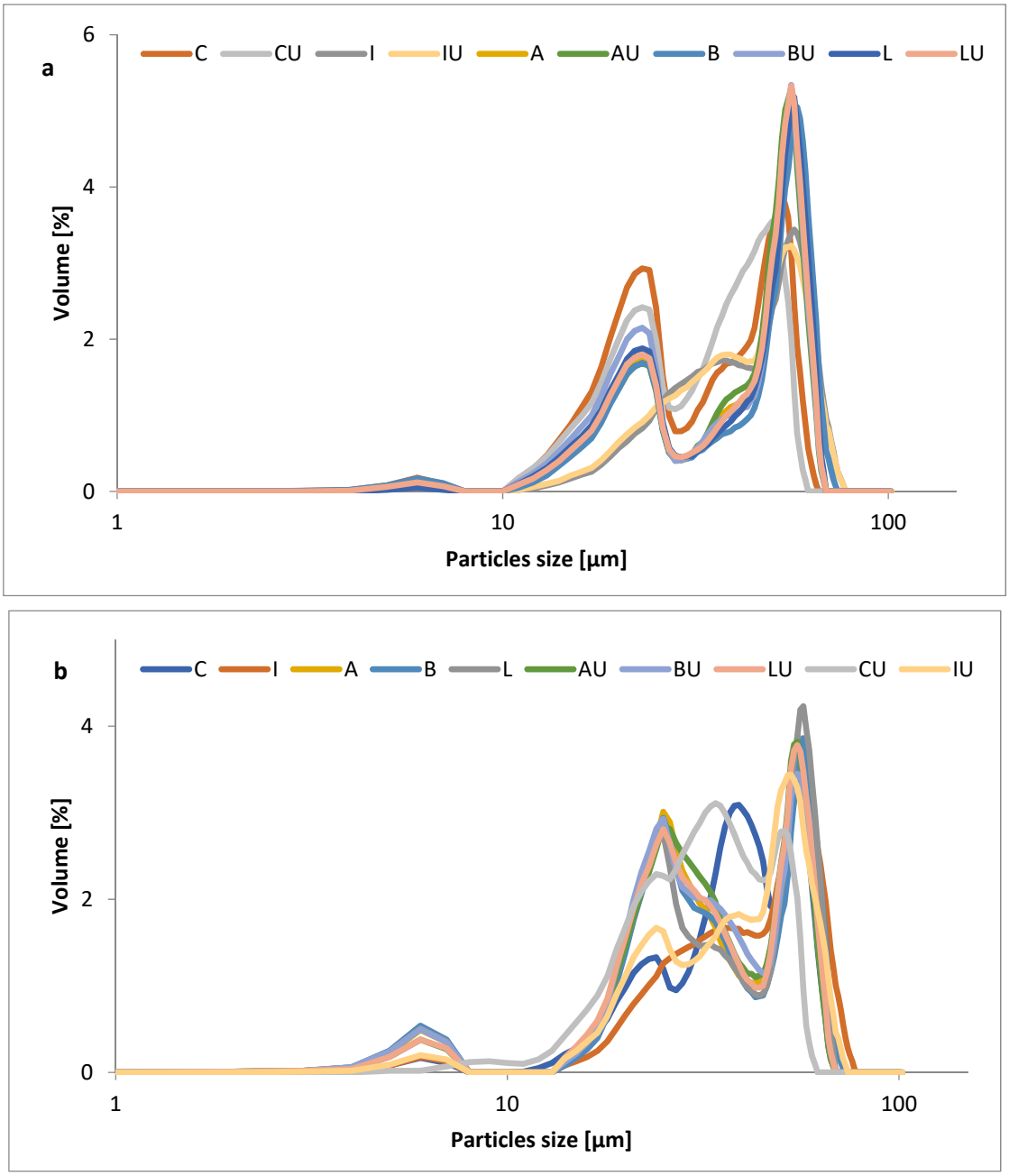


Figure 3. The distribution of particle size: (**a**) before the maturation of ice cream mixes; (**b**) after the maturation of ice cream mixes (for 24 h; at 4 °C).

Considering the size of the particles, the particlesize distribution of the ice cream mix before and after maturation was analysed (Figure 3a,b). Before maturation, the trimodal or fourfold particle size distribution were noted (Figure 3a, Table 2).

Additionally, looking at the range of peaks in the figures, samples with the addition of stabilisers (regardless of ultrasound treatment) and control samples (C and CU) were approximately the same. Only samples I and IU (with the addition of iota carrageenan) had distinctive distributions of peaks in the figure. After 24 h of maturation, in all ice cream mixes, the four-fold particle size distribution was observed based on four characteristic peaks in the figure (Figure 3b, Table 1). Only sample I (with the iota carrageenan) did not change the sort of distribution of particles. Moreover, the distribution of characteristic peaks in the figure was the same for samples with the addition of hydrolysates of iota carrageenan. For the control samples (C and CU) and for the sample with the addition of

Before the maturation process (directly after preparing the ice cream mixes), the mean diameter of D_{50} ranged from 14.68 to 42.15 µm (Tables 1 and 2). The lowest value was noted for the control sample, CU, with the ultrasound treatment. For all ice cream mixes with the addition of stabilisers, the mean diameter was significantly higher (from 28.70 to 42.15 µm) than the previously mentioned CU sample. Nonetheless, it was observed that the ultrasound treatment reduced the size of particles in all samples no matter the addition of stabilisers. For instance, sample B (with enzymatic (β-galactosidase) hydrolysates of iota carrageenan)) without ultrasound homogenisation had a D_{50} at 42.15 µm (it was the highest result), while the same sample but with ultrasound treatment achieved a level of 34.29 µm. According to that, the decrease in the size of the particles by acoustic cavitation was beneficial. The statistical analysis resulted in significant differences between samples before maturation (C and CU), so based on that it may be said that the

iota carrageenan (I and IU) the range of peaks was different in comparison to the previously mentioned samples.

3.3. Analysis of Rheological Properties of Ice Cream Mixes

The relation between shear rate and apparent viscosity can be described by different rheological models (Ostwald de Waele, Bingham, Power Law, Herschel–Buckley). Data obtained from the ice cream mixes (before and after maturation) showed that the lowest values of the chi-square (from 15 to 22,143) and the highest values of the correlation coefficient (from 0.9981 to 1) were observed for the Herschel–Buckley model. For this reason, this model was selected to characterise the flow behaviour of ice cream mix samples.

The increase in consistency K was observed for ice cream samples with the addition of stabilisers in comparison to control samples (Figure 4a). However, the consistency values of samples prepared with iota carrageenan (I, IU) were four- to seven-fold higher than those observed for ice cream mixes obtained with the addition of other stabilisers. The effect of ultrasound treatment on the consistency index was only significant for ice cream mixes with the enzymatic β-galactosidase hydrolysates of iota carrageenan (B, BU) and iota carrageenan (I, IU). The consistency index of these samples decreased after ultrasound treatment. The consistency and viscosity of ice cream mixes with iota carrageenan without ultrasound treatment (I) increased after 24 h of maturation. A similar effect was observed for the ice mix, BU, after maturation. Additionally, the high-value standard deviation and coefficient of variation (15%) of the K parameter may indicate that the sample with iota carrageenan after maturation was less homogenous than other mixes, which was also visible on the images (Figure 4—IU b). The consistency index did not differ for other ice cream mixes after maturation.

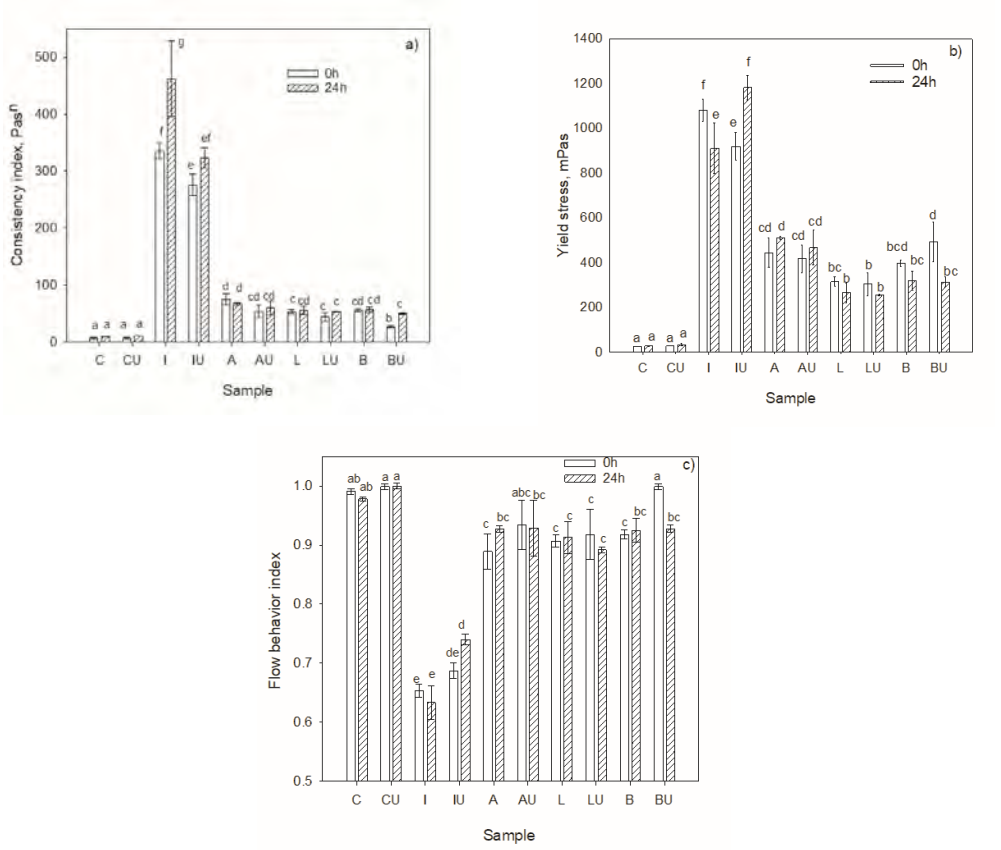


Figure 4. The parameters of the Herschel–Buckley model of the ice cream mix before and after maturation (for 24 h; at 4 °C): (**a**) the consistency index, (**b**) yield stress, (**c**) the flow behaviour index. The different superscript letters represent significant differences in the means of the same parameter ($p < 0.05$).

3.4. The Microstructure of Ice Cream Mixes

To support previous analysis and results, microscopic photos were also taken. The microscopic analysis is presented in Figure 5 with regard to the maturation process.

3.4. The Microstructure of Ice Cream Mixes

To support previous analysis and results, microscopic photos were also taken. The microscopic analysis is presented in Figure 5 with regard to the maturation process.

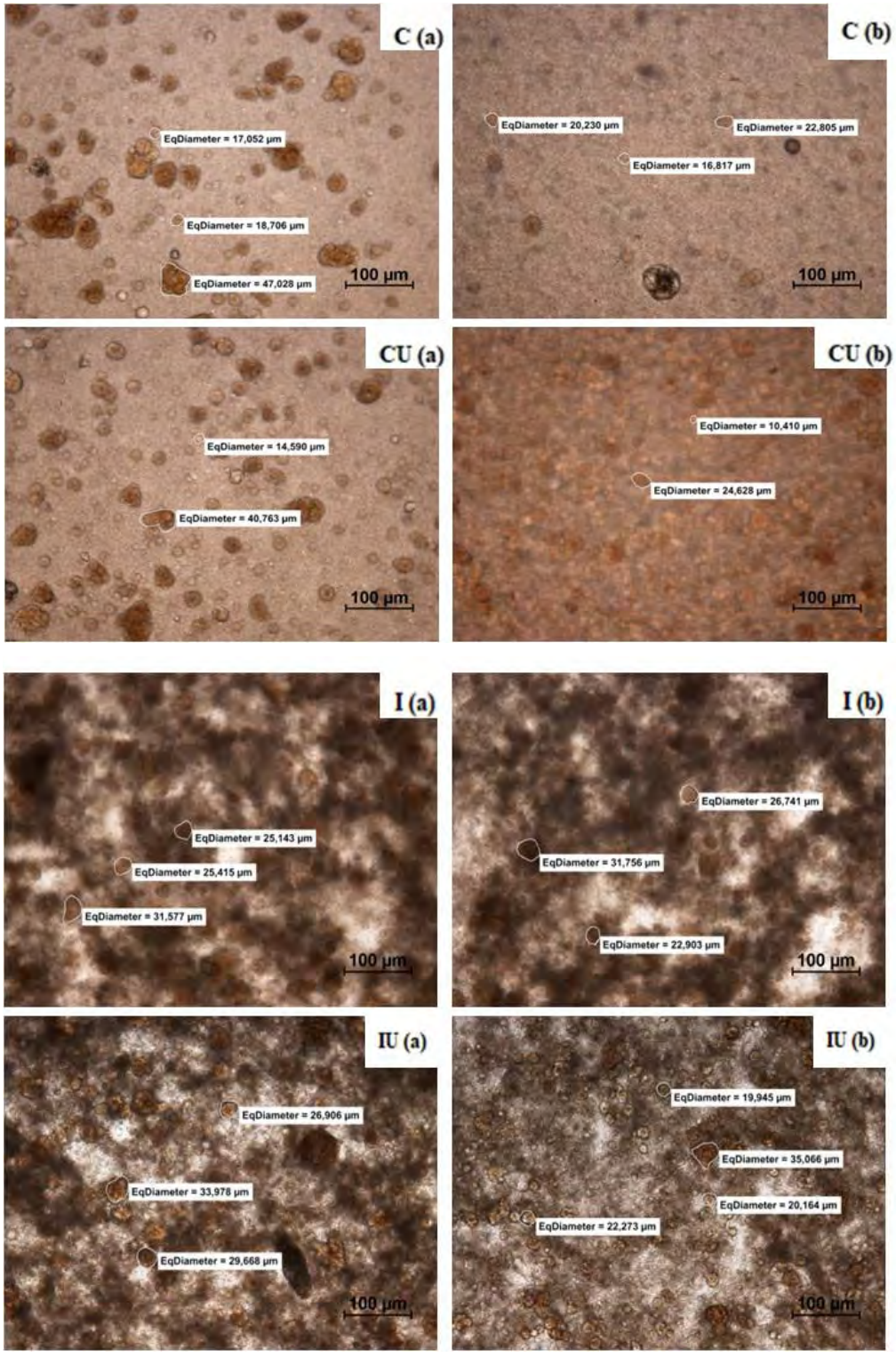


Figure 5. *Cont.*

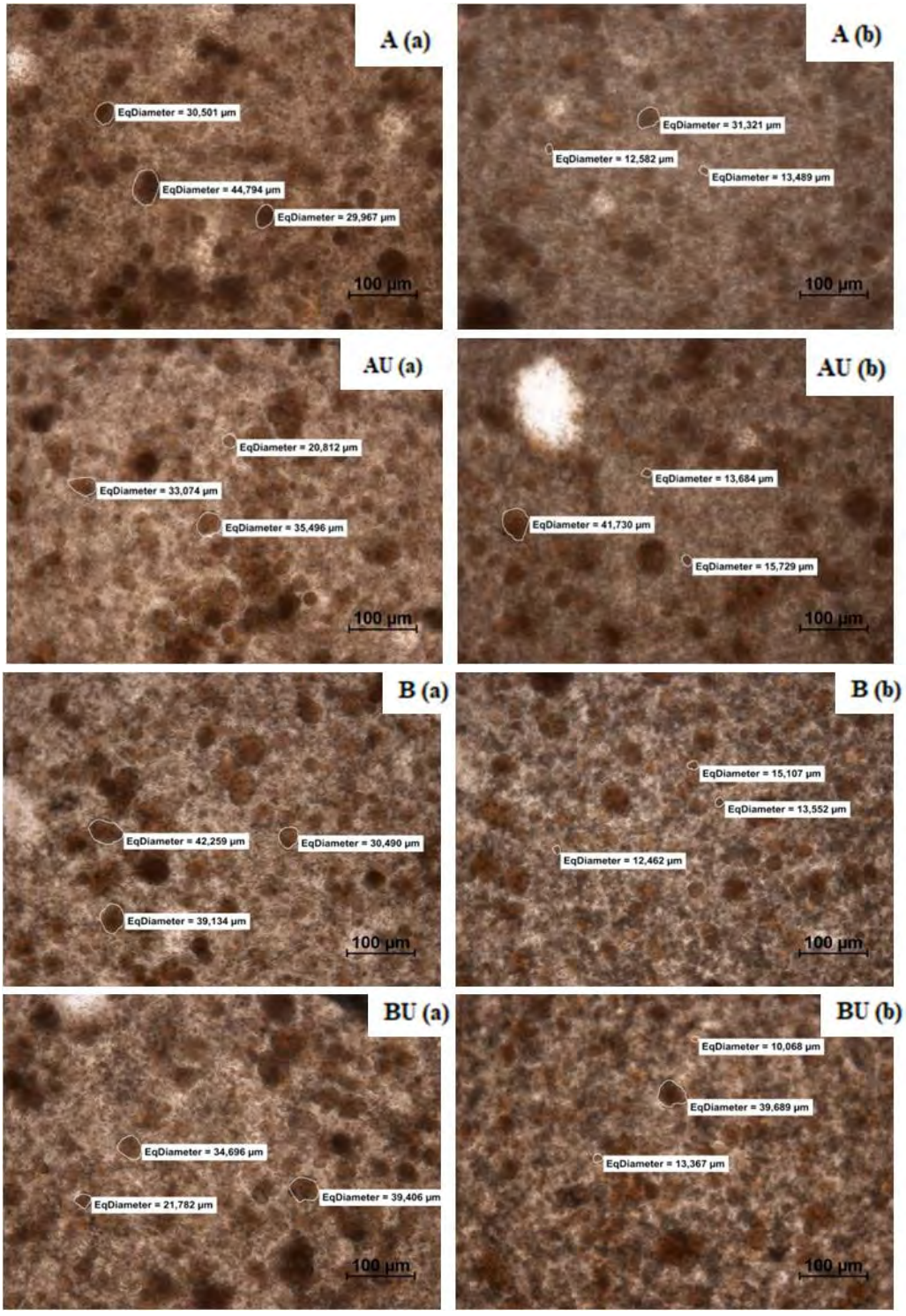


Figure 5. *Cont.*

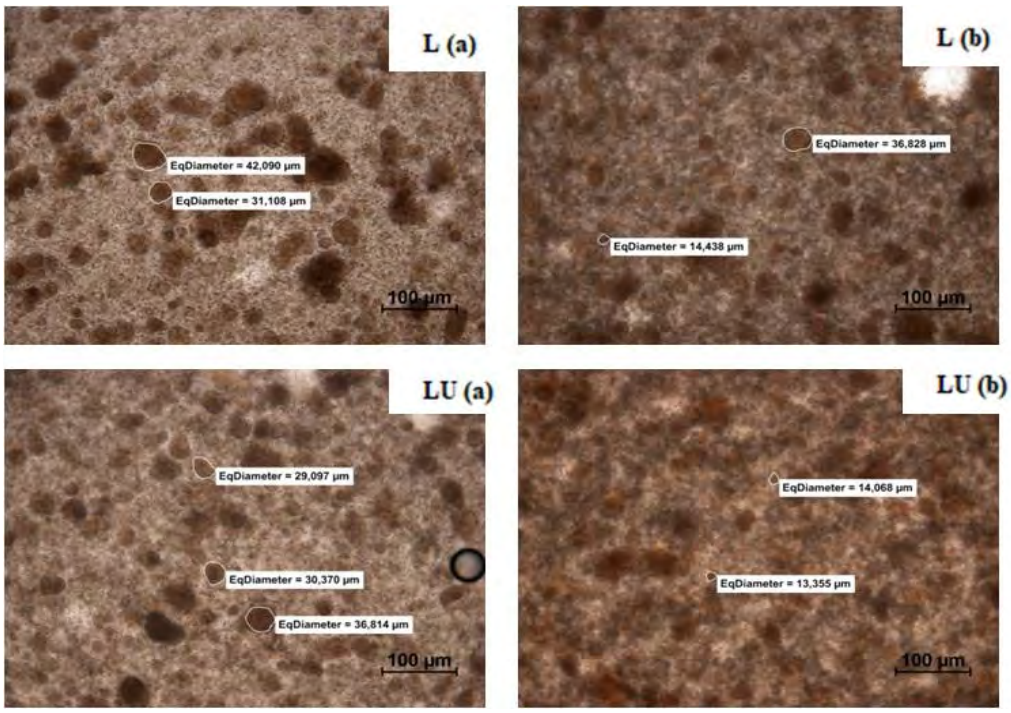


Figure 5. The microscopy analysis of ice cream mixes before (**a**) and after maturation (**b**) (for 24 h; at 4 °C).

Figure 5. The microscopy analysis of ice cream mixes before (**a**) and after maturation (**b**) (for 24 h; at 4 °C).

Overall, the ice cream mixes were described by an irregular distribution of fat particles and the agglomeration of particles were visible; however, the images prove that both controls with and without ultrasound treatment look different from samples with the addition of the stabiliser. As already pointed out, those samples also present the highest stability with behaviour similar to Newtonian fluids. Taking into consideration the ultrasound treatment in ice cream mixes, a reduction in particles and different orders in the structure were observed.

Overall, the ice cream mixes were described by an irregular distribution of fat particles and the agglomeration of particles were visible; however, the images prove that both controls with and without ultrasound treatment look different from samples with the addition of the stabiliser. As already pointed out, those samples also present the highest stability with behaviour similar to Newtonian fluids. Taking into consideration the ultrasound treatment in ice cream mixes, a reduction in particles and different orders in the structure were observed.

For instance, in the control sample before the maturation with and without ultrasound homogenisation, the changes were visible. In the sample before maturation creating the double emulsion was noticed. Before the maturation, the particles were surrounded by bigger agglomerates (Figure 5). After the maturation, the number of agglomerates decreased in comparison to single smaller particles which also was confirmed by the TSI value (Figure 1). This is owing to the fact that the control sample characterised the lowest TSI value and the same better stability of the emulsion. Additionally, in the sample CU (Tables 1 and 2) after the maturation, the D_{50} was significantly smaller than before the maturation step.

For instance, in the control sample before the maturation with and without ultrasound homogenisation, the changes were visible. In the sample before maturation creating the double emulsion was noticed. Before the maturation, the particles were surrounded by bigger agglomerates (Figure 5). After the maturation, the number of agglomerates decreased in comparison to single smaller particles which also was confirmed by the TSI value (Figure 1). This is owing to the fact that the control sample characterised the lowest TSI value and the same better stability of the emulsion. Additionally, in the sample CU (Tables 1 and 2) after the maturation, the D_{50} was significantly smaller than before the maturation step.

Additionally, the addition of stabilisers affected the structure of ice cream mixes. After microscopy analysis, we can suppose that all undesirable changes occurred. Looking at samples before maturation with iota carrageenan or with its hydrolysates in comparison to the control sample, no matter of ultrasound treatment, the distinctive conformation of particles was noticed. For samples I and IU (Tables 1 and 2) before and after the maturation time, the size of particles did not change significantly, however, the sample IU stood out among more uniform structures than other samples. According to the Back Scattering (Figure 2a,b), the destabilisation of samples occurred which can be explained by agglomerates in all mentioned samples. In samples with the addition of hydrolysates of iota carrageenan, the reduction in particles was observed no matter the ultrasound treatment. The particles before the maturation were characterised by bigger size which was confirmed by median D_{50} (Table 2). After maturation, a significant size reduction was observed in the presented photos which also was proved by the D_{50} parameter. Moreover, the bigger

Additionally, the addition of stabilisers affected the structure of ice cream mixes. After microscopy analysis, we can suppose that all undesirable changes occurred. Looking at samples before maturation with iota carrageenan or with its hydrolysates in comparison to the control sample, no matter of ultrasound treatment, the distinctive conformation of particles was noticed. For samples I and IU (Tables 1 and 2) before and after the maturation time, the size of particles did not change significantly, however, the sample IU stood out among more uniform structures than other samples. According to the Back Scattering (Figure 2a,b), the destabilisation of samples occurred which can be explained by agglomerates in all mentioned samples. In samples with the addition of hydrolysates of iota carrageenan, the reduction in particles was observed no matter the ultrasound treatment. The particles before the maturation were characterised by bigger size which was confirmed by median D_{50} (Table 2). After maturation, a significant size reduction was observed in the presented photos which also was proved by the D_{50} parameter. Moreover, the bigger

particles were observed based on the microscopic analysis after the maturation, but it can be the effect of destabilisation according to the Back Scattering (Figure 2a,b). We can also suppose based on the images that flocculation and aggregation occurred.

4. Discussion

4.1. Analysis of the Stability of Ice Cream Mixes

Based on the TSI value, it may be concluded that the effect of ultrasound cavitation declined the stability of ice cream mixes. In the research of Kot et al. [17], the homogenisation process also contributed to exacerbating the stability of vegan ice cream mixes. The TSI value for samples after homogenisation treatment ranged from 3.0 to 6.5. According to the results in the presented paper, the TSI value for samples after the ultrasound homogenisation did not achieve more than 4.2. Based on that, it may be deduced that ultrasound homogenisation is a more beneficial method for the stability of ice cream mixes than traditional homogenisation owing to the fact that the air bubbles which occur during the homogenisation may be the reason for decreasing the stability of the emulsion in the ice cream mix. According to the research of O'Sullivan et al. [18], the ultrasound treatment contributed to enhancing the stability of the emulsion prepared based on the pea protein isolate. Additionally, an improvement in the stability of the emulsion contributed to the improvement in the interfacial layer after ultrasonic irradiation. The authors also concluded that not only did the ultrasound treatment influence this parameter, but it also affected the time of treatment, before or after emulsification. So, based on the mentioned examples, it may be inferred that the influence of ultrasound on stability depends on various factors. Moreover, in the event of ice cream mixes, which is the multiphase product, the choice of parameters in this method may be more difficult and also hard to control.

As was mentioned in the part of the results, two different phenomena of destabilisation have occurred: coalescence and sedimentation. Coalescence often occurs due to particle size increases, while sedimentation is explained as the migration of particles [19]. For instance, in the research by Voronin et al. [20] milk ice cream mixes, after high-pressure jet processing, were prone to creaming and rapid separation was visible. On the other hand, in the work by Aslan and Dogan [16], it was found that ultrasound treatment was resistant to the coalescence of the emulsion owing to the fact that the smaller droplet size, created by ultrasound, was associated with the creaming index. The emulsion was also more stable when the creaming index was low.

4.2. An Analysis of Particle Sizes of Ice Cream Mixes

In the result part, it was highlighted that the size of particles was decreased. Additionally, in the study by O'Sullivan et al. [21], a pivotal reduction in particle size in the emulsion, based on the pea protein isolate with the ultrasound treatment, was observed. This phenomenon was attributed to the disruption of non-covalent associative forces, such as electrostatic and hydrophobic interactions and hydrogen bonding. Such mentioned forces maintained aggregates of protein in the solution through high levels of hydrodynamic shear and turbulences during ultrasonic cavitations. On the other hand, in comparison to the research of Kot et al. [17], where traditional homogenisation was used in vegan ice cream mixes, the reduction in the particle size, at the lowest value of D_{50} after maturation, was 6.33 µm. In the presented study, the lowest particle size for samples after the ultrasound treatment was 9.76 µm. Furthermore, in the study by Sert and Mercan [22], a different sort of homogenisation was used—high-pressure homogenisation, for preparing the sheep milk ice cream mix. The higher the pressure of homogenisation the lower and more satisfying the particle size achieved. The lowest D_{50} value was at a level of 6.49 µm. Based on the mentioned studies, ultrasound homogenisation did not reduce the particle size significantly, like other sorts of homogenisation. However, the sizes of the particles in the ice cream mix do not play a crucial role in forming a crystal structure. The uniform structure of the prepared emulsion is considered more important [14]. Nonetheless, it must not be forgotten that ultrasound is much more economical than conventional high-pressure

homogenisation or other mixing methods. Ultrasound is believed to be energy-saving and at the same time, offers more flexibility in its implementation [23].

The changes in the size and distribution of particles, in this step of preparing ice cream, may contribute to creating a more or less desirable structure of ice crystals in the final product. In comparison to other research, for instance, in the research conducted by Warren and Hartel [24], a bimodal and a trimodal distribution of particle size were observed in the ice-cream mix. Such distribution may have reflected increased partial coalescence of fat droplets in ice cream mixes. In our research, the phenomena of coalescence also were noted, which may be connected with increasing diversity in particle distribution among ice cream mix samples. Moreover, in the study by Huppertz et al. [25], it was noted that the ice cream mix had a comparable particle size distribution, after high-pressure homogenisation. In the case of our study, ultrasound homogenisation contributed to more diversity in ice cream samples.

4.3. An Analysis of Rheological Properties of Ice Cream Mixes

The Hershey–Buckley model, which was used to describe the rheological properties in ice cream mixes, was used to describe the ice cream mixes obtained with different methods of homogenisation [17,26]. According to the obtained results, the higher values of yield stress may indicate the presence of firmer structures [27]. The addition of stabilisers to mixes caused a significant increase in yield stress.

The flow behaviour index characterises the type of fluids. The samples with an n value close to 1 show behaviour similar to Newtonian fluids. The smaller values of n (lower than 1) indicate a departure from Newtonian behaviour [28]. All ice cream mixes, with the addition of stabilisers, showed non-Newtonian shear-thinning (pseudoplastic) behaviour. After maturation, the low behaviour values index did not vary for the same type of ice cream mix with the exception of the BU sample. Moreover, the effect of ultrasound treatment on the n index was not significant for most samples. The differences between the two variants of control and the stabilized samples are also visible in the images (Figure 4).

4.4. The Microstructure of Ice Cream Mixes

Microscopic analysis was immensely pivotal to seeing changes in fat globules and the sort of destabilisation. Voronin et al. [20], proved that the destabilisation of fat aggregates was visible in the milk ice cream mixes. Overall, it may be concluded that maturation as a step in ice cream production displays a considerably pivotal role in creating the structure of ice cream mixes. Moreover, the reduction in the size of the particles was achieved, which may also have influenced conformation after maturation. According to research by Ahn et al. [29], the observation of the microstructure of an emulsion can provide a significant clue to the understanding of the structure of the emulsion and its relationship with stability. In this research, it was noticed that the interactions with proteins and phospholipids can modulate the stability of the emulsion, which may later contribute to functionality, such as the digestibility of dairy-based emulsions. Furthermore, according to the previous studies [30,31], the way of preparation but also the composition (including the chosen: stabilisers iota carrageenan and its hydrolysates) had concurrently a great impact on forming ice crystals structure of ice cream.

5. Conclusions

In the presented study, ultrasound homogenisation contributed to a decrease in the stability of ice cream mixes. The samples after the ultrasound treatment had a higher value of TSI. The elevated value of TSI was noted for sample IU (with the addition of iota carrageenan) and BU (hydrolysate of iota carrageenan) and was at a level of 4. The addition of stabilisers in ice cream mixes improved stability during maturation. Moreover, the ultrasound treatment in ice cream mixes reduced the particle size; however, it was visible after maturation. Additionally, the combination of ultrasound and the use of stabilisers gave better results in reducing the size of the particle, than only using stabilisers and

the smallest particles were noticed in the BU sample—9.76 µm. The effect of ultrasound treatment on the consistency index was only significant for ice cream mixes IU and BU and the consistency index of these samples decreased after ultrasound treatment. Based on the microscopic photos, the agglomeration of fats and considerable diversification was visible in all ice cream mixes. While analysing the microstructure, the effect of ultrasound treatment was not noticeable for most samples and only in the IU sample, after maturation, were the differences visible.

Due to the specific mechanism of ultrasound homogenisation, it may be contemplated as an interesting tool for ice cream applications. This method can be effectively used for reducing the size of particles. Moreover, it may be used in improving the stability of the emulsion in comparison to the traditional form of homogenisation. This method has the potential in enhancing the quality and performance of vegan ice cream mixes. However, although a plethora of advantages to using this method in production are acknowledged, wide-ranging research is still required to further industrial applicability.

Author Contributions: Conceptualization, A.K. and A.K.-D.; methodology, A.K. and E.J.; software, A.K.; validation, A.K., A.K.-D., E.J.; formal analysis, A.K. and A.K.-D.; investigation, A.K., A.K.-D. and E.J.; resources, E.J.; data curation, A.K., A.K.-D. and E.J.; writing—original draft preparation, A.K., A.K.-D. and E.J.; writing—review and editing, A.K., A.K.-D. and E.J.; visualization, A.K.; supervision, E.J.; project administration, A.K.-D. All authors have read and agreed to the published version of the manuscript.

Funding: The research equipment purchased as part of the "Food and Nutrition Centre—modernisation of the WULS campus to create a Food and Nutrition Research and Development Centre (C·Zi·Z)" was co-financed by the European Union from the European Regional Development Fund under the Regional Operational Programme of the Mazowieckie Voivodeship for 2014–2020 (Project No. RPMA. 01.01.00-14-8276/17).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Fiol, C.; Prado, D.; Romero, C.; Laburu, N.; Mora, M.; Alava, I.J. Introduction of a new family of ice creams. *Int. J. Gastron. Food Sci.* **2017**, *7*, 5–10. [CrossRef]
2. Biasutti, M.; Venir, E.; Marino, M.; Maifreni, M.; Innocente, N. Effects of high pressure homogenisation of ice cream mix on the physical and structural properties of ice cream. *Int. Dairy J.* **2013**, *32*, 40–45. [CrossRef]
3. Lomolino, G.; Zannoni, S.; Zabara, A.; Da Lio, M.; De Iseppi, A. Ice recrystallization and melting in ice cream with different proteins levels and subjected to thermal fluctuation. *Int. Dairy. J.* **2020**, *100*, 104557. [CrossRef]
4. Goff, H.D. Colloidal aspects of ice cream. A review. *Int. Dairy J.* **1997**, *7*, 363–373. [CrossRef]
5. Mesa, J.; Hinestroza-Córdoba, L.I.; Barrera, C.; Seguí, L.; Betoret, E.; Betoret, N. High homogenization pressures to improve food quality, functionality and sustainability. *Molecules* **2020**, *25*, 3305. [CrossRef]
6. Carrillo-Lopez, L.M.; Garcia-Galicia, I.A.; Tirado-Gallegos, J.M.; Sanchez-Vega, R.; Huerta-Jimenez, M.; Ashokkumar, M.; Alarcon-Rojo, A.D. Recent advances in the application of ultrasound in dairy products: Effect on functional, physical, chemical, microbiological and sensory properties. *Ultrason. Sonochem.* **2021**, *73*, 105467. [CrossRef]
7. Laborde, J.-L.; Bouyer, C.; Caltagirone, J.-P.; Gerard, A. Acoustic bubble cavitation at low frequencies. *Ultrasonics* **1998**, *36*, 589–594. [CrossRef]
8. Chemat, F.; Zill-e-Huma; Khan, K.M. Application of ultrasound in food technology: Processing, preservation and extraction. *Ultrason. Sonochem.* **2011**, *18*, 813–835. [CrossRef]
9. Akdeniz, V.; Akalin Sibel, A. New approach for yoghurt and ice cream production: High-intensity ultrasound. *Trends Food Sci. Technol.* **2019**, *86*, 392–398. [CrossRef]
10. Gallo, M.; Ferrara, L.; Naviglio, D. Application of Ultrasound in Food Science and Technology: A perspective. *Foods* **2018**, *7*, 164. [CrossRef]
11. Arzeni, C.; Martinez, K.; Zema, P.; Arias, A.; Perez, O.E.; Pilsof, A.M.R. Comparative study of high intensity of ultrasound effects on food proteins functionality. *J. Food Eng.* **2012**, *108*, 463–472. [CrossRef]

12. Bhargava, N.; Mor, R.S.; Kumar, K.; Sharanagat, V.S. Advances in application of ultrasound in food processing: A review. *Ultrason. Sonochem.* **2021**, *70*, 105293. [CrossRef]
13. Hii, C.L.; Ong, S.P.; Yap, J.Y.; Putranto, A.; Mangindaan, D. Hybrid drying of food and bioproducts: A review. *Dry. Technol.* **2021**, *39*, 1554–1576. [CrossRef]
14. Firouz, M.S. Application of high-intensity ultrasound in food processing for improvement of food quality. In *Design and Optimization of Innovative Food Processing Techniques Assisted by Ultrasound. Developing Healthier and Sustainable Food Products*; Barba, F., Cravotto, G., Chemat, F., Lorenzo Rodriguez, J.M., Sichetti Munekata, P.E., Eds.; Elsevier Inc.: Amsterdam, The Netherlands, 2021; pp. 155–159. [CrossRef]
15. Firouz, M.S.; Farahmandi, A.; Hosseinpour, S. Recent advances in ultrasound application as a novel technique in analysis, processing and quality control of fruits, juices and dairy products industries: A review. *Ultrason. Sonochem.* **2019**, *57*, 73–88. [CrossRef]
16. Aslan, D.; Dogan, M. The influence of ultrasound on the stability of dairy-based, emulsifier-free emulsions: Rheological and morphological aspect. *Eur. Food Res. Technol.* **2017**, *244*, 409–421. [CrossRef]
17. Kot, A.; Kamińska-Dwórznicka, A.; Galus, S.; Jakubczyk, E. Effects of different ingredients and stabilisers on properties of mixes based on almond drink for vegan ice cream production. *Sustainability* **2021**, *13*, 12113. [CrossRef]
18. O'Sullivan, J.; Beevers, J.; Park, M.; Greenwood, R.; Norton, I. Comparative assessment of the effect of ultrasound treatment on protein functionality pre- and post-emulsification. *Colloids Surf. A Physicochem. Eng. Asp.* **2015**, *484*, 89–98. [CrossRef]
19. Mengual, O.; Meunier, G.; Cayre, I.; Puech, K.; Snabre, P. Characterisation of instability of concentrated dispersion by a new optical analyser: The TURBISCAN MA 1000. *Colloids Surf. A Physicochem. Eng. Asp.* **1999**, *152*, 111–123. [CrossRef]
20. Voronin, G.L.; Roberts, R.; Felix, T.L.; Coupland, J.N.; Harte, F.M. Effect of high-pressure-jest processing on the physicochemical properties of low-fat ice cream mix. *J. Dairy Sci.* **2020**, *103*, 6003–6014. [CrossRef]
21. O'Sullivan, J.; Murray, B.; Flynn, C.R.; Norton, I. The effect of ultrasound on the structural, physical and emulsifying properties of animal and vegetable proteins. *Food Hydrocoll.* **2016**, *53*, 141–154. [CrossRef]
22. Sert, D.; Mercan, E. High-pressure homogenisation of sheep milk ice cream mix: Physicochemical and microbiological characterization. *LWT* **2021**, *151*, 1124148. [CrossRef]
23. Zhang, R.; Luo, L.; Yang, Z.; Ashokkumar, M.; Hemar, Y. Formation by high ultrasound of aggregated emulsions stabilised with milk protein concentrate (MPC70). *Ultrason. Sonochem.* **2021**, *81*, 105852. [CrossRef]
24. Warren, M.M.; Hartel, R.W. Effects of emulsifier, overrun and dasher speed on ice cream microstructure and melting properties. *J. Food Sci.* **2018**, *83*, 639–647. [CrossRef]
25. Huppertz, T.; Smiddy, M.A.; Goff, H.D.; Kelly, A.L. Effects of high pressure treatment of mix on ice cream manufacture. *Intern. Dairy J.* **2011**, *21*, 718–726. [CrossRef]
26. Innocente, N.; Biasutti, M.; Venir, E.; Spaziani, M.; Marchesini, G. Effect of high-pressure homogenization on droplet size distribution and rheological properties of ice cream mixes. *J. Dairy Sci.* **2009**, *92*, 1864–1875. [CrossRef]
27. Rao, M.A. *Rheology of Fluid And Semisolid Foods: Principles and Applications*; Springer Science & Business Media: New York, NY, USA, 2007. [CrossRef]
28. Mostafavi, F.S.; Tehrani, M.M.; Mohebbi, M. Rheological and sensory properties of fat reduced vanilla ice creams containing milk protein concentrate (MPC). *J. Food Meas. Charact.* **2017**, *11*, 567–575. [CrossRef]
29. Ahn, N.; Park, J.; Chai, C.; Imm, J. The interaction of milk sphingomyelin and proteins on stability and microstructure of dairy emulsions. *J. Dairy Sci.* **2022**, *105*, 3832–3845. [CrossRef]
30. Kamińska-Dwórznicka, A.; Łaba, S.; Jakubczyk, E. The effects of selected stabilizers addition on physical properties and changes in crystal structure of whey ice cream. *LWT* **2022**, *154*, 112841. [CrossRef]
31. Kot, A.; Kamińska-Dwórznicka, A.; Antczak, A.; Jakubczyk, E.; Matwijczuk, A. Effect of ι-carrageenan and its acidic and enzymatic hydrolysates on ice crystal structure changes in model sucrose solution. *Colloids Surf. A Physicochem. Eng. Asp.* **2022**, *643*, 128744. [CrossRef]

Check for updates

Received: 27 March 2023 | Revised: 7 August 2023 | Accepted: 20 September 2023

DOI: 10.1111/jfpe.14472

ORIGINAL ARTICLE

Journal of Food Process Engineering WILEY

The influence of ultrasound homogenization on recrystallization during the storage of vegan ice cream

Anna Kot | Anna Kamińska-Dwórznicka

Department of Food Engineering and Process Management, Institute of Food Sciences, Warsaw University of Life Sciences (WULS-SGGW), Warsaw, Poland

Correspondence
Anna Kot, Department of Food Engineering and Process Management, Institute of Food Sciences, Warsaw University of Life Sciences (WULS-SGGW), Nowoursynowska 159C, Warsaw 02-776, Poland.
Email: anna_kot1@sggw.edu.pl

Funding information
Regional Operational Programme of the Mazowieckie Voivodeship for 2014-2020, Grant/Award Number: RPMA.01.01.00-14-8276/17

Abstract

This article investigated the influence of ultrasound homogenization and stabilizers (iota carrageenan and its hydrolysates) in the production of vegan ice cream. The cryoscopic temperature, osmotic pressure, overrun, melting time, and microstructure analysis were investigated. Vegan ice cream were stored at −18°C and the recrystallization process was analyzed based on images taken after 24 h, 1 month, and 3 months. According to the results, it was noted that ultrasound homogenization contributed to obtaining smaller ice crystals in ice cream, in comparison to the samples after traditional homogenization. The average diameter of ice crystals in the sample US pretreated, after 3 months of storage was less than 21 μm. The overrun value ranged from 9.22% to 32.36% and the melting time for samples did not exceed 34 min. The analysis of the cryoscopic temperature and osmotic pressure showed that ultrasound contributed to increasing the cryoscopic temperature and lowering the osmotic pressure.

Practical Applications

The presented study shows an alternative to dairy products for the vegan ice cream market. Changing mechanical homogenization to ultrasound homogenization in vegan ice cream production might be an effective step for ice cream producers. Owing to the fact that the use of ultrasound during production may reduce the impact of using stabilizers on the recipe of vegan ice cream. Finally, such changes will not result in additional steps in ice cream production which may not generate additional costs.

KEYWORDS
crystal structure, recrystallization, ultrasound homogenization, vegan ice cream

1 | INTRODUCTION

Based on current knowledge, ice cream is one of the most popular items within the worldwide food sector and this sector is growing fast. The term ice cream is connected with a complex colloidal system consisting of air cells, ice crystals, and fat droplets which are dispersed into the serum phase (Clarke, 2004; Goff & Hartel, 2013; Góral et al., 2018). Furthermore, vegan ice cream refers to a frozen and aerated mixture based on plant protein, vegetable fat, and additions such as stabilizers or emulsifiers, without any animal-based ingredients. Interest in such frozen dessert is rapidly growing over the last few years (Kot & Kamińska-Dwórznicka, 2022). The implementation of vegan ice cream in our diet is related to a plethora of reasons such as health, ethics, and ecology. First and foremost, the combination of lifestyle-related health restrictions, such as lactose intolerance or a cow's milk allergy, contributed to limiting the milk products and substituting it with a vegan one in daily life. Therefore, it has given rise to new foods and products on the market (Aboulfazli et al., 2016;

 © 2023 Wiley Periodicals LLC.

Cadena et al., 2012; Vanga & Raghavan, 2018). Moreover, the majority of consumers are adopting and successfully adhere to vegan or vegetarian diets. Last but not least, it should not be forgotten that people's awareness of ecology is usually responsible for their food choices. It is known that a plant-based diet is essential to counteract climate change. Due to this fact, the exclusion of a meat-based diet can contribute to the promotion of a more sustainable diet by using natural resources. Taking all these points into consideration there exists a need to produce vegan-labeled ice creams that will measure up to such expectations (Alvaro, 2017; Krizanova et al., 2021; Williams et al., 2023).

In frozen foods, such as ice cream, any changes in the physical state of water cause undesirable changes in stability, structure, and texture during distribution or storage. Such modifications pose a problem that results in a quality loss at the final part of the cold chain due to recrystallization phenomena (Ndoye & Alvarez, 2015; Pham & Mawson, 1997). The recrystallization phenomenon is a process related to the changes in characteristics of ice crystals such as shape, number, size, or orientation. The diffusing unfrozen water from the serum phase to the crystal structure contributes to the enhancement of the growth of crystals, and, consequently, to recrystallization. This process occurs because of two mechanisms: accretion and migration. The first one is connected by joining together two or more crystals to form single and larger crystals. This process requires the close proximity of crystals. The second one, migration, is based on the melting process which yields the movement of the melted liquid to the surface of larger crystals. This mechanism is triggered by temperature (Adapa et al., 2000; Goff & Hartel, 2013; Hartel, 1988; Zhu et al., 2019). Taking all this into consideration, it is advisable to obtain ice crystals between 10 to 20 μm to achieve a favorable texture, on grounds that ice crystals larger than 50 μm make the quality of ice cream coarse or grainy (Gaukel et al., 2014; Kamińska-Dwórznicka et al., 2019; Smith et al., 2000). Additionally, the recrystallization process is also related to the capacity of stabilizers to form cryogens or entangled networks. In accordance with gel firmness, it is associated with the possibility of inhibiting ice crystal growth and changing the morphology (Blond, 1988; Regand & Goff, 2002). One effective and commonly known way to prevent the recrystallization process is the use of stabilizers. Accordingly, to alter the behavior of water, stabilizers are able to modify water-binding capacity, freezing rates, rheological properties, and ice crystal formation (Adapa et al., 2000).

However, the need for finding other methods to prevent the recrystallization process is driven by non-thermal technologies. Such technologies may reduce the number of ingredients in the recipe of ice cream or obtain eligible results in the final product by creating a combination of stabilizers. Nonetheless, it should be pointed out that the ingredients in ice cream and their correlations are able to determine the final nature of ice cream. According to this, changes in the production process may be related to preparing the ice cream mix even in the process of freezing. That is the main reason the presented article focuses on using ultrasound homogenization instead of the traditional one (Carrillo-Lopez et al., 2021).

Overall, the ultrasound technique is an up-and-coming technology due to the fact that it is relatively cheap, quite simple, fast, non-toxic, and energy-saving. Moreover, based on current trends, it is vital to use green technologies, which may provide safe food and increase the effectiveness or efficiency of conventional technologies (Akdeniz & Akalin, 2019; Chemat & Khan, 2011). Ultrasound homogenization is an innovative method of producing an emulsion, also the ice cream mix emulsion. First and foremost, using ultrasound homogenization may contribute to narrowing particle size distribution and improving stability. The fact that forces such as turbulence, mixing, and shear are generated during acoustic cavitation that disrupt fat globules and simultaneously reduce their sizes. Additionally, ultrasound homogenization presents lower investment costs and ease of cleaning in comparison to traditional homogenization (Akdeniz & Akalin, 2019; Firouz, 2021). According to this, the appropriate amount of fat and its structure may affect the ice crystals' size, creating less space for crystal formation and smaller ice crystals will be obtained (Adapa et al., 2000). Consequently, it would contribute to inhibiting the recrystallization phenomena in ice cream, obtaining smaller crystals, and thus improve the quality of the product.

The new knowledge presented in this article is a step forward to creating the most advantageous method to prevent the recrystallization process. Owing to the fact that little is known about the influence of ultrasound homogenization on the ice crystal structure, especially in vegan ice cream, the objective of this article was to evaluate the impact of ultrasound homogenization in comparison to the traditional method and the addition of stabilizers such as iota carrageenan and its hydrolysates on the physical properties and crystal structure of vegan ice cream.

2 | MATERIALS AND METHODS

2.1 | The preparation of the hydrolysates of iota carrageenan

How the hydrolysis of iota carrageenan was conducted was described in this article by Kot, Kamińska-Dwórznicka, Antczak, et al. (2022). To perform acid hydrolysis, 0.1 M hydrochloric acid and 0.1 M sodium hydroxide were used. Three variants of acid hydrolysis were performed: 0, 1, and 3 h of acid hydrolysis. For enzymatic hydrolysis, two sorts of enzymes were applied: β-galactosidase and its cheaper and commercial equivalent—lactase. For β-galactosidase hydrolysis, the four variants of samples were prepared (2, 24, 48, and 72 h), while for lactase hydrolysis two variants (0 and 24 h of hydrolysis) were prepared. Molecular mass distribution was estimated through SEC (size-exclusion chromatography) analysis and only hydrolysates with the highest reduction of molecular mass were used for further analysis. Moreover, to confirm that the IRI (Ice Recrystallization Inhibition) activity of obtained hydrolysates of iota carrageenan depends on the functional group's position changes, FTIR (Fourier Transform Infrared Spectroscopy) analysis was performed. Based on these results only samples with the highest molecular mass reduction and the longest time of hydrolysis were used for further research as a stabilizer in ice cream.

17454530, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfpe.14472 by Warsaw University of Life Sciences, Wiley Online Library on [06/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

2.2 | The preparation of ice cream

2.2.1 | The materials for the recipe for ice cream

The ingredients used to prepare the ice cream mixes were: 66.5% roasted almond original drink (Enerbio, Rossmann, Hanover, Germany), 16% almond syrup (Monin, Bourges, France), 12% inulin (Orafti BENEO, Tienen, Belgium), 5% pea protein (Nuturalys S85 plus, Roquette, Lestrem, France), 0.4% emulsifier E471 (Fooding Shanghai, Shanghai, China), 0.08% LBG—Locust Bean Gum (Fooding Shanghai, Shanghai, China), 0.02% xanthan gum (Fooding Shanghai, Shanghai, China), 0.01% iota carrageenan (Fluka, Sigma-Aldrich, St. Louis, MI, USA) or 0.005% newly obtained: the acid hydrolysates of iota carrageenan and enzymatic hydrolysis by β-galactosidase and enzymatic hydrolysis by commercial lactase.

The characteristics of the prepared samples of ice cream mixes:

- **C**—the control sample (without stabilizers);
- **I**—the sample with stabilizers (the combination of iota carrageenan, LBG, and xanthan gum);
- **A**—the sample with stabilizers (the combination of acid hydrolysates of iota carrageenan, LBG, and xanthan gum);
- **B**—the sample with stabilizers (the combination of enzymatic β-galactosidase hydrolysates of iota carrageenan, LBG, and xanthan gum);
- **L**—the sample with stabilizers (the combination of enzymatic commercial lactase hydrolysates of iota carrageenan, LBG, and xanthan gum);
- **H**—the sample after traditional homogenization treatment;
- **U**—the sample after ultrasound homogenization treatment.

Each variant of ice cream mixes was prepared twice, per 2 L.

2.2.2 | The production of ice cream mixes

According to the recipe, dry and liquid ingredients were weighed separately. After this, all components were mixed using a Bosch MaxoMixx 750 W blender (Bosch, Gerlingen, Germany). Then, the pasteurization process was performed using a Vorwerk thermomixer used at a temperature of 85°C within 1.5 min and cooled to 25°C.

The two methods of homogenization were used:

- Traditional homogenization using the homogenizer IKA T 25 digital ULTRA-TURRAX 20 rpm (IKA®-Werke GmbH & Co. KG, Staufen, Germany) through 2.5 min.
- Ultrasound homogenization using a homogenizer Ultrasonic Liquid Processor VCX 500 (Sonics & Materials, Inc., Newtown, CT, USA) with a diameter probe (Model CV334). 250 mL of ice cream mixes for each trial. The frequency of 20 kHz and exposure time of 5 min was used. The used frequency of ultrasound was also tested in accordance with other papers such as this article by O'Sullivan et al. (2016) for the ultrasound homogenization on soy and wheat protein isolates and this article by da Silva et al. (2019) during the ultrasound homogenization of cupuaçu juice.

After the homogenization step, all prepared ice cream mixes were submitted to the maturation process for 24 h at 4°C (fridge, Whirlpool, Poland).

2.2.3 | The freezing of ice cream

The freezing of ice cream mixes was performed in an ice cream maker Neumaker Gelato 5 K SC (Hemer, Germany) until the ice cream temperature was −7°C around 15 min. Then the samples were placed in plastic containers and stored at −18°C for 24 h, 1 month, and 3 months (freezer, Whirlpool, Poland).

2.3 | The ice Cream's physical analysis

2.3.1 | Cryoscopic temperature and osmolality

The cryoscopic temperature and also osmolality of ice cream mixes were determined using an osmometer Marcel os3000 (Warsaw, Poland). The accuracy of measurement of the freezing temperature was 0.002°C and for osmolality, it was 1%. According to the instructions of the devices, 100 μL of ice cream mixes after the maturation process was put in Eppendorf tubes and measured until the device was stabilized. The analysis was performed in duplicate.

2.3.2 | Melting time

The melting behavior of ice cream was determined using a cooled metal ring (11 cm in height and 2 cm in diameter, volume 35 mL), stored at −25°C for 24 h before measurement. After this time, the ring was filled with ice cream directly after the freezing process and then stored for 24 h at −25°C. After storage, the ring was placed on the funnel with two pins located at the ends of the ring at a controlled temperature of 25°C. The first drop of melted ice cream was recorded as the melting time of the sample (Dłużewska et al., 2003; Góral et al., 2018). The analysis was performed in duplicate.

2.3.3 | Determination of the overrun

The overrun of ice cream was determined according to the following formula and was performed in duplicate (Dłużewska et al., 2003; Góral et al., 2018):

$$\text{Overrun} = \frac{W_1 - W_2}{W_2} \cdot 100\%, \quad (1)$$

where W_1—the mass of the unit volume of the mixture (g) and W_2—the mass of the unit volume of ice cream (g).

17454530, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfpe.14472 by Warsaw University of Life Sciences, Wiley Online Library on [06/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

2.3.4 | Microstructural analysis of ice crystals

The microstructure of ice crystals was analyzed based on the images taken after 24 h, 1 month, and 3 months of storage at −18°C. To prepare samples, a small amount of ice cream was taken from the center of the plastic box (from at least 3 different locations, and a minimum of 3 cm away from the surface), then put on a cool slide using a spatula and covered with a cool slip glass on the top of the sample. All samples were prepared in a freezing chamber and transferred to a microscope with the cooling system Linkam Scientific PE 94.

The recrystallization process was analyzed based on images taken using the Olympus model BX43F (Tokyo, Japan) microscope with a cooling system with liquid nitrogen—Linkam Scientific Instruments LTD model LNP96-S (Tokyo, Japan) and camera Olympus model SC50 (Tokyo, Japan). The obtained images were analyzed using the Olympus cellSens Dimension Desktop program. Around 300 ice crystals were marked for one sample, and then the area, equivalent diameter, and standard deviation were calculated. Analysis of the recrystallization process was performed in duplicate.

2.3.5 | Statistical analysis

For the melting time, overrun, cryoscopic temperature, and osmolality, a statistical analysis was performed. The data have expressed a mean with standard deviations (±SD) in Table 1. The results were analyzed using the analysis of variance (ANOVA). Tukey's test was used to determine if the differences between the parameters of the ice cream samples were significant. The statistical appraisal was performed using STATISTICA 13.3 software (Statsoft Polska, Kraków, Poland). The significance of the test was set at $\alpha = 0.05$.

The frequency distribution of the ice crystal size was figured using Microsoft Excel 2011 macro data analysis. The relative frequency of any class interval was calculated as the number of crystals in that class (class frequency) divided by the total number of crystals and expressed as a percentage (Figures 1–3). According to the method described by Regand & Goff, 2003, the parameter X_{50} was analyzed as the mean diameter (D_A) for 50% of the crystals in the sample. The mean diameter (D_A) and standard deviations (SD) of each class were also calculated (Table 2). The method has previously been described (Goff & Hartel, 2013; Kamińska-Dwórznicka et al., 2015; Kamińska-Dwórznicka et al., 2016; Kot et al., 2020).

3 | RESULTS AND DISCUSSION

3.1 | The cryoscopic temperature and osmotic pressure of ice cream mixes

The measurement of the cryoscopic temperature is one of the pivotal characteristics of ice cream mixes due to the fact that it determines the nature of cooling the water during freezing or later during the hardening and storage of ice cream (Adapa et al., 2000).

TABLE 1 The physical analysis of vegan ice cream.

Sample	Cryoscopic temperature, °C	Osmotic pressure, mOsm/kg	Melting time, min.	Overrun, %
C	−1.852 ± 0.042^{a}	997 ± 4^{efg}	26.24 ± 0.14^{bcde}	11.44 ± 0.30^{ab}
CH	−1.861 ± 0.012^{a}	1002 ± 7^{fg}	24.38 ± 1.50^{bcd}	12.06 ± 0.86^{ab}
CU	−1.834 ± 0.041^{a}	986 ± 4^{def}	29.54 ± 1.39^{de}	10.86 ± 2.49^{a}
I	−1.918 ± 0.115^{a}	969 ± 3^{c}	32.10 ± 1.45^{e}	32.36 ± 1.41^{e}
IH	−1.856 ± 0.005^{a}	999 ± 3^{fg}	20.18 ± 0.17^{ab}	8.15 ± 1.20^{a}
IU	−1.813 ± 0.029^{a}	976 ± 1^{cd}	24.36 ± 1.08^{bcd}	14.05 ± 1.28^{abc}
A	−1.824 ± 0.018^{a}	982 ± 3^{cde}	24.07 ± 1.86^{bcd}	22.13 ± 0.84^{cd}
AH	−1.858 ± 0.011^{a}	1000 ± 7^{fg}	16.19 ± 1.26^{a}	15.19 ± 0.05^{abcd}
AU	−1.839 ± 0.013^{a}	999 ± 4^{efg}	22.31 ± 1.23^{abc}	14.60 ± 2.97^{abc}
B	−1.804 ± 0.010^{a}	969 ± 4^{bc}	23.25 ± 1.41^{bcd}	32.21 ± 2.76^{e}
BH	−1.844 ± 0.027^{a}	993 ± 2^{efg}	15.37 ± 1.32^{a}	16.39 ± 3.31^{abcd}
BU	−1.771 ± 0.011^{a}	953 ± 7^{ab}	26.12 ± 3.80^{bcde}	19.55 ± 2.57^{bcd}
L	−1.894 ± 0.004^{a}	1020 ± 2^{h}	24.10 ± 0.14^{bcd}	23.09 ± 2.96^{d}
LH	−1.873 ± 0.011^{a}	1008 ± 6^{gh}	29.22 ± 3.14^{cde}	9.22 ± 3.13^{a}
LU	−1.757 ± 0.042^{a}	946 ± 3^{a}	33.05 ± 2.16^{e}	11.08 ± 0.59^{a}

Abbreviations: C, control sample (without stabilizers); I, sample with stabilizers (the combination of iota carrageenan, LBG, and xanthan gum); A, sample with stabilizers (the combination of acid hydrolysates of iota carrageenan, LBG, and xanthan gum); B, sample with stabilizers (the combination of enzymatic β-galactosidase hydrolysates of iota carrageenan, LBG, and xanthan gum); L, sample with stabilizers (the combination of enzymatic commercial lactase hydrolysates of iota carrageenan, LBG, and xanthan gum); H, after the traditional homogenization treatment, U, after the ultrasound homogenization treatment.

Note: Different superscript letters (a−h) in columns represent significant differences in the means of the same parameter ($p < 0.05$). Values represent means ± standard deviations.

17454530, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfpe.14472 by Warsaw University of Life Sciences, Wiley Online Library on [06/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

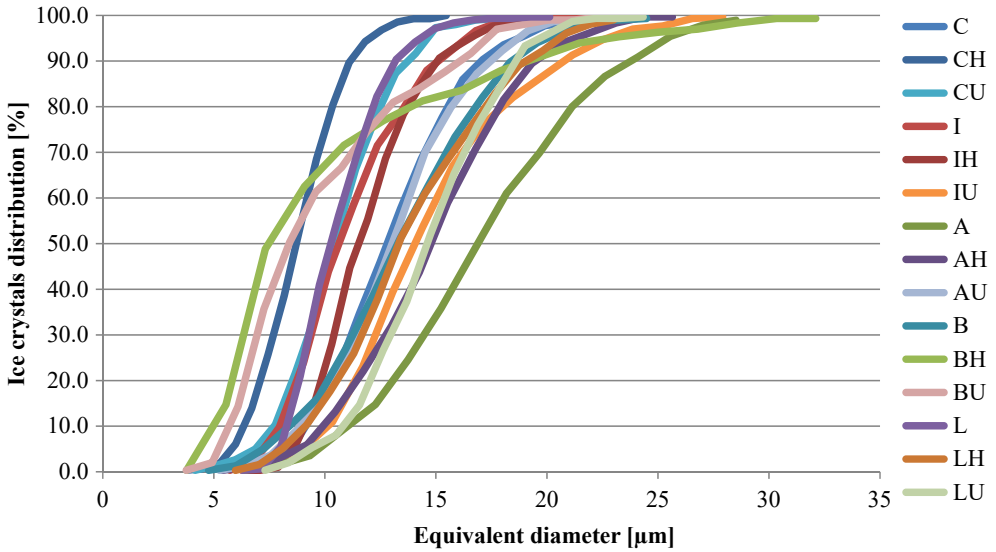


FIGURE 1 Ice crystal size distribution in ice cream after 24 h of storage at −18°C. C, control sample (without stabilizers); I, sample with stabilizers (the combination of iota carrageenan, LBG, and xanthan gum); A, sample with stabilizers (the combination of acid hydrolysates of iota carrageenan, LBG, and xanthan gum); B, sample with stabilizers (the combination of enzymatic β-galactosidase hydrolysates of iota carrageenan, LBG, and xanthan gum); L, sample with stabilizers (the combination of enzymatic commercial lactase hydrolysates of iota carrageenan, LBG, and xanthan gum); H, after the traditional homogenization treatment; U, after the ultrasound homogenization treatment, 24 h—after 24 h of storage.

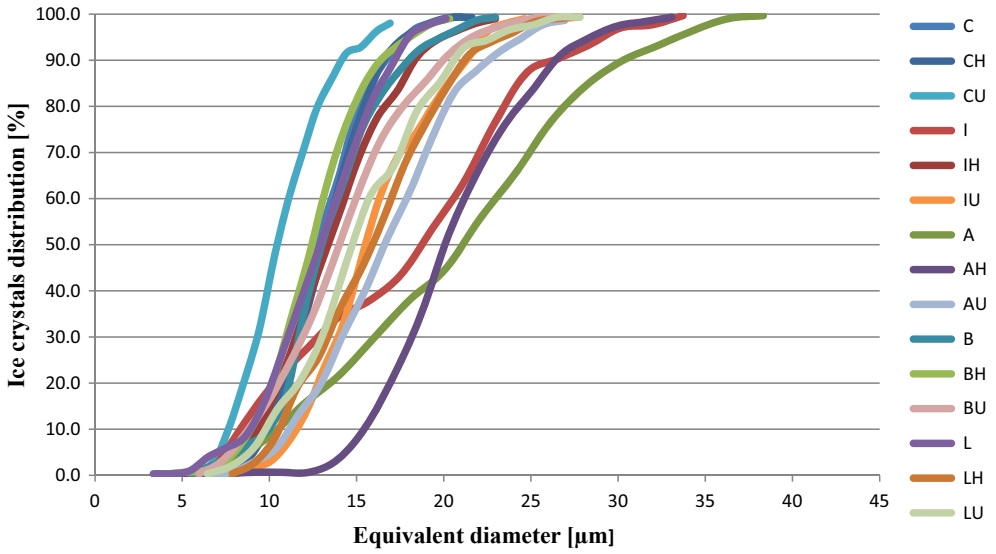


FIGURE 2 Ice crystal size distribution in ice cream after 1 month of storage at −18°C. C, control sample (without stabilizers); I, sample with stabilizers (the combination of iota carrageenan, LBG, and xanthan gum); A, sample with stabilizers (the combination of acid hydrolysates of iota carrageenan, LBG, and xanthan gum); B—sample with stabilizers (the combination of enzymatic β-galactosidase hydrolysates of iota carrageenan, LBG, and xanthan gum); L, sample with stabilizers (the combination of enzymatic commercial lactase hydrolysates of iota carrageenan, LBG, and xanthan gum); H, after the traditional homogenization treatment; U, after the ultrasound homogenization treatment; 1 m, after 1 month of storage.

In the presented study, the range for the cryoscopic temperature was from −1.918 to −1.757°C (Table 1). Based on statistical analysis, the type of used stabilizer and homogenization treatment did not significantly influence the cryoscopic temperature. Looking at the results of cryoscopic temperature, it was noted that the sample with the addition of iota carrageenan (I) and the sample with the addition of enzymatic commercial lactase hydrolysates of iota carrageenan (L) was characterized by the lowest value of temperature, at a level of −1.918 and −1.894°C. Moreover, despite the different sorts of stabilizers used, it was noticed that the value of cryoscopic temperature was no different in comparison to the control sample. On the other hand, for other variants of ice cream, traditional homogenization was

17454530, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfpe.14472 by Warsaw University of Life Sciences, Wiley Online Library on [06/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

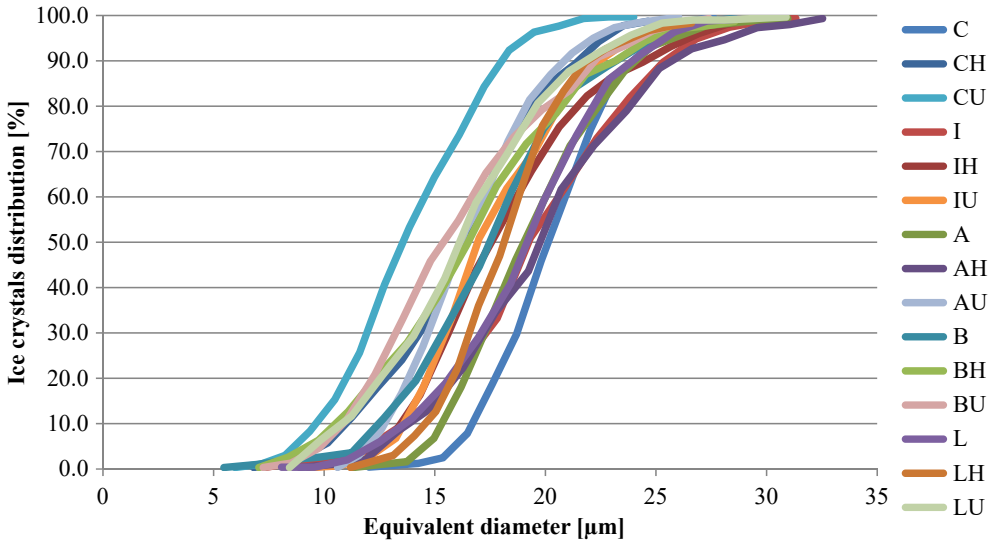


FIGURE 3 Ice crystal size distribution in ice cream after 3 months of storage at −18°C. C, control sample (without stabilizers); I, sample with stabilizers (the combination of iota carrageenan, LBG, and xanthan gum); A, sample with stabilizers (the combination of acid hydrolysates of iota carrageenan, LBG, and xanthan gum); B, sample with stabilizers (the combination of enzymatic β-galactosidase hydrolysates of iota carrageenan, LBG, and xanthan gum); L, sample with stabilizers (the combination of enzymatic commercial lactase hydrolysates of iota carrageenan, LBG, and xanthan gum); H, after the traditional homogenization treatment; U, after the ultrasound homogenization treatment; 3 m—after 3 months of storage.

the reason which contributed to lowering this parameter and, at the same time, ultrasound homogenization influenced the increasing cryoscopic temperature (Table 1). The cryoscopic temperature has been investigated by Muse and Hartel (2004) during the analysis of ice cream produced with different types of sweeteners and emulsifiers. It was noted that cryoscopic temperature ranges from −1.8 to −4.6°C. Moreover, much of the available literature on ultrasound presents that ultrasound is a pivotal tool in improving heat transfer efficiency in the freezing process (Chemat et al., 2015). This statement could be attributed to the higher cryoscopic temperature of presented ice cream mixes after the homogenization treatment in comparison to traditional homogenization and samples without any treatment, on the grounds that ultrasound might accelerate the freezing process in those samples resulting in higher temperatures. Additionally, looking at Table 1, it may be noticed that changes between samples were dependent on the type of added stabilizers not only from the pretreatment owing to the fact that the molecular mass of the used stabilizers (iota carrageenan and its hydrolysates) may influence the freezing point of prepared ice cream mixes. In our previous research (Kot, Kamińska-Dwórznicka, Antczak, et al., 2022), it was proven that the molecular mass was dependent on the sort of hydrolysis. On the other hand, a small difference between iota carrageenan and its hydrolysates was noticed. Such results may be caused by the used ultrasound due to the fact that the iota carrageenan was less resistant to flow and the same accumulated higher shear force which leads to more frequent ruptures as the cavitation bubbles collapse than in the shorter polymer (Tecson et al. 2021). Finally, the mass of iota carrageenan and presumably the mechanism were similar to hydrolysates of iota carrageenan. Moreover, such dependents give the information that used hydrolysates might be more stable and less vulnerable on the ultrasound treatment than iota carrageenan.

In the case of osmotic pressure, the value was from 946 to 1020 mOsm/kg. (Table 1). Traditional homogenization for all prepared ice cream gave a higher value of osmotic pressure, while the ultrasound treatment lowered this parameter. Therefore, it may be concluded that ultrasound reduces the moisture-binding capacity of used stabilizers on ice cream mixes. In the case of used stabilizers, there were no significant differences between samples. Only for sample L (with the addition of enzymatic commercial lactase hydrolysates of iota carrageenan) was the osmotic pressure higher in comparison to the sample after the homogenization treatment (LH) (Table 1). Baer and Czmowski (1985) observed that the osmotic pressure of milk vanilla ice cream ranged from 1426 to 1705 mOsm/kg. The lower value of osmolality in our ice cream samples might be the reason for the different number and sort of soluble ingredients that were contained in this ice cream mix.

3.2 | The melting time of ice cream

The melting time of ice cream is a crucial factor in assessing the quality of this product. Based on the melting time, there is the ability to assess the correctness of selected technology or for instance, freezing parameters. There exist many factors that contribute to the melting time of ice creams such as the amount of air incorporated, the network of fat globules, or the nature of ice crystals (Muse & Hartel, 2004).

In the presented article, the melting time for samples of vegan ice cream ranged from 15.37 to 33.05 min. According to the statistical

17454530, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfpe.14472 by Warsaw University of Life Sciences, Wiley Online Library on [06/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 2 Ice crystal sizes in ice cream after 24 h, 1, and 3 months of storage at −18°C.

Time of storage and variant of ice cream		Average diameter D_A in the class with the highest frequency [μm] ± SD	The minimal size of ice crystals [μm]	The maximal size of ice crystals [μm]
C	24 h	13.01 ± 3.06	6.90	22.71
	1 month	13.07 ± 2.66	7.02	20.55
	3 month	20.41 ± 2.80	14.59	27.26
CH	24 h	10.73 ± 2.17	4.45	17.72
	1 month	13.26 ± 2.69	6.54	21.62
	3 months	16.28 ± 3.81	7.33	25.71
CU	24 h	10.61 ± 2.41	4.32	17.25
	1 month	10.74 ± 2.57	4.68	17.10
	3 months	12.88 ± 3.27	6.13	22.16
I	24 h	11.14 ± 2.83	5.73	24.56
	1 month	17.96 ± 6.83	9.50	27.73
	3 months	20.25 ± 4.57	9.38	32.25
IH	24 h	11.86 ± 2.40	6.28	20.00
	1 month	13.59 ± 3.58	4.92	24.09
	3 months	18.48 ± 4.15	10.71	27.87
IU	24 h	14.75 ± 4.12	6.30	29.26
	1 month	15.81 ± 3.63	8.56	23.30
	3 months	17.67 ± 3.62	10.46	29.57
A	24 h	17.32 ± 4.70	6.63	28.34
	1 month	18.78 ± 7.41	8.54	30.56
	3 months	19.41 ± 3.49	13.53	28.06
AH	24 h	15.10 ± 3.73	7.48	25.10
	1 month	20.38 ± 4.35	7.79	31.80
	3 months	19.99 ± 4.64	10.21	32.72
AU	24 h	13.13 ± 3.24	5.61	23.39
	1 month	16.69 ± 4.32	7.29	26.32
	3 months	16.47 ± 3.12	10.87	25.82
B	24 h	13.46 ± 3.87	5.05	22.50
	1 month	13.37 ± 3.32	5.69	22.00
	3 months	17.50 ± 4.17	7.24	28.09
BH	24 h	10.55 ± 5.97	5.78	19.56
	1 month	12.56 ± 2.91	6.25	20.61
	3 months	17.52 ± 4.71	9.37	27.39
BU	24 h	10.80 ± 3.92	5.45	17.83
	1 month	14.28 ± 4.21	7.05	23.84
	3 months	16.06 ± 4.39	7.48	28.21
L	24 h	10.61 ± 2.04	7.35	17.49
	1 month	13.23 ± 3.28	5.37	20.90
	3 months	19.33 ± 3.91	10.44	28.59
LH	24 h	13.76 ± 3.75	6.60	23.36
	1 month	15.89 ± 4.18	7.87	28.92
	3 months	18.19 ± 3.02	11.60	26.03
LU	24 h	14.53 ± 3.04	7.59	22.15
	1 month	15.23 ± 4.20	6.44	29.15
	3 months	16.54 ± 4.16	8.97	28.62

Abbreviations: C, control sample (without stabilizers); I, sample with stabilizers (the combination of iota carrageenan, LBG, and xanthan gum); A, sample with stabilizers (the combination of acid hydrolysates of iota carrageenan, LBG, and xanthan gum); B, sample with stabilizers (the combination of enzymatic β-galactosidase hydrolysates of iota carrageenan, LBG, and xanthan gum); L, sample with stabilizers (the combination of enzymatic commercial lactase hydrolysates of iota carrageenan, LBG, and xanthan gum); H, after the traditional homogenization treatment; U, after the ultrasound homogenization treatment.

17454530, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfpe.14472 by Warsaw University of Life Sciences, Wiley Online Library on [06/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

appraisal, the significant differences between samples were noticed, and the influence of the sort of homogenization treatment and used stabilizers was confirmed (Table 1). The longest melting time and the same most favorable results were noted for sample LU (with the addition of enzymatic commercial lactase hydrolysates of iota carrageenan and after ultrasound homogenization). The lowest value of this parameter was observed for sample BH (with the addition of the enzymatic hydrolysates by β-galactosidase of iota carrageenan and after traditional homogenization treatment) (Table 1). Additionally, it was noticed that in most of the prepared ice cream samples (except for the sample with the addition of iota carrageenan) ultrasound homogenization contributed to longer melting times regardless of the used stabilizers or their absence. Even in the control sample (after ultrasound homogenization treatment) CU, the effect of ultrasound homogenization was visible. The melting time for this ice cream was at a level of 29.54 min, while for the sample after traditional homogenization treatment at 24.38 min. Besides, in the presented article, the water-holding capacity of ice cream did not measure, in the literature, such the positive effect of ultrasound may be explained by increasing water-holding capacity in products which resulted in prolonged melting time in ice cream (Akdeniz & Akalin, 2019; Carrillo-Lopez et al., 2021). Additionally, presumably, ultrasound treatment created a more preferable emulsion which had small fat globules and well-incorporated air bubbles. Therefore, it might be the reason for prolonging the melting time of ice cream. Moreover, this kind of system contributed to better conditions to form ice crystal structures after freezing with small ice crystals (Kamińska-Dwórznicka et al., 2022, 2023). In the case of the addition of stabilizers, the hydrolysates of iota carrageenan (samples: A, B, and L) presented a close melting time (from 23.25 to 24.10 min) but in comparison to the sample with the iota carrageenan (I) (31.1 min) (Table 1) the melting time was shorter. In the paper by Pintor and Totosus (2012), also the addition of iota carrageenan with a combination of two other stabilizers: locust bean gum and carboxymethylcellulose was tested in ice cream. It also resulted in an increased melting rate in ice cream. According to it, the addition of iota carrageenan is more beneficial in the case of the melting time of vegan ice cream. Moreover, it is known that fats-melting ice cream is undesirable among consumers and also such products tend to become heat-shocked readily (Goff & Hartel, 2013). Longer melting time can be perceived as a satisfying result which may increase the quality of ice cream. Research conducted by Tüker and Dogan (2021) on the effect of ultrasound homogenization on the structural and sensorial attributes of ice cream proved that ultrasound had a favorable impact on the melting properties of ice cream. The melting time was longer for the sample after ultrasound treatment than in the control sample with no US. Moreover, it is vital to highlight the addition of inulin to obtain a vegan ice cream since in accordance with research by Akın et al. (2007), in probiotic ice cream the addition of inulin influenced melting time. It had occurred that inulin prolonged the melting time of ice cream. The reason for this was the inulin's ability to link with water molecules which could prevent the free movement of water (Akın et al., 2007). Last but not least, there is one more pivotal factor that may also be responsible for the better melting time of ice cream. It is known that the greater amount of fat destabilization may increase the resistance to the flow of the serum phase which gives a slower time of melting (Regand & Goff, 2003). Additionally, fat destabilization is necessary to stabilize the structure of ice cream. The partial coalescence of fat is responsible for stabilizing air cells and fat networks in the unfrozen matrix of ice cream mixes (Liu et al., 2022). In previous research by Kot et al. (2021) and Kot, Kamińska-Dwórznicka, and Jakubczyk (2022), vegan ice cream mixes with the same recipe such as those presented were prepared, and fat destabilization was confirmed. Coalescence, sedimentation, and creaming occurred during the maturation time of ice cream mixes. Taken together, there are many factors contributing to the melting properties of ice cream. Albeit, in the presented study, ultrasound homogenization had a greater influence on samples of vegan ice cream and may be a promising tool for further research.

3.3 | The overrun of ice cream

The overrun of ice cream is a pivotal characteristic because air incorporation affects the quality of the product such as texture or stability and, additionally, it can contribute to the economic production of ice cream (Seo & Oh, 2022). During the freezing process, the air cells are incorporated into ice cream mixes which leads to an increase in the volume of this matrix (Akbari et al., 2019).

Table 1 presents the results obtained from the overrun analysis. It was observed that the overrun of prepared vegan ice cream did not exceed 33%. The lowest overrun was noticed for sample IH (with the addition of iota carrageenan and after traditional homogenization treatment) at a level of 8.15%, while the highest overrun was 32.36% for I (with the addition of iota carrageenan). Based on the ANOVA test, it was found that the type of used stabilizers and homogenization contributed to significant differences between samples. With the addition of stabilizers in vegan ice cream the overrun of samples increased. In the case of homogenization treatment (traditional or ultrasound), the overrun decreased. The most surprising aspect of this data on the overrun of prepared ice cream is that the sort of homogenization did not unambiguously influence the ice cream due to the fact that the differences between the homogenization sample and the ultrasound sample were not significant. It is known that the appropriate amount of fat can stabilize the air system bubbles in ice cream. In our recipe of ice cream, with the small participation of fat, the system was less stable and, therefore, acoustic cavitation and traditional homogenization may contribute to breaking the soft structure of air bubbles in ice cream. Looking at the control sample, such a mentioned perception is visible (Table 1). Flores and Goff (1999) observed that changes in the incorporation of air in ice cream resulted in a change in microstructure, especially in ice crystal size distribution. This may be due to the fact that foam structure may be a physical barrier that reduces the possibility of collision between crystals such as confining crystals to a thinner unfrozen phase. On the other hand, it was also found that in ice cream with an overrun of less than 50%, the influence on ice crystallization was not observed. In our research,

17454530, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfpe.14472 by Warsaw University of Life Sciences, Wiley Online Library on [06/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

correlation was not noticed (Tables 1 and 2). Moreover, for vegan ice cream, such results which were obtained in our research, are extremely beneficial. Additionally, incorporation at this level might influence better conditions to create the ice crystal structure during freezing and increase melting time.

Ice cream overrun is strictly connected to a great extent to the protein content in the ice cream mix. Góral et al. (2018) highlighted that while measuring coconut ice cream, the overrun ranged from 8.76% to 15.31%. Presumably, the lower value of this parameter may be the total absence of animal protein and also the low protein content in the coconut milk that was used. Additionally, in the research by Akesowan (2009), in ice cream with the addition of the soy protein isolate, the overrun was decreasing with the increasing amount of plant protein. Moreover, one possible explanation for lower overrun in samples after ultrasound treatment will be the exposure time and used frequency of ultrasound during homogenization. Tüker and Dogan (2021) found that an overrun of ice cream after the ultrasound treatment ranged from 33.17% to 43.74%. The differences between samples were visible depending on the mentioned parameters. Furthermore, Sert and Mercan (2021) also concluded that the sort of pressure of HPH (high-pressure homogenization) contributed to the distinctive value of overrun in sheep milk ice cream. Such observations might be a suggestion to develop the study in connection with a parameter of ultrasound homogenization to obtain better results in the overrun of vegan ice cream.

3.4 | The recrystallization process in ice cream

Ice crystal size analysis was examined based on the value of average diameter (D_A) (Table 2) and distributions obtained from the image analysis which were characterized by the values for the ice crystal equivalent diameter at 50% of the cumulative distribution (X_{50}) (Figures 1–3). The samples of vegan ice cream were examined after 24 h, 1 month, and 3 months of storage at −18°C to estimate the progress of the recrystallization process.

The presented results, after 24 h of storage, indicated that for all prepared vegan ice cream, the average diameter of ice crystals did not exceed 18 μm (Table 2). The lowest diameter of crystals was observed for ice cream BH (with the addition of enzymatic hydrolysates by β-galactosidase of iota carrageenan and after traditional homogenization treatment), at a level of 10.55 μm and the highest D_A was noticed for sample A (with the acid hydrolysates of iota carrageenan), at a level of 17.32 μm. Moreover, for samples such as CU (the control sample after the ultrasound treatment) and AU (samples with the addition of acid hydrolysates of iota carrageenan), the effect of ultrasound was observed due to the fact the size of crystals was smaller than after traditional homogenization. For other variants, this effect was not noticed, therefore it will not be possible to indicate the same tendency in all ice cream. In previous research by Kot et al. (2021), it was observed that ultrasound homogenization increased the consistency index in ice cream mixes. According to Aslan and Dogan (2017), the shear forces which were generated by acoustic cavitation in emulsions were responsible for breaking oil droplets into small ones and allowed the better distribution of proteins on new interface systems. Therefore, also in the presented ice cream mixes, the ultrasound contributed to different interface systems in comparison to the effect of traditional homogenization. Consequently, by creating less space for ice crystal formation, smaller ice crystals were obtained. According to the value of the ice crystal diameter at 50% of the cumulative distribution of the sample—the X_{50} diameter ranged from 7 to 17 μm (Figure 1). Kot et al. (2020) found that after 24 h of storage, the average diameter (D_A) of ice crystals in vegan ice cream with stabilizers such as locust bean gum and xanthan gum, was 17.21 μm, while in the control sample, it achieved a value of 51.69 μm. In comparison to the presented results, it may be concluded that additional processes such as traditional homogenization, ultrasound homogenization, and iota carrageenan or its hydrolysates contributed to obtaining even smaller crystals after 24 h of storage than samples with only stabilizers. Moreover, Kamińska-Dwórznicka et al. (2020) in strawberry sorbets with the addition of kappa and iota carrageenans as stabilizers proved that after freezing the hydrocolloids positively influenced the diameter of the crystal (D_A). For the stabilized ice cream, the average diameter did not exceed 14 μm. Additionally, in the study on the influence of US-assistant freezing on mango sorbet, it was proved that the frequency of 21.5 Hz of ultrasound contributed to obtaining the smallest diameters of ice crystals lower than 10 μm in comparison to a conventional freezer (Kamińska-Dwórznicka et al., 2023).

After 1 month of storage of vegan ice cream at a temperature of −18°C it may be concluded that the recrystallization process was noticeable (Table 2). Looking at the average diameter of ice crystals in presented samples after this time, the lowest value was observed at a level 10.74 μm for sample CU (control sample after the ultrasound treatment), while the highest value for sample AH (the sample with the acid hydrolysates of iota carrageenan and after traditional homogenization treatment). Moreover, in comparison to the obtained results after 24 h of storage, sample I (with the addition of iota carrageenan) characterized the highest growth of ice crystals, almost 7 μm. While for sample B (with the addition of the enzymatic hydrolysates by β-galactosidase of iota carrageenan), recrystallization phenomena were not recorded. As mentioned in the previous paragraph, in the samples such as CU (the control sample after ultrasound treatment) and AU (samples with the addition of acid hydrolysates of iota carrageenan), the effect of ultrasound was observed also after 1 month of storage. Additionally, based on the value of the ice crystal diameter at 50% of the cumulative distribution of the sample (X_{50} diameter), it extended from 10 to 20 μm (Figure 2). At this stage of storage, the effect of ultrasound homogenization on the recrystallization process was visible in comparison to samples after traditional homogenization or only with a mix of stabilizers for instance, looking at the control sample (CU) and samples with the addition of acid hydrolysates of iota carrageenan (AU). Kamińska-Dwórznicka et al. (2022) provided an analysis of the influence of a combination of carrageenans (kappa or iota) with LBG and guar gum, on whey ice cream during storage. After 1 month of storage at −18°C, it was noticed that the addition of iota carrageenan with LBG and guar gum contributed to obtaining crystals

17454530, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfpe.14472 by Warsaw University of Life Sciences, Wiley Online Library on [06/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

around 25 μm. According to the ice crystal diameter at 50% of the cumulative distribution of the sample (X_{50}), the sample with iota carrageenan addition had the smallest size of crystals and the X_{50} parameter after 3 months of storage was at a level of 23 μm. This result showed that iota carrageenan, despite its ability to create intra-molecular combinations with calcium ions, will also be an appropriate stabilizer in vegan ice cream.

The analysis of the structure of ice crystals after 3 months of storage indicated a striking observation for used stabilizers and homogenization treatment. First and foremost, for all samples, the average diameter of ice crystals was less than 21 μm (Table 2). As was already mentioned, the small ice crystal sizes (10 to 20 μm) are crucial to obtaining a lucrative texture of ice cream, and the presented results unambiguously proved this statement. The sample of ice cream after ultrasound treatment CU but without the addition of stabilizers achieved the lowest size of crystals owing to the fact that the average diameter D_A was at 12.88 μm. According to the value of the X_{50} diameter, it ranged from 14 to 20 μm (Figure 3). What should be highlighted here is the fact that in all samples where ultrasound treatment has been applied the size of crystals was lower in comparison to traditional homogenization or samples with stabilizers. In addition, in samples with ultrasound treatment and with the addition of stabilizers also the average diameter was lower, for instance, sample IH (the sample with iota carrageenan and after the traditional homogenization treatment) with comparisons to sample IU (the sample with iota carrageenan and after ultrasound homogenization treatment). The reason for these results may be the positive effect of ultrasound on the structure of ice cream mixes. Consequently, less space is available to form ice crystals, and a smaller and more favorable structure is achieved. Additionally, the collapse of air bubbles may initiate ice nucleation by creating local zones of high pressure in a very short time. Simultaneously, the force that was generated by the collapse of cavitation bubbles may be responsible for the fragmentation of bigger ice crystals into smaller ones (Akdeniz & Akalin, 2019; Cheng et al. 2015). Looking at the results in samples I, A, B and L (Table 2), where only stabilizers were used without any homogenization treatment, sample B had lower size crystals while other results were similar. Such an effect may be the reason for the better water-binding capacity of these hydrolysates than in others. According to our previous research (Kot, Kamińska-Dwórznicka, Antczak, et al., 2022), it may be considered that not molecular mass but the difference in structure was responsible for a better IRI effect. Because, based on FTIR analysis, it was proven that the enzymatic hydrolysates of iota carrageenan were more flexible, and the vibration intensity of the —OH groups in model solutions was seen in comparison to acid hydrolysates or pure iota carrageenan. Nonetheless, it may not be forgotten that such small differences were the results of small amounts of such stabilizers in the recipe. Maybe further studies are needed with different dependencies to prove this mentioned effect.

Some authors, Pereira et al. (2011), have mainly been interested in questions concerning the quality and structure of ice cream with the plant protein (soy extract) as a substitute in ice cream. As was noted, the ice cream containing the soy extract in the formulation led to a larger amount of small ice crystals and, additionally, a lower amount of ice crystal growth. For all samples that contained soy in the recipe, the ice crystal size (X_{50}) was less than 50 μm. Additionally, the results were divided into size categories which showed that the substitution of skim milk powder for the soy extract in ice cream contributed to ice crystals from 10 to 40 μm. Moreover, looking at ice crystal growth (%) before and after cycling temperature, the ice cream with a higher addition of the plant substitute characterized lower growth, at 22.08%. Additionally, in the research by Islam et al. (2015), it was noted that using ultrasound applied during freezing and frozen storage improved the quality of the mushrooms. Results indicated that ultrasound initiated the nucleation of ice crystals and reduced the mean size of ice crystals in comparison to the control sample. The size of ice crystals in the sample after ultrasound treatment was from 0 to 80 μm, while in the sample without the ultrasound from 50 to 180 μm. As was mentioned in the paper by Xu, Zhang, Bhandari, Cheng, and Sun (2015), the ultrasound during immersion freezing contributed to the smallest ice crystals and the most compact and dense with the smallest pores in the sample of red radish, based on SEM images.

Goff and Hartel (2013) pointed out that large ice crystals are perceived as rough particles, which detrimentally resulted in the palate and sensorial texture of ice cream. According to this statement, it is pivotal to pay attention not only to the size of ice crystals but also to their appearance. Additionally, the observation of the ice crystal structure also provides information about the progress of the recrystallization phenomena. Figure 4 and Figure 5 provide an overview of the appearance of ice crystals during storage after 24 h and 3 months of storage at −18°C (only representative photos were chosen to show in the article, avoiding the photos after 1 month).

Overall, it may be noticed that the shape of ice crystals was regular and round after 24 h of storage (Figure 4). After this time, there were no visible changes between samples and the same between the sort of used homogenization treatment, for instance in Figure 4, for samples IH_24h (the sample with iota carrageenan and after traditional homogenization treatment) and IU_24h (the sample with iota carrageenan and after ultrasound homogenization treatment). The changes in the microstructure of ice cream were visible only in the size of the ice crystals such as confirmed in Table 2. There were no observed differences in shape in samples of ice cream, especially between samples after the different types of used homogenization processes. It is known that ultrasound is responsible for crystal fragmentation and hence promotes the production of smaller and pure crystals in uniform size (Chávez-Martínez et al. 2020). For example, research by Zhu et al. (2020) proved that ice crystals formed by an ultrasound-assisted freezing system were smaller and more uniform in distribution in samples of potatoes based on SEM images, especially with the higher number of ultrasonic frequencies used, a greater reduction was observed. On the other hand, in our research, as was mentioned, the ultrasound may only affect the size of crystals, but the shape was not changed. This may be treated as a positive influence due to the fact that the recrystallization process was inhibited but no undesirable changes in the structure of ice crystals occurred.

Samples of vegan ice cream after 3 months of storage were characterized by different sizes as a result of recrystallization, which was

17454530, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfpe.14472 by Warsaw University of Life Sciences, Wiley Online Library on [06/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

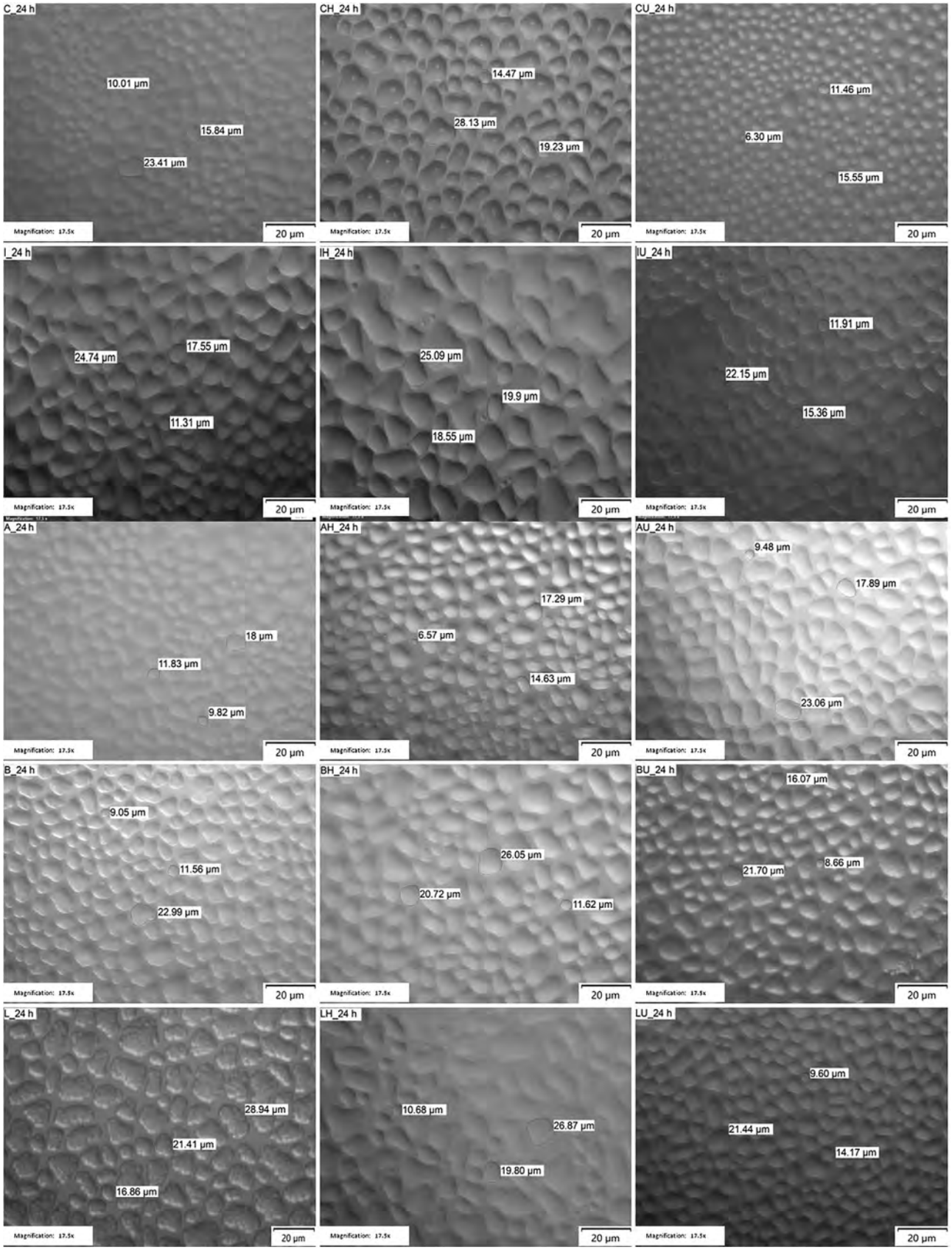


FIGURE 4 Ice crystal images after 24 h of storage at −18°C. C, control sample (without stabilizers); I, sample with stabilizers (the combination of iota carrageenan, LBG, and xanthan gum); A, sample with stabilizers (the combination of acid hydrolysates of iota carrageenan, LBG, and xanthan gum); B, sample with stabilizers (the combination of enzymatic β-galactosidase hydrolysates of iota carrageenan, LBG, and xanthan gum); L, sample with stabilizers (the combination of enzymatic commercial lactase hydrolysates of iota carrageenan, LBG, and xanthan gum); H, after the traditional homogenization treatment; U, after the ultrasound homogenization treatment; 24 h, after 24 h of storage.

17454530, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfpe.14472 by Warsaw University of Life Sciences, Wiley Online Library on [06/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

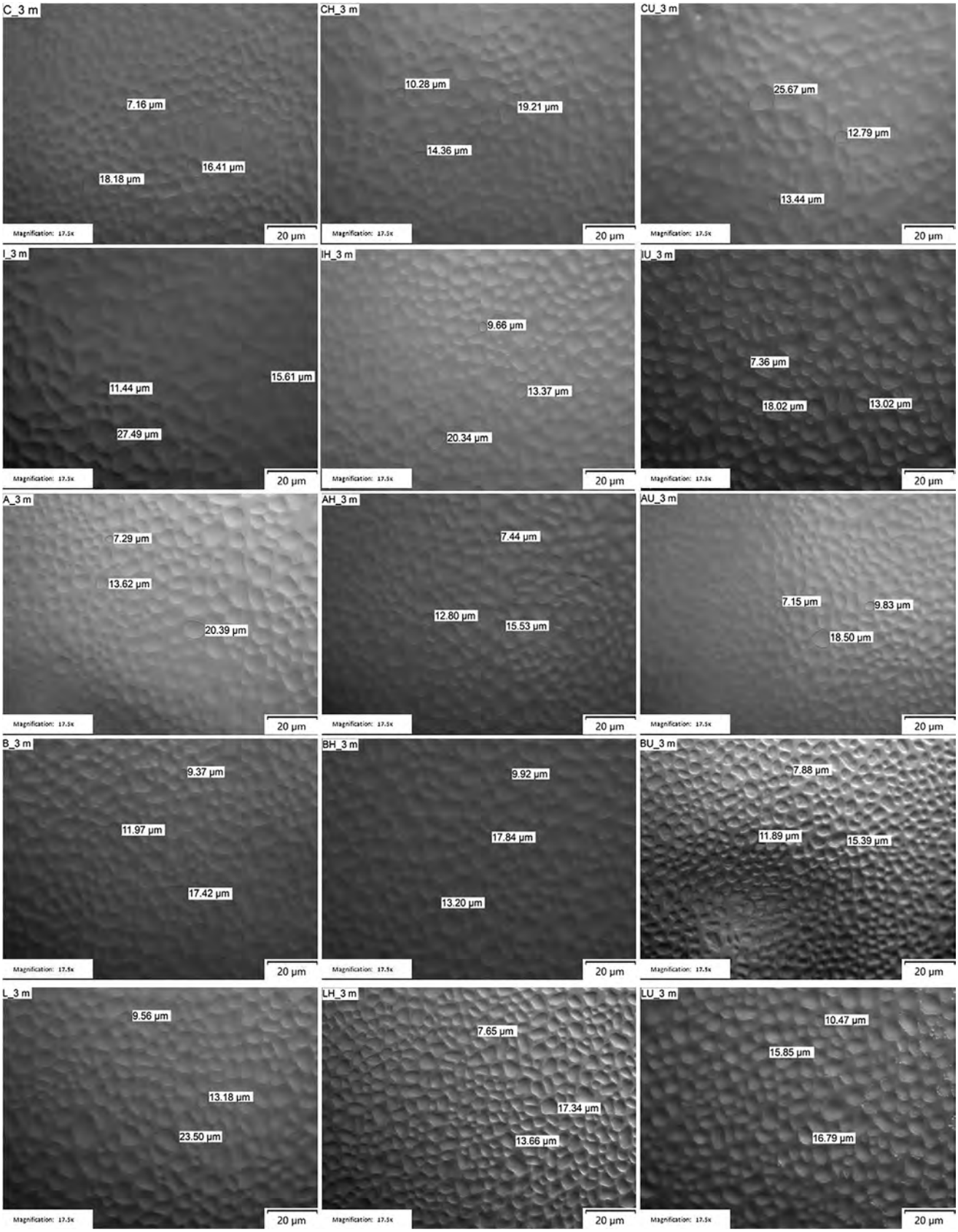


FIGURE 5 Ice crystal images after 3 months of storage at −18°C. C, control sample (without stabilizers); I, sample with stabilizers (the combination of iota carrageenan, LBG, and xanthan gum); A, sample with stabilizers (the combination of acid hydrolysates of iota carrageenan, LBG, and xanthan gum); B, sample with stabilizers (the combination of enzymatic β-galactosidase hydrolysates of iota carrageenan; LBG, and xanthan gum); L, sample with stabilizers (the combination of enzymatic commercial lactase hydrolysates of iota carrageenan, LBG, and xanthan gum); H, after the traditional homogenization treatment; U, after the ultrasound homogenization treatment, 3 m, after 3 months of storage.

17454530, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfpe.14472 by Warsaw University of Life Sciences, Wiley Online Library on [06/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

confirmed by an analysis of average diameter (Figure 5), (Table 2). The ice crystals for all samples were regular and round. The changes between used homogenization treatments (traditional and ultrasound) were not visible based on the images as it was marked after 24 h of storage. Therefore, it can also be concluded that ultrasound homogenization did not change the shape of crystals but only influenced their size. owing to the fact that the shape of samples after ultrasound treatment and after traditional treatment was similar. According to the images, the growth of ice crystals was not as prominent for example in comparison samples I_24h and sample I_3m, therefore, recrystallization in all ice cream samples did not develop during the storage (Figures 4 and 5), which was also confirmed by average diameter (D_A) (Table 2). Lomolino et al. (2020) in the research were concerned about the size and shape of ice crystals due to the crucial consequences of thermal stress for ice cream. It was asserted that a vegan version of ice cream with an addition of inulin or potato protein, characterized the heterogenous growth of ice crystals and a non-uniformity was visible in the crystals' structure. Moreover, based on the results after thermal stress, it was observed that stabilizers such as locust bean gum and carrageenan impacted the recrystallization phenomenon, with the reduction of crystal growth. In our research, the used iota carrageenan and its hydrolysates were responsible for the inhibition of recrystallization. Additionally, in the research conducted by Xin et al. (2014), on the influence of ultrasound-assisted freezing on broccoli, it was observed that ultrasound simultaneously contributed to the reduced freezing time and formation of smaller ice crystals. Therefore, the structural integrity of the cell's structure is better than in immersion freezing without ultrasound. In the research by Xu, Zhang, Bhandari, Cheng, and Islam (2015), it was seen that differences in the structure after the ultrasound treatment also depend on ultrasonic power intensities. Therefore, it is vital to choose the best parameter of ultrasound treatment owing to the fact that insufficient or excessive effects of ultrasound may lead to the formation of large ice crystals.

4 | CONCLUSION

The present article showed that ultrasound homogenization might bring a positive IRI (Ice Recrystallization Inhibition) effect during the storage of vegan ice cream. In the samples after the ultrasound treatment, after 3 months of storage, the average diameter did not exceed 18 μm. For samples after traditional mechanical homogenization and used stabilizers, the average diameter was higher −21 μm. The most striking observations were the samples without stabilizers and after ultrasound treatment which obtained the smallest ice crystals in comparison to samples with stabilizers and after ultrasound.

It showed a promising tool (US homogenization) to inhibit the recrystallization process without using stabilizers in the vegan recipe of ice cream. The overrun of ice cream showed that ultrasound homogenization and traditional homogenization decrease air incorporation. A better overrun was noticed in samples without homogenization and with the addition of stabilizers, especially with the addition of iota carrageenan (32.36%). According to the results of melting time, it was concluded that ultrasound homogenization prolongs the melting of ice cream in comparison to traditional homogenization. The melting time for ultrasound homogenization ranged from 23.25 to 33.05 min, while for traditional homogenization from 15.37 to 29.22 min. Moreover, it may be used interchangeably with stabilizers, and the same effect will be achieved. Looking at the cryoscopic temperature and osmotic pressure values, ultrasound homogenization decreases this parameter. In this case, the addition of iota carrageenan and its hydrolysates would be more favorable. For samples with the addition of stabilizers, the cryoscopic temperature ranged from −1918 to −1804°C, while for samples after ultrasound treatment, the cryoscopic temperature ranged from −1839 to −1757°C.

Finally, it might be concluded that changing traditional homogenization to ultrasound homogenization in vegan ice cream production would be advisable. Not only did the recrystallization process inhibit, but it also brought positive results on the physical aspects of ice cream such as melting time. Moreover, such changes will not result in additional steps in ice cream production, which may not generate additional costs for ice cream producers.

FUNDING INFORMATION

Research equipment was purchased as part of the "Food and Nutrition Centre-modernization of the WULS campus to create a Food and Nutrition Research and Development Centre (C ˙Zi ˙Z)" co-financed by the European Union from the European Regional Development Fund under the Regional Operational Programme of the Mazowieckie Voivodeship for 2014–2020 (Project No. RPMA.01.01.00-14-8276/17).

CONFLICT OF INTEREST STATEMENT

There is no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Anna Kot https://orcid.org/0000-0003-3850-1743

REFERENCES

Aboulfazli, F., Shori, A. B., & Baba, A. S. (2016). Effects of the replacement of cow milk with vegetable milk on probiotics and nutritional profile of fermented ice cream. *LWT—Food Science and Technology*, *70*, 261–270.

Adapa, S., Schmidt, K. A., Jeon, I. J., Herald, T. J., & Flores, R. A. (2000). Mechanisms of ice crystallization and recrystallization in ice cream: A review. *Food Reviews International*, *16*(3), 259–271.

Akbari, M., Eskandari, M. H., & Davoudi, Z. (2019). Application and functions of fat replacers in low-fat ice cream: A review. *Trends in Food Science & Technology*, *86*, 34–40.

Akdeniz, V., & Akalin, A. S. (2019). New approach for yoghurt and ice cream production: High-intensity ultrasound. *Trends in Food Science & Technology*, *86*, 392–398.

Akesowan, A. (2009). Influence of soy protein isolate on physical and sensory properties of ice cream. *Thai Journal of Agricultural Science*, *42*(1), 1–6.

Akın, M. B., Akın, M. S., & Kırmacı, Z. (2007). Effects of inulin and sugar levels on the viability of yoghurt and probiotic bacteria and the

17454530, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfpe.14472 by Warsaw University of Life Sciences, Wiley Online Library on [06/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

physical and sensory characteristics in probiotic ice-cream. *Food Chemistry*, *104*, 93–99.

Alvaro, C. (2017). Ethical veganism, virtue, and greatness of the soul. *Journal of Agricultural and Environmental Ethics*, *30*(6), 765–781.

Aslan, D., & Dogan, M. (2017). The influence of ultrasound on the stability of dairy-based, emulsifier-free emulsions: Rheological and morphological aspect. *European Food Research and Technology*, *244*, 409–421.

Baer, R. J., & Czmowski, T. P. (1985). Use of the osmometer for quality control of ice cream mix. *Journal of Food Protection*, *48*(11), 976–978.

Blond, G. (1988). Velocity of linear crystallization of ice in macromolecular systems. *Cryobiology*, *25*, 61–66.

Cadena, R. S., Cruz, A. G., Faria, J. A. F., & Bolini, H. M. A. (2012). Reduced fat and sugar vanilla ice creams: Sensory profiling and external preferences mapping. *Journal of Dairy Science*, *95*, 4842–4850.

Carrillo-Lopez, L. M., Garcia-Galicia, I. A., Tirado-Gallegos, J. M., Sanchez-Vega, R., Huerta-Jimenez, M., Ashokkumar, M., & Alarcon-Rojo, A. D. (2021). Recent advances in the application of ultrasound in dairy products: Effect on functional, physical, chemical, microbiological and sensory properties. *Ultrasound Sonochememistry*, *73*, 105467.

Chávez-Martínez, A., Reyes-Villagrana, R. A., Rentería-Monterrubio, A. L., Sánchez-Vega, R., Tirado-Gallegos, J. M., & Bolivar-Jacobo, N. A. (2020). Low and high-intensity ultrasound in dairy products: Applications and effects on physicochemical and microbiological quality. *Foods*, *9*, 1688.

Chemat, F. Z., & Khan, K. M. (2011). Application of ultrasound in food technology: Processing, preservation and extraction. *Ultrasound Sonochemistry*, *18*, 813–835.

Chemat, X., Zhang, M., Xu, B., Adhikari, B., & Sun, J. (2015). The principles of ultrasound and its application in freezing related processes of food materials: A review. *Ultrasound Sonochemistry*, *27*, 576–585.

Cheng, J., Ma, Y., Li, X., Yan, T., & Cui, J. (2015). Effects of milk protein-poly-saccharide interactions on the stability of ice cream model systems. *Food Hydrocolloids*, *45*, 327–336.

Clarke, C. (2004). *The science of ice cream*. The Royal Society of Chemistry.

da Silva, L. F. R., L. F., da Silva Gomes, A., Castro, D. R. G., das Chagas do Amaral Souza, F., Mar, J. M., Souza da Silva, L., Sanches, E. A., de Araújo Bezerra, J., Bakry, A. M., & Campelo, P. H. (2019). Ultrasound-assisted homogenisation and gum Arabic combined to physicochemical quality of cupacu juice. *Journal of Food Processing and Preservation*, *43*(9), e14072.

Dłużewska, E., Gazda, B., & Leszczyński, K. (2003). Wpływ wybranych hydrokoloidów polisacharydowych na jakość koncentratów lodów owocowych. *Journal Food Science and Nutrition Acta Scientiarium Polonorum Technologia Alimentaria*, *2*(1), 97–107.

Firouz, M. S. (2021). Application of high-intensity ultrasound in food processing for improvement of food quality. In F. Barba, G. Cravotto, F. Chemat, J. M. Lorenzo Rodriguez, & P. E. Sichetti Munekata (Eds.), *Design and optimization of innovative food processing techniques assisted by ultrasound. Developing healthier and sustainable food products* (pp. 155–159). Elsevier Inc.

Flores, A. A., & Goff, H. D. (1999). Ice crystals size distribution in dynamically frozen model solutions and ice cream as affected by stabilisers. *Journal of Dairy Science*, *82*, 1399–1407.

Gaukel, V., Leiter, A., & Spiess, W. E. L. (2014). Synergism of different fish antifreeze proteins and hydrocolloids on recrystallization inhibition of ice in sucrose solutions. *Journal of Food Engineering*, *141*, 44–50.

Goff, H. D., & Hartel, R. W. (2013). *Ice cream* (7th ed.). New York.

Góral, M., Kozłowicz, K., Pankiewicz, U., Góral, D., Kluza, F., & Wójtowicz, A. (2018). Impact of stabilizers on the freezing process, and physicochemical and organoleptic properties of coconut milk-based ice cream. *LWT—Food Science and Technology*, *92*, 516–522.

Hartel, R. W. (1988). Mechanism and kinetics of recrystallization in ice cream. In D. S. Reid (Ed.), *The properties of water in foods ISOPOW 6* (pp. 287–319). Blackie Academic & Professional (an imprint of Thomson Science).

Islam, M. N., Zhang, M., Fang, Z., & Sun, J. (2015). Direct contact ultrasound assisted freezing of mushroom (Agaricus bisporus): Growth and size distribution of ice crystals. *International Journal of Refrigeration*, *57*, 46–53.

Kamińska-Dwórznicka, A., Gondek, E., Łaba, S., Jakubczyk, E., & Samborska, K. (2019). Characteristics of instrumental methods to describe and assess the recrystallization process in ice cream systems. *Food*, *8*, 117.

Kamińska-Dwórznicka, A., Janczewska-Dupczyk, A., Kot, A., Łaba, S., & Samborska, K. (2020). The impact of ʟ- and κ-carrageenan addition on freezing process and ice crystals structure of strawberry sorbet frozen by various methods. *Journal of Food Science*, *1*(85), 50–56.

Kamińska-Dwórznicka, A., Kot, A., Jakubczyk, E., Buniowska-Olejnik, M., & Nowacka, M. (2023). Effect of ultrasound-assisted freezing on the crystal structure of mango sorbet. *Crystals*, *13*, 396.

Kamińska-Dwórznicka, A., Łaba, S., & Jakubczyk, E. (2022). The effects of selected stabilizers addition on physical properties and change in crystal structure of whey ice cream. *LWT—Food Science and Technology*, *154*, 112841.

Kamińska-Dwórznicka, A., Matusiak, M., Samborska, K., Witrowa-Rajchert, D., Gondek, E., Jakubczyk, E., & Antczak, A. (2015). The influence of kappa carrageenan and its hydrolysates on the recrystallization process in sorbet. *Journal of Food Engineering*, *167*, 162–165.

Kamińska-Dwórznicka, A., Skrzypczak, P., & Gondek, E. (2016). Modification of kappa carrageenan by β-galactosidase as a new method to inhibit recrystallization. *Food Hydrocolloids*, *61*, 31–35.

Kot, A., & Kamińska-Dwórznicka, A. (2022). Vegan ice cream: A review. *Przemysł Spożywczy*, *12*(76), 30–34.

Kot, A., Kamińska-Dwórznicka, A., Antczak, A., Jakubczyk, E., & Matwijczuk, A. (2022). Effect of ʟ-carrageenan and its acidic and enzymatic hydrolysates on ice crystal structure changes in model sucrose solution. *Colloids and Surfaces A: Physicochemicala and Engineering Aspects*, *643*, 128744.

Kot, A., Kamińska-Dwórznicka, A., & Barańska, A. (2020). Study of the properties of vegan ice cream based on almond drink. *Zeszyty Problemowe Postępów Nauk Rolniczych*, *600*, 21–30.

Kot, A., Kamińska-Dwórznicka, A., Galus, S., & Jakubczyk, E. (2021). Effects of different ingredients and stabilisers on properties of mixesbased on almond drink for vegan ice cream production. *Sustainability*, *13*, 12113.

Kot, A., Kamińska-Dwórznicka, A., & Jakubczyk, E. (2022). Study on the influence of ultrasound homogenisation on the physical properties of vegan ice cream mixes. *Applied Sciences*, *12*, 8492.

Krizanova, J., Rosenfeld, D. L., Tomiyama, A. J., & Guardiola, J. (2021). Pro-environmental behaviour predicts adherence to plant-based diets. *Appetite*, *163*, 105243.

Liu, X., Sala, G., & Scholten, E. (2022). Effect of fat aggregate size and percentage on the melting properties of ice cream. *Food Research International*, *160*, 111709.

Lomolino, G., Zannoni, S., Zabara, A., da Lio, M., & de Iseppi, A. (2020). Ice recrystallisation and melting in ice cream with the different proteins levels and subjected to thermal fluctuation. *International Journal of Dairy Science*, *100*, 104557.

Muse, M. R., & Hartel, R. W. (2004). Ice cream structural elements that affect melting rate and hardness. *Journal of Dairy Science*, *87*, 1–10.

Ndoye, F. T., & Alvarez, G. (2015). Characterization of ice recrystallization in ice cream during storage using the focused beam reflectance measurement. *Journal of Food Engineering*, *148*, 24–34.

O'Sullivan, J., Park, M., & Beevers, J. (2016). The effect of ultrsoud upon the physicochemical and emulsifying properties of wheat and soy protein isolates. *Journal of Cereal Science*, *69*, 77–84.

Pereira, G.d. G., de Resende, J. V., de Abreu, L. R., de Oliveira Giarola, T. M., & Perrone, I. T. (2011). Influence of the partial substitution of skim milk powder for soy extract on ice cream structure and quality. *European Food Research and Technology*, *232*, 1093–1102.

17454530, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfpe.14472 by Warsaw University of Life Sciences, Wiley Online Library on [06/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Pham, Q. T., & Mawson, R. F. (1997). Moisture migration and ice recrystallization in frozen foods. In M. C. Erickson & Y. C. Hung (Eds.), *Quality in frozen foods* (pp. 67–91). Springer Science+Business Media.

Pintor, A., & Totosus, A. (2012). Ice cream properties affected by lambda-carrageenan or iota-carrageenan interactions with locust bean gum/-carboxymethylcellulose mixtures. *International Food Research Journal*, *19*(4), 1409–1414.

Regand, A., & Goff, H. D. (2002). Effect of biopolymers on structure and ice recrystallization in dynamically frozen ice cream model systems. *Journal of Dairy Science*, *85*, 2722–2732.

Regand, A., & Goff, H. D. (2003). Structure and ice recrystallization in frozen stabilized ice cream model systems. *Food Hydrocolloids*, *17*, 95–102.

Seo, C. W., & Oh, N. S. (2022). Functional application of Maillard conjugate derived from a κ-carrageenan/milk protein isolate mixture as stabilizer in ice cream. *LWT—Food Science and Technology*, *161*, 113406.

Sert, D., & Mercan, E. (2021). Hog-pressure homogenisation of sheep milk ice cream mix: Physicochemical and microbiological characterisation. *LWT—Food Science and Technology*, *151*, 112148.

Smith, A. K., Goff, H. D., & Kakuda, Y. (2000). Microstructure and rheological properties of whipped cream as affected by heat treatment and addition of stabilizer. *International Dairy Journal*, *10*, 295–330.

Tecson, M. G., Abad, L. V., Ebajo, V. D. Jr., & Camacho, D. H. (2021). Ultrasound-assisted depolymerization of kappa-carrageenan and characterization of a degradation product. *Ultrasonic Sonochemistry*, *73*, 105540.

Tüker, D. A., & Dogan, M. (2021). Effect of ultrasound homogenization on the structural and sensorial attributes of ice cream: Optimization with Taguchi and data envelopment analysis. *Joutnal of Food Measurement and Characterization*, *15*, 4888–4898.

Vanga, S. K., & Raghavan, V. (2018). How well do plant-based alternatives fare nutritionally compared to cow's milk? *Journal of Food Science and Technology*, *55*(1), 10–20.

Williams, E., Vardavoulia, A., Lally, P., & Gardner, B. (2023). Experiences of initiating and maintaining a vegan diet among young adults: A qualitative study. *Appetite*, *180*, 106357.

Xin, Y., Zhang, M., & Adhikari, B. (2014). The effects of ultrasound-assisted freezing on the freezing time and quality of broccoli (*Brassica oleracea* L. var. botrytis L.) during immersion freezing. *International Journal of Refrigeration*, *41*, 82–91.

Xu, B.-G., Zhang, M., Bhandari, B., Cheng, X.-F., & Islam, M. N. (2015). Effect of ultrasound-assisted freezing on the physic-chemical properties and volatile compounds of red radish. *Ultrasound Sonochemistry*, *27*, 316–324.

Xu, B.-G., Zhang, M., Bhandari, B., Cheng, X.-F., & Sun, J. (2015). Effect of ultrasound immersion freezing on the quality attributes and water distributions of wrapped red radish. *Food Bioprocess Technology*, *8*, 1366–1376.

Zhu, Z., Zhang, P., & Sun, D.-W. (2020). Effects of multi-frequency ultrasound on freezing rates and quality attributes of potatoes. *Ultrasound Sonochemistry*, *60*, 104733.

Zhu, Z., Zhou, Q., & Sun, D.-W. (2019). Measuring and controlling ice crystallization in frozen foods: A review of recent developments. *Trends in Food Science & Technology*, *90*, 13–25.

How to cite this article: Kot, A., & Kamińska-Dwórznicka, A. (2023). The influence of ultrasound homogenization on recrystallization during the storage of vegan ice cream. *Journal of Food Process Engineering*, e14472. https://doi.org/10.1111/jfpe.14472

17454530, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfpe.14472 by Warsaw University of Life Sciences, Wiley Online Library on [06/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

ORIGINAL ARTICLE
http://journal.pan.olsztyn.pl

Polish Journal of Food and Nutrition Sciences
2023, Vol. 73, No. 2, 196–204
DOI: 10.31883/pjfns/166382
ISSN 1230-0322, e-ISSN 2083-6007

Effect of ι-Carrageenan and Its Hydrolysates on the Stability of Milk Ice Cream Mixes

Anna Kot[1]*, Ewa Jakubczyk[1], Magdalena Buniowska-Olejnik[2], Anna Kamińska-Dwórznicka[1]

[1]Department of Food Engineering and Process Management, Institute of Food Sciences, Warsaw University of Life Sciences (WULS-SGGW), Nowoursynowska 159C, 02-776, Warsaw, Poland
[2]Department of Dairy Technology, Institute of Food Technology and Nutrition, University of Rzeszow, Ćwiklinskiej 2D St., 35601 Rzeszow, Poland

The objective of this research was to determine the influence of ι-carrageenan and its acid and enzymatic hydrolysates on the physical properties of milk ice cream mixes. The main factors considered were the Turbiscan stability index, backscattering profile, particle size distribution and median diameter (D_{50}), the consistency index and the flow behavior index of ice cream mix before and after 24 h of maturation at 4°C. The microstructure of emulsion was also analysed based on confocal laser scanning microscopy (CLSM). The addition of ι-carrageenan resulted in lower stability of emulsion compared to emulsions with its acid and enzymatic hydrolysates. The sedimentation, coalescence and flocculation were observed based on the backscattering profile and CLSM images. The addition of stabilisers contributed to an increase in D_{50} of ice cream mix from 17.56 to 37.05–45.50 µm before maturation and from 34.73 to 46.73 µm after maturation. The ι-carrageenan after commercial lactase treatment improved the stability of milk ice cream mixes by increasing the consistency index to 0.104 and a flow behaviour index to 0.702. Finally, it may be concluded that the stabilisers used – ι-carrageenan and its hydrolysates – significantly influenced the physical properties of milk ice cream mixes and, hence, can be used as beneficial ingredients in the recipe of milk ice cream mixes.

Key words: ice cream mix emulsion, particle size, confocal laser scanning microscopy

INTRODUCTION

The colloidal structure of ice cream has recently been an object of research interest for the global audience. It is known that the final physicochemical structure of ice cream may be regarded as a four-phase system, which includes air bubbles, fat globules, ice crystals and an unfrozen serum phase. In this unfrozen serum phase, there exist the unadsorbed casein micelles in a suspension of sugars, salts, whey proteins and high molecular weight polysaccharides. The structure of ice cream characterizes its complexity as a result of several stages of manufacturing such as pasteurisation, homogenisation, maturation, freezing and hardening. The colloidal aspects of ice cream, such as interactions between protein and emulsifier, the partial coalescence of fat or interactions between protein and partially coalesced fat, allow for a better understanding of this unique system [Gelin *et al*., 1994; Goff, 1997, 2002; Goff & Hartel, 2013; Konstantas *et al*., 2019].

During the manufacturing process, the ice cream mix emulsion is foamed, the dispersed phase of air bubbles is formed, and frozen to obtain another dispersed phase of ice crystals. The ice cream mix contains fat droplets, coated with a protein-emulsifier layer as a dispersed phase. The ice cream mix can also be classified as emulsion-filled gel, in which macromolecular gel contains dispersed fat particles (fillers). Therefore, the structure and rheological properties of the ice cream mix can be affected

***Corresponding Author:**
e-mail anna_kot1@sggw.edu.pl (A. Kot)

Submitted: 1 December 2022
Accepted: 15 May 2023
Published on-line: 2 June 2023

© Copyright by Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences
© 2023 Author(s). This is an open access article licensed under the Creative Commons Attribution-NonCommercial-NoDerivs License (http://creativecommons.org/licenses/by-nc-nd/4.0/).

as a result of distinctive interactions of the filler gel matrix [Chen *et al*., 2019; Daw & Hartel, 2015; Goff, 1997; Innocente *et al*., 2009; Voronin *et al*., 2020].

Not only is the formation of the ice phase significant but stability is also equally crucial in the structure of ice cream. Desirable stability includes no changes in the size distribution or spatial arrangement of droplets. Stability plays a crucial role in controlling the shelf-life of ice cream or its texture and mouthfeel during consumption. It may be understood as the ability to resist undesirable changes in physicochemical properties. Loss of stability may occur due to various processes, such as creaming, flocculation, coalescence, partial coalescence, Ostwald ripening or phase inversion [Cheng *et al*., 2015; Dickinson, 1994, 2010; Pal, 2019]. The initial stability of emulsion in the ice cream mix is obtained by simultaneous adsorption of proteins and emulsifiers during the homogenisation process. Subsequently, during the maturation process, changes in the physical state occur in the liquid phase, not only in the emulsified fat but also in the adsorbed layer of mono- and diglycerides. Additionally other changes take place during maturation except for the mentioned process, such as the hydration of milk proteins and stabilisers, the crystallization of fat globules or the rearrangement of the surfactant membrane. They all contribute to a smoother texture and consequently better quality of ice cream [Gelin *et al*., 1994; Goff, 1997; Mendez-Velasco & Goff, 2012; Segall & Goff, 2002b].

Furthermore, the stability of emulsion in the ice cream mix may be considered through the prism of stabiliser addition. Despite the increasing viscosity, one of the main functions of stabilisers is to stabilise the emulsion and prevent separation [Akbari *et al*., 2019; Lomolino *et al*., 2020; Pintor & Totosaus, 2012; Seisun, 2010]. An example of such stabilisers is carrageenan that has been proved effective in preserving quiescent stability/shear instability in the emulsion with a low protein surface concentration [Segall & Goff, 2002a]. This happens as the migration of the milk protein from the solution to the oil-water interface in the emulsion is inhibited by carrageenans. Due to specific interaction with the milk proteins, carrageenans are employed in milk ice cream production. The favourable interactions between carrageenan and milk proteins depend on the number of sulphate groups in carrageenan structure and environmental conditions [Pintor & Totosaus, 2012]. ι-Carrageenan is one of the three forms of carrageenan (next to κ- and λ-carrageenan) that exhibits the specific ability to form a soft elastic gel in the presence of calcium ions, and stands out through other forms owing to the fact that it can form intra-molecular bonds between sulphate groups of anhydro-D-galactose and D-galactose *via* calcium as a divalent cation [Kiran-Yildrim *et al*., 2021; Thrimawithana *et al*., 2010]. Additionally, there are pieces of evidence that hydrolysates of carrageenan have a better ability to stabilize ice cream than native carrageenan. For instance, in the research by Kamińska-Dwórznicka *et al*. [2015] or Kot *et al*. [2022], it was noticed that hydrolysates of κ- and ι-carrageenan had more favourable effect on ice recrystallization inhibition (IRI) then κ-carrageenan in ice cream and ι-carrageenan in model solutions of ice cream, respectively.

This work focused on the influence of ι-carrageenan and its hydrolysates on the physical properties, such as stability, particle size distribution, consistency index and the flow behavior index, of milk ice cream mixes. Knowledge concerning these stabilisers in such products before freezing is lacking. Moreover, our previous research, in which the addition of ι-carrageenan and its acid and enzymatic hydrolysates allowed achieving desired size of crystals in a model milk solution, gave rise to the idea of using such stabilisers in this work [Kot *et al*., 2022]. This research might provide a valuable insight into the interactions between hydrocolloids and milk ice cream mixes.

MATERIALS AND METHODS

Materials

Pure ι-carrageenan powder was obtained from Sigma-Aldrich (St. Louis, MO USA). β-Galactosidase (1,000 U/mg, from *Escherichia coli*) and lactase (min. activity 5,200 NLU/g) for ι-carrageenan hydrolysis were purchased from Sigma-Aldrich and Serowar s.c. (Szczecin, Poland), respectively. The ingredients used to prepare the ice cream mixes were acquired from Mlekovita, Wysokie Mazowieckie, Poland (milk 0.5%, skimmed milk in powder); Orafti BENEO, Tienen, Belgium (inulin); Diamant, Poznań, Poland (white sugar); and Fooding Shanghai, Shanghai, China (emulsifier E471, locust bean gum (LBG), xanthan gum).

The preparation of hydrolysates of ι-carrageenan

The hydrolysis of ι-carrageenan was carried out according to the procedure described in our previous paper [Kot *et al*., 2022]. Briefly, ι-carrageenan was dissolved in distilled water heated up to 40°C to obtain a 0.4 mg/mL solution. The enzymatic hydrolysis was carried out using β-galactosidase for 72 h, at 37°C and a ratio of enzyme to ι-carrageenan solution of 1:1,000 (*v/v*) or using lactase for 24 h, at 5°C and enzyme to substrate ratio of 1:250 (*v/v*). For both hydrolyses, the reaction was stopped by neutralisation at 48°C for 5 min. To perform acid hydrolysis of ι-carrageenan, the substrate was dissolved in 0.1 M hydrochloric acid solution (pH 3). The solution was heated at 60°C for 3 h and then neutralised. All hydrolysates were stored frozen at –18° and thawed just before analysis. To characterise the hydrolysates, their molecular weight was determined by size-exclusion chromatography (SEC) according to the procedure described by Kamińska-Dwórznicka *et al*. [2015] using the Shimadzu high-performance liquid chromatography system consisting of a RID-10A detector, an LC-20 CE pump, a CTO-20A heater (Shimadzu, Kyoto, Japan) and equipped with a PolySep-GFC-P Linear column (300 mm x 7.8 mm, Phenomenex, Torrance, CA, USA). The molecular weight of samples after the hydrolysis by β-galactosidase ranged from 3.20×10^6 to 3.80×10^6 Da; that of hydrolysates obtained by commercial lactase from 3.50×10^6 to 3.60×10^6 Da, and that of acid hydrolysates from 1.48×10^6 to 1.94×10^6 Da.

The preparation of ice cream mixes

The ingredients of the ice cream mixes are presented in Table 1. The control sample (C) was prepared without stabilisers. The ice cream mix with the combination of ι-carrageenan, LBG

Table 1. The composition (%) of ice cream mixes without stabilisers (control, C), with ι-carrageenan (I) and with hydrolysates obtained by acid (A), β-galactosidase (B) and commercial lactase (L) treatment of ι-carrageenan.

Ingredient	C	I	A	B	L
Milk 0.5	76.0	75.49	75.495	75.495	75.495
Inulin	10.0	10.0	10.0	10.0	10.0
Milk powder	7.0	7.0	7.0	7.0	7.0
White sugar	7.0	7.0	7.0	7.0	7.0
Emulsifier E471	0.4	0.4	0.4	0.4	0.4
Locust bean gum	–	0.08	0.08	0.08	0.08
Xanthan gum	–	0.02	0.02	0.02	0.02
ι-Carrageenan	–	0.01	–	–	–
Acid hydrolysate of ι-carrageenan	–	–	0.005	–	–
ι-Carrageenan hydrolysate obtained by β-galactosidase treatment	–	–	–	0.005	–
ι-Carrageenan hydrolysate obtained by lactase treatment	–	–	–	–	0.005

and xanthan gum was coded as I. In turn, samples A, B and L instead of ι-carrageenan contained its acid hydrolysate or hydrolysates obtained using β-galactosidase and commercial lactase, respectively. All components were mixed using a Bosch Maxo -Mixx 750W blender (Bosch, Gerlingen, Germany). The next step involved pasteurization conducted in a Vorwerk thermomixer (Vorwerk, Wuppertal, Germany), at 85°C within 1.5 min. After this process, the mixtures were cooled to 25°C. Finally, the obtained ice cream mixes were matured for 24 h at 4°C in a refrigerator (Whirlpool, Warszawa, Poland). Two liters of ice cream mix were prepared for each experimental variant in duplicate.

Stability analysis of ice cream mixes

The analysis of the stability of ice cream mixes was performed using a Turbiscan Lab Expert device (Formulation SA, Toulouse, France). The Turbisoft 2.0.0.33 software was used to evidence the date of backscattering (BS) and calculate the Turbiscan stability index (TSI). The ice cream mixes were analysed before and after maturation in three replications.

The analysis of particle size distribution of ice cream mixes

The particle size distribution of the ice cream mixes before and after maturation was measured by laser diffraction using a Cilas 1190 analyser (Cilas, Orléans, France). Few drops of emulsion of milk ice cream mixes were suspended in water at an obscuration of 10%. The results were presented as the median diameter (D_{50}) and as diagrams of particle size distribution. Analyses of all samples were performed in three replications.

The rheological analysis of ice cream mixes

The rheological properties of the milk ice cream mixes were examined using the Haake Mars 40 rheometer (Thermo Scientific Inc., Karlsruhe, Germany) in a rotational mode within a shear rate of 0–100 s^{-1} at a constant temperature of 25°C. The analyses were performed in triplicate. The apparent viscosity (η_{app}) curve as a function of shear rate ($\dot{y}$) in the semi-logarithmic scale (the flow curves) was plotted by the RheoWin v.4.86. Job Manager (Thermo Scientific). Based on the obtained results, only Ostwald de Waele model (1) was used to describe the flow curves and to determine the flow behaviour index.

$$\eta_{app} = K\dot{y}^{n-1} \quad (1)$$

where: K is the consistency index (Pa×s^n), $\dot{y}$ is the shear rate (s^{-1}), and n is the flow behavior index (dimensionless).

The adequacy of fitted model was estimated using regression analysis which delivered the correlation coefficient (R).

Characterization of the ice cream mix emulsion using confocal laser scanning microscopy (CLSM)

The microstructure of the emulsion of the ice cream mix (before and after the maturation step) was analysed using a confocal laser scanning microscope FLUOVIEW FV300 (Olympus, Tokyo, Japan), according to the method described by Ahn *et al.* [2022]. The fluorescence dye – Nile red – was used to label lipids in the samples. An aliquot of 200 µL of the emulsion for all ice cream mixes and 3 µL of the Nile red solution (1 µg/mL) were mixed. Then, the samples were stained on glass slides, covered with a coverslip and observed at excitation and emission wavelengths of 630 and 660 nm, respectively. From every sample of ice cream mix, 6 photos were made and only representative ones were chosen for to results.

Statistical analysis

The results were expressed as a mean with standard deviations. The analysis of variance (ANOVA) was performed for the TSI,

the D_{50} and data from rheological properties. The analysis was performed using STATISTICA 13.3 software (Statsoft Polska, Kraków, Poland) with test significance at α=0.05. The differences between homogenous groups were assessed using the Tukey's honestly significant difference (HSD) test.

RESULTS AND DISCUSSION

The stability of ice cream mixes

The stability of milk ice cream mixes was scrutinised with the turbidimetric method, and respective results are presented as the TSI in Table 2 and as backscattering profile in Figure 1 (for control mix and mix with acid hydrolysate of ι-carrageenan) and in Figures S1–S3 in Supplementary Materials (for other ice cream mixes). The addition of stabilisers (ι-carrageenan and its hydrolysates) contributed to changes in the stability of milk ice cream mixes. The TSI ranged from 1.9 to 2.8. The lowest (p<0.05) value was noted for the sample with the addition of acid hydrolysate of ι-carrageenan (A). Moreover, it was noticed that there was no significant difference (p≥0.05) in the TSI of ice cream mixes with enzymatic hydrolysates of ι-carrageenan (B, after β-galactosidase treatment and L, after lactase treatment) and control sample without stabilisers (C). Nonetheless, the most striking observation was that the addition of ι-carrageenan contributed to the highest TSI value, which indicates slightly worse stability of the mix with ι-carrageenan compared to the other samples. Based on TSI, it may be stated that only the addition of acid hydrolysate of ι-carrageenan (A) contributed to significant improvement in the stability of ice cream mixes compared to the control sample. In research by Seo & Oh [2022], the TSI of ice cream mixes stabilized by the κ-carrageenan/milk protein isolate or Maillard conjugate derived from the reaction of the κ-carrageenan/milk protein isolate during 120 h of storage was less than 1.8.

The backscattering profiles (Figure 1, Figures S1–S3) showed that during maturation time, two phenomena occurred in the milk ice cream mixes. Firstly, it was noticed that the same type of destabilization, such as coalescence/flocculation, occurred in the control sample (C) and in the sample with the addition of ι-carrageenan (I) and that the intensity of backscattering

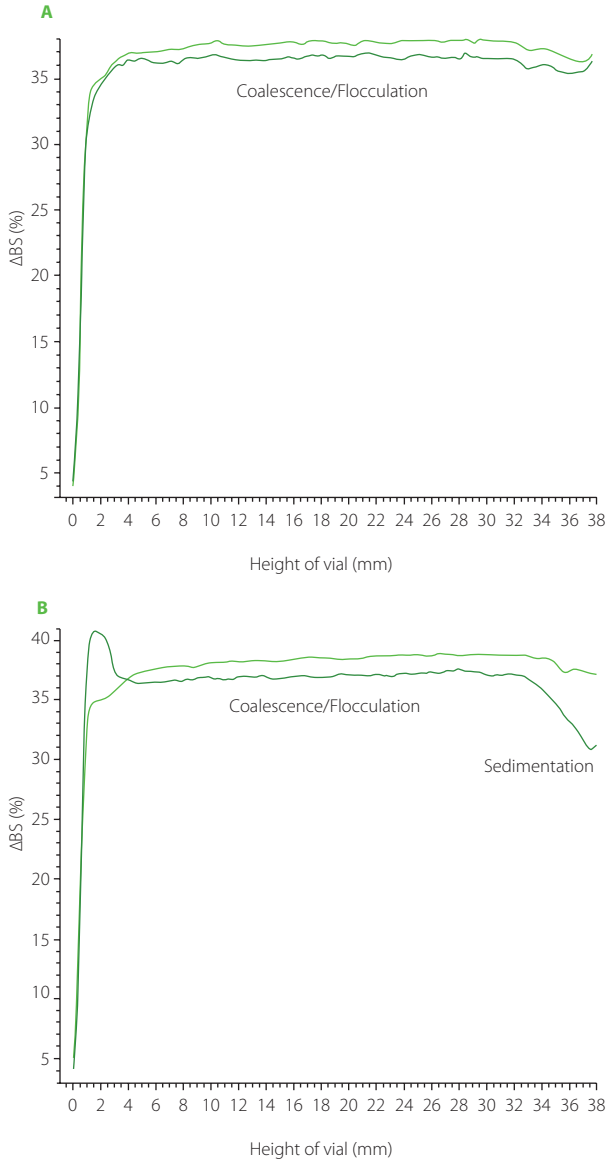


Figure 1. Backscattering (BS) profile of ice cream mixes without stabilisers (control sample) (**A**) and stabilised by the combination of acid hydrolysate of ι-carrageenan, locust bean gum and xanthan gum (**B**) before and after maturation. The dark green line presents measurement before maturation and the light green line presents measurement after maturation.

Table 2. The results of physical properties analysis of milk ice cream mixes with stabilisers before and after maturation.

Ice cream mix	TSI	Before maturation				After maturation			
		D_{50} (μm)	*K*	*n*	R	D_{50} (μm)	*K*	*n*	R
C	2.2±0.2^{b}	17.56±0.31^{c}	0.009±0.001^{d}	0.900±0.001^{a}	0.99	23.24±2.40^{c}	0.012±0.012^{b}	0.838±0.007^{a}	0.99
I	2.8±0.1^{a}	40.60±2.97^{ab}	0.061±0.005^{b}	0.738±0.022^{c}	0.99	35.57±2.63^{b}	0.059±0.058^{ab}	0.761±0.011^{ab}	0.99
A	1.9±0.1^{c}	45.50±2.37^{a}	0.059±0.005^{bc}	0.765±0.018^{bc}	0.99	46.73±0.19^{a}	0.058±0.057^{ab}	0.771±0.031^{ab}	0.99
B	2.2±0.1^{b}	37.05±2.34^{b}	0.048±0.001^{c}	0.798±0.005^{b}	0.99	34.73±0.90^{b}	0.050±0.049^{b}	0.803±0.016^{ab}	0.99
L	2.2±0.1^{b}	41.08±1.79^{ab}	0.083±0.006^{a}	0.727±0.014^{c}	0.99	35.16±1.23^{b}	0.104±0.103^{a}	0.702±0.083^{b}	0.99

Values are presented as mean ± standard deviations. Different superscript letters in column represent significant differences in the means (p<0.05). C, control ice cream mix (without stabilisers); I, ice cream mix with ι-carrageenan, locust bean gum (LBG) and xanthan gum; A, B and L, ice cream mixes with hydrolysates obtained by acid, β-galactosidase and commercial lactase treatment of ι-carrageenan, respectively, LBG and xanthan gum; TSI, Turbiscan stability index, D_{50}, median diameter, *K*, consistency index ($Pa{\cdot}s^{n}$), *n*, flow behaviour index; R, correlation coefficient.

was changing across the whole height of the vial. Additionally, observations of CLSM images of these samples before and after maturation (Figure 2) confirmed destabilisation of fat in mixes, as indicated by agglomerates visible in images Ia, Ib (the sample with the ι-carrageenan) and Ca, Cb (the control sample). Furthermore, except for coalescence/flocculation, also sedimentation occurred in the milk ice cream mixes with the addition of acid hydrolysates of ι-carrageenan (Figure 1); as evidenced by significant changes in the intensity of backscattering on the top and bottom and also slightly in the middle of the vial. These processes of destabilisation may take place simultaneously (droplets coalescing during sedimentation) or one by one (for instance, firstly small droplets grow by coalescence before sedimenting). The separation in ice cream mix with ι-carrageenan hydrolysate (β-galactosidase treatment) before maturation was captured in image Ba (Figure 2). The order of this process may depend on the droplet diameter, dispersed phase concentration or viscosity [Frising *et al*., 2008]. What should be highlighted here is the fact that after the maturation, the median diameter (D_{50}) for samples with the addition of ι-carrageenan or enzymatic hydrolysates decreased (Table 2). It is common knowledge that coalescence or flocculation occurs when the particle size increases not decreases [Goff *et al.,*1989]. However, as mentioned above, the agglomerates of fat of ice cream mixes were visible in CLSM images (Figure 2). In the research by Cheng *et al.* [2015], flocculation occurred despite a decrease in the average particle size after the maturation of ice cream mixes with different polysaccharide contents. In our research, despite the content of stabilisers and emulsifiers contributing to a decrease in particle size, the change was not significant enough to inhibit the mentioned type of destabilisation.

It was proven that a certain amount of fat destabilisation is covetable in frozen products, such as ice cream [Berger & White, 1971; Goff *et al*., 1989; Koxhlot *et al*., 2001, Liu *et al*., 2022]. As a result of a combination of sheer forces and ice crystallisation during the freezing process, fat globules are mechanically damaged and agglomeration or coalescence takes place. Consequently, such desirable destabilisation contributes to dry appearance, slow meltdown, good shape retention and finally a firmer texture. Albeit, the excessive coalescence of fat may be associated with poor whipping properties or a buttery texture. Furthermore, it is known that the ice cream mix is not only an example of dairy emulsion but also a foamed dairy emulsion [Stanley *et al*., 1996]. It means that the stability of such products is connected with the presence of air bubbles. In such cases, preventing air bubbles to grow in size or adjacent to coalescing should be considered in the whole conception of the stability of ice cream mixes. Additionally, Liu *et al*. [2022], proved that the higher the amount of fat aggregates in the serum phase, the greater the possibility of a 3D destabilization network formation in ice cream. In our research, destabilisation occurred in ice cream mixes; thus, it may be concluded that fat destabilisation at this step of production could produce conditions for the formation of more favourable and uniform ice crystal structures while freezing. Finally, in the present study, the ice cream mixes remained stables despite TSI value.

The particle sizes of ice cream mixes

The median diameter (D_{50}) of particles of milk ice cream mixes before and after maturation was presented in Table 2, and the particle size distribution in Figure 3. Before maturation, the D_{50} ranged from 17.56 to 45.50 μm. The lowest

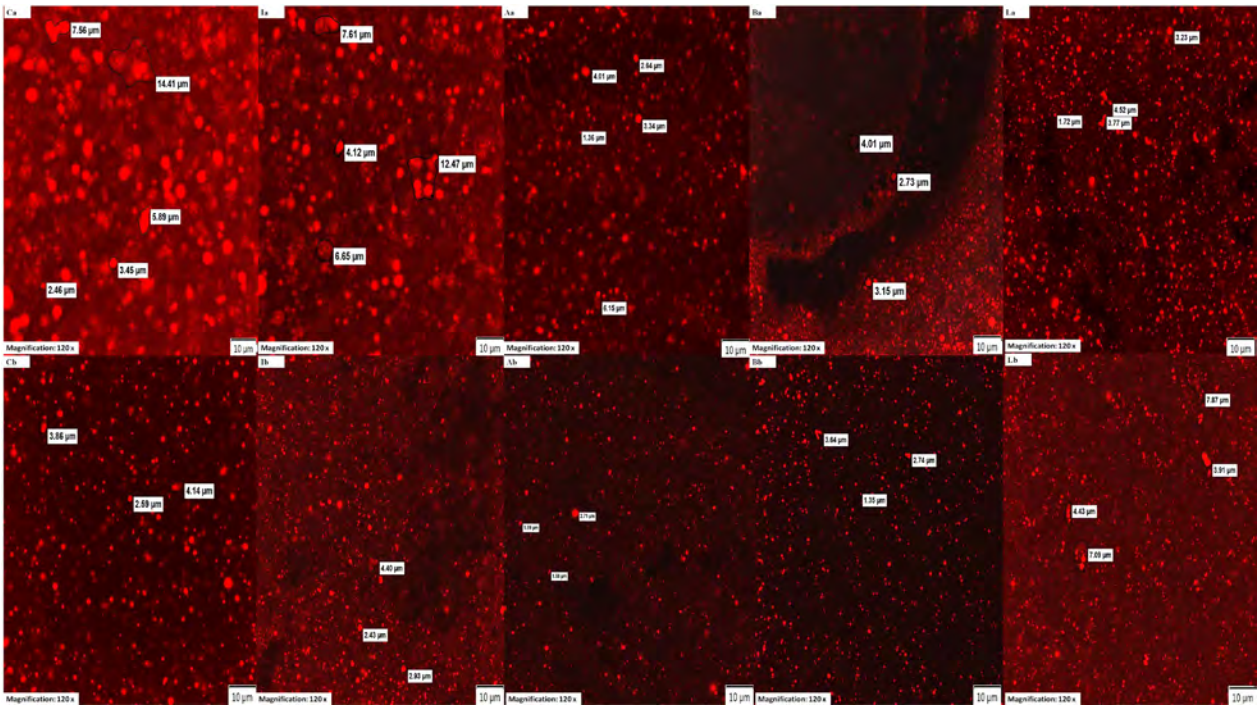


Figure 2. Confocal laser scanning microscopy images of ice cream mixes before (a) and after (b) maturation. C, control ice cream mix (without stabilizers); I, ice cream mix with ι-carrageenan, locust bean gum (LBG) and xanthan gum; A, B and L, ice cream mixes with hydrolysates obtained by acid, β-galactosidase and commercial lactase treatment of ι-carrageenan, respectively, LBG and xanthan gum.

(p<0.05) value of this parameter was noted for the control sample without stabilisers (C), whereas the highest one – for the ice cream mix with the addition of acid hydrolysate of ι-carrageenan (A); however, the values determined for the mixes with ι-carrageenan and with its hydrolysate obtained using lactase did not differ significantly (p≥0.05) from that of sample A. Overall, before the maturation, the addition of stabilisers contributed to the increase in the median diameter of ice cream mix particles. After maturation, the D_{50} ranged from 23.24 to 46.73 µm. The tendency of the variation of this parameter was the same as before maturation. In the control sample (C), the median diameter was at 23.34 µm and in the sample with the addition of acid hydrolysate of ι-carrageenan (A) it reached almost 47 µm. Nonetheless, some changes occurred compared to the values before maturation. The decrease in the size of particles was observed in the ice cream mixes with the addition of enzymatic hydrolysates of ι-carrageenan (B and L) and in the sample with ι-carrageenan (I). On the other hand, a slight increase of D_{50} was noticed in the mix with the acid hydrolysates of ι-carrageenan (A) and the control sample (C). As a result of maturation, the greatest reduction of median diameter (by almost 6 µm) was observed for the sample with the ι-carrageenan hydrolysate obtained using lactase (Table 2). Such changes are desirable to obtain the expected quality of the final product, especially in the context of the formation of ice crystals. In our previous study, vegan ice cream mixes with the same stabilisers were analysed [Kot *et al*., 2021]. Similar results were noticed, *i.e.*, the D_{50} of ice cream mixes decreased as a result of maturation, and only in the control sample was an increase observed. On the other hand, the size of the particles was smaller than in the present paper; the range of median diameter after maturation was from 17.23 to 28.50 µm. Overall, the reason for the large size of particles in ice cream mixes may be the addition of an emulsifier to the recipe. Based on the research by Liu *et al*. [2022], the fat aggregate size and also the fat aggregate percentage increased with the increased amount of the added emulsifier, such as Tweet 80, P4780 and whey protein isolate, to ice cream mixes. In the sample with the addition of 0.25% of emulsifier, the size of fat aggregates was from 1.6 to 66.3 µm. Bolliger *et al*. [2000] also discussed using emulsifiers in ice cream mixes and reported that the increasing amount of emulsifier contributed to the increased fat globule size or aggregate size of fat globules. Furthermore, in the samples without emulsifiers, no stable agglomerates were formed, while the only stable agglomerates were present in ice cream mixes with the addition of this ingredient. Alternatively, Alvarez *et al*. [2005] showed that maturation did not affect the size distribution of fat globules. The authors attributed it to a result of no noticeable destabilisation of emulsions during maturation due to the fact that the changes in the structure of emulsion, such as rearrangement of fat globule membranes, contributed to the lesser stability during maturation.

It is known that the changes in the structure of ice cream mixes, such as the arrangement of fat droplets, affect their texture as well as the melting stability of the final product

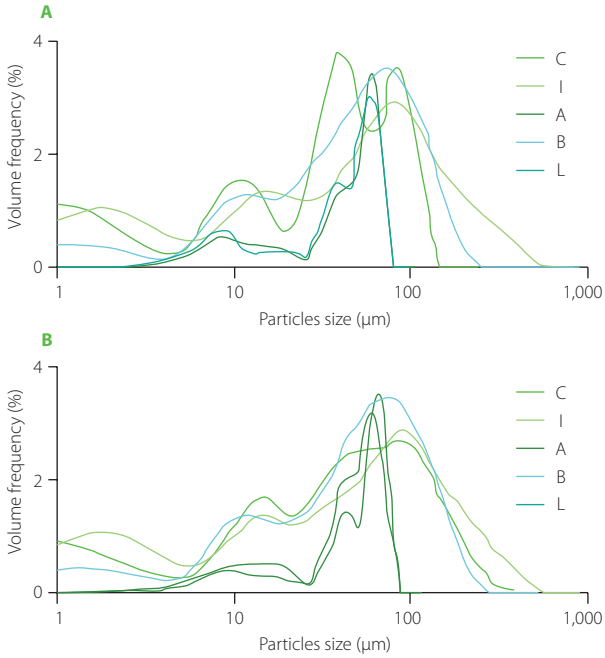


Figure 3. Particle size distribution of ice cream mixes before (**A**) and after (**B**) maturation. C, control ice cream mix (without stabilizers); I, ice cream mix with ι-carrageenan, locust bean gum (LBG) and xanthan gum; A, B and L, ice cream mixes with hydrolysates obtained by acid, β-galactosidase and commercial lactase treatment of ι-carrageenan, respectively, LBG and xanthan gum.

[Mendez-Velasco & Goff, 2012]. The uniform distribution of droplets may be the reason for kinetic instabilities such as creaming or sedimentation [Pal, 2019]. Therefore, the particle size distribution of ice cream mixes was analysed and the results are shown in Figure 2. Considering particle size distribution of the samples before maturation, four peaks were observed for the control sample (with particle size ranges of 0–2, 7–30, 50–80 and 100–120 µm), and the ice cream mix with the addition of hydrolysates obtained using acid and lactase (8–10, 20–40, 60–70, and 80–90 µm). While for the ice cream mixes with the addition of ι-carrageenan and ι-carrageenan after β-galactosidase treatment, three peaks were noted (particle size ranges for I sample: 2–5, 15–30 and 90–110 µm and for L sample: 2–5, 7–15 and 80-110 µm. Moreover, the range and location of peaks were similar for samples C, I, and B. The samples A and L, with the hydrolysates obtained using acid and lactase, respectively, had also similarities in particle size distribution. After maturation, four peaks in particle size distribution were noticed only for the control sample, whereas three-peaks were detected for the other samples (Figure 2). Among the samples with stabilisers, the significant differences in particle size distribution were only visible for the samples with the addition of ι-carrageenan after acid treatment (A) and after lactase treatment (L). In both mixes, particles in the size ranges 10–40, 50–70 and 80–90 µm were dominant. The frequency of particles with diameter less than 5 µm was low. The more frequent particles between 10 to 100 µm could represent fat agglomerates or fat coalescence. The same observation was made for molten ice creams; single fat droplets with a size of 1–1.5 µm and fat

clusters with a diameter of around 10 μm were determined [Liu *et al*., 2022]. Mendez-Velasco & Goff [2012] provided a deep analysis of the influence of the fat structure and properties of ice cream. Unsaturated monoglycerides (from sunflower oil) and saturated monoglycerides (from palm kernel oil) added to ice cream were tested. Based on particle size distribution, it was concluded that ice cream with unsaturated lipids formed larger fat networks with the higher number of droplets as stable particles, while ice cream with saturated lipids destabilized readily with smaller aggregates. The particles with diameters of 0.01–1 and around 100 μm dominated in ice cream with unsaturated lipids, while distribution of particle size was more homogenous in the ice cream with saturated lipids. In our study, the mix of saturated and unsaturated acids (mono- and diglycerides of fatty acids) was used as an emulsifier, which may explain such a wide range of particle sizes in all samples.

The rheological properties of ice cream mixes

The flow behavior and mouthfeel of ice cream are referred to as rheological properties. The rheological behaviour of milk ice cream mixes was described using the Ostwald de Waele model. The accuracy of this model fitting was high, as indicated by high correlation coefficients (Table 2). The rheological parameters of ice cream mixes before and after maturation are shown in Table 2. Data obtained for the samples before maturation shows that consistency index (*K*) ranged from 0.009 to 0.083. The addition of stabilisers contributed to an increase in its values owing to the fact that the lowest value was noted for the control sample. Considering the type of stabilizer used, the highest *K* was determined for the ice cream mix with the addition of ι-carrageenan after lactase treatment (L). In the case of the flow behaviour index of the samples before maturation, the lowest value was determined for L (0.727), but the values achieved for ice cream mix with ι-carrageenan and its acid hydrolysate were similar and did not differ significantly ($p \geq 0.05$) from that determined for L sample. The highest ($p < 0.05$) *n* was determined for the control sample (0.900), which allows concluding that higher *K* was obtained from the lower flow behaviour index. In the research by Atalar *et al*. [2021], flow curves plotted for the hazelnut vegan ice cream mixes were described using the Ostwald de Waele model, and the values of their flow behaviour index ranged from 0.61 to 0.76. Similar *n* values (0.65–0.73) were computed for vanilla ice creams with various contents of milk protein concentrate [Mostafavi *et al*., 2017].

After maturation, the same trend in the variation of *K* and *n* between samples as before maturation was observed. The highest *K* value (0.104) was determined for the ice cream mix with the addition of ι-carrageenan after lactase treatment and the lowest one (0.012) for the control sample. In the case of the flow behaviour index, the lowest value (0.702) was noted for the L sample and the highest one (0.838) for the control sample. Soukoulis *et al*. [2008] showed that the consistency index of the ice cream mixes with the addition of different hydrocolloids and κ-carrageenan ranged from 0.498 to 1.951, which was due to the distinctive ability of κ-carrageenan to form a gel with other hydrocolloids. Thus, the combination of carrageenan with guar gum or carboxymethyl-cellulose should be more favourable than the combination with xanthan gum or sodium alginate, which usually forms weak gel networks. Referring to the views of Alvarez *et al*. [2005], after maturation, an increase in viscosity was observed in milk ice cream mixes. Such a change was understandable due to the hydration of protein and stabilisers during maturation, which results in a change in viscosity.

According to the presented results for all ice cream mixes (before and after maturation), the *n* values were less than 1 (Table 2). It means that the ice cream mixes showed non-Newtonian shear-thinning (pseudoplastic) behaviour [Rao, 2007]. Previous research reported that ice cream mixes exhibited pseudoplastic behaviour due to the aggregated fat globules and polysaccharide stabilisers [Akbari *et al*., 2019]. Consequently, the viscosity decreased with an increasing shear rate.

After maturation, only the samples with the addition of enzymatic hydrolysates of ι-carrageenan (B and L) tended to increase their consistency index (Table 2). It may suggest the same mechanism of rheological behaviour in both ice cream mixes. Our previous study [Kot *et al*., 2022] proved that the mentioned enzymatic hydrolysates of ι-carrageenan were more flexible in comparison to ι-carrageenan or acid hydrolysate of ι-carrageenan in model solutions of ice cream. It may be the reason why the consistency of samples B and L was improved.

Microscopic analysis

The microscopic observation of emulsion may provide pivotal clues to understanding the relationship between microstructure and the stability of the emulsion [Ahn *et al.,* 2022]. The images from the CLSM analysis of the milk ice cream mix before and after maturation are presented in Figure 2. To observe the changes in the stability of samples, Nile red was used to stain the fat droplets. In all samples both before and after maturation, single particles of fat were visible with a size of around 5 μm. This is in agreement with the particle size distribution; the first peak was observed for the particles <10 μm (Figure 3) but larger fat agglomerates were visible in microscopic images as well (Figure 3). Their presence in ice cream mixes was also proven by previous analyses of the TSI and D_{50}. The sizes of agglomerates were around 10 μm or more. Such molecules were visible in the control sample and ice cream mix with the addition of ι-carrageenan before maturation (Figure 3 Ca and Ia, respectively). Additionally, fat droplet deformation was observed in the image of the control sample before maturation. This confirmed flocculation, which was suggested above based on the backscattering profile. Moreover, as can be seen from image Ba, phase separation in the sample with ι-carrageenan after β-galactosidase treatment (before maturation) occurred, which also confirmed the destabilisation of this milk ice cream mix. Frising *et al*. [2008] noticed in the picture of sedimentation of water-in-oil emulsion that droplets which were not evenly distributed tended to gather in more or fewer agglomerates. It means that flocculation may occur in this place later. In

our study, such phenomena could also be observed during maturation (Figure 2). Melted ice cream was also observed by confocal scanning laser microscopy after dynamic freezing [Voronin *et al*., 2020]. Based on the results, the control sample and the sample with the addition of polysorbate 80 (C-P80) were characterized by destabilized fat globules and partial coalescence, especially in the sample with the addition of an emulsifier. The same method was used to observe the protein and fat globules in ice cream mixes with polysaccharides [Cheng *et al*., 2015]. According to the results, flocculation was visible in the examined samples. In our research, the same or similar behaviour of fat destabilisation was observed.

CONCLUSION

The presented study showed that ι-carrageenan and its hydrolysates had a strong influence on the physical properties of milk ice cream mixes. They affected the stability of the emulsion during maturation. The hydrolysates of ι-carrageenan improved the stability of mixes contrary to ι-carrageenan as indicated by TSI. On the other hand, the addition of ι-carrageenan to the mixes affected two different types of destabilisation: coalescence and flocculation, while in the samples with hydrolysates of ι-carrageenan additionally, sedimentation was noted. Before and after maturation, aggregates of fats occurred and particles of distinctive sizes were observed in the ice cream mixes. The used stabilisers contributed to increasing the sizes of particles while maturation decreased it. The addition of the enzymatic hydrolysate of ι-carrageenan after commercial lactase treatment beneficially influenced the rheological properties of the ice cream mix.

It may be concluded that ι-carrageenan and its hydrolysates could serve as effective stabilisers in milk ice cream. However, further investigations are needed to explain the mechanism of their action as stabilisers and the behavior of fat in the presented recipe during maturation. The study ultimately proved that the type of stabilisers, in this case, ι-carrageenan and its hydrolysates, contributed to the destabilisation of ice cream mixes during maturation. Such a conclusion may be useful in planning the ice cream production process or predicting the formation of ice crystal structure.

SUPPLEMENTARY MATERIALS

These are available at http://journal.pan.olsztyn.pl/Effect-of-Iota-Carrageenan-and-Its-Hydrolysates-on-the-Stability-of-Milk-Ice-Cream,166382,0,2.html. Figure S1. Backscattering (BS) profile of ice cream mixes stabilised by the combination of ι-carrageenan, locust bean gum and xanthan gum before and after maturation. Figure S2. Backscattering (BS) profile of ice cream mixes stabilised by the combination of ι-carrageenan hydrolysate obtained using β-galactosidase, locust bean gum and xanthan gum before and after maturation. Figure S3. Backscattering (BS) profile of ice cream mixes stabilised by the combination of ι-carrageenan hydrolysate obtained using commercial lactase, locust bean gum and xanthan gum before and after maturation.

RESEARCH FUNDING

Research equipment was purchased as part of the "Food and Nutrition Centre - modernisation of the WULS campus to create a Food and Nutrition Research and Development Centre (CŻiŻ)" co-financed by the European Union from the European Regional Development Fund under the Regional Operational Programme of the Mazowieckie Voivodeship for 2014-2020 (Project No. RPMA.01.01.00-14-8276/17).

CONFLICT OF INTERESTS

Authors declare no competing interests.

ORCID IDS

M. Buniowska-Olejnik https://orcid.org/0000-0003-3795-4003
E. Jakubczyk https://orcid.org/0000-0002-4080-8851
A. Kamińska-Dwórznicka https://orcid.org/0000-0002-2835-2265
A. Kot https://orcid.org/0000-0003-3850-1743

REFERENCES

1. Ahn, N., Park, J-H., Chai, Ch., Imm, J-Y. (2022). The interaction of milk sphingomyelin and proteins on stability and microstructure of dairy emulsions. *Journal of Dairy Science*, *105*(5), 3832-3845. https://doi.org/10.3168/jds.2021-21253
2. Akbari, M., Eskandari, M.H., Davoudi, Z. (2019). Application and functions of fat replacers in low-fat ice cream: A review. *Trends in Food Science & Technology, 86*, 34-40. https://doi.org/10.1016/j.tifs.2019.02.036
3. Alvarez, V.B., Wolters, C.L., Vodovotz, Y., Ji, T. (2005). Physical properties of ice cream containing milk protein concentrates. *Journal of Dairy Science, 88*(3), 862-871. https://doi.org/10.3168/jds.S0022-0302(05)72752-1
4. Atalar, I., Kurt, A., Gul, O., Yazici, F. (2021). Improved physicochemical, rheological and bioactive properties of ice cream: Enrichment with higher pressure homogenized hazelnut milk. *International Journal of Gastronomy and Food Science*, *24*, art. no. 100358. https://doi.org/10.1016/j.ijgfs.2021.100358
5. Berger, K.G., White, G.W. (1971). An electron microscopical investigation of fat destabilization in ice cream. *International Journal of Food Science and Technology*, *6*(2), 285-294. https://doi.org/10.1111/j.1365-2621.1971.tb01617.x
6. Bolliger, S., Goff, H.D., Tharp, B.W. (2000). Correlation between colloidal properties of ice cream mix and ice cream. *International Dairy Journal, 10*(4), 303-309. https://doi.org/10.1016/S0958-6946(00)00044-3
7. Chen, W., Liang, G., Li, X., He, Z., Zeng, M., Gao, D., Qin, F., Goff, H.D., Chen, J. (2019). Effect of soy proteins and hydrolysates on fat globule coalescence and meltdown properties of ice cream. *Food Hydrocolloids, 94*, 279-286. https://doi.org/10.1016/j.foodhyd.2019.02.045
8. Cheng, J., Ma, Y., Li, X., Yan, T., Cui, J. (2015). Effects of milk protein-polysaccharide interactions on the stability of ice cream model systems. *Food Hydrocolloids, 45*, 327-336. https://doi.org/10.1016/j.foodhyd.2014.11.027
9. Daw, E., Hartel, R.W. (2015). Fat destabilization and melt-down of ice creams with increased protein content. *International Dairy Journal, 43*, 33-41. https://doi.org/10.1016/j.idairyj.2014.12.001
10. Dickinson, E. (1994). Emulsion stability. In K. Nishinari, E. Doi (Eds.). *Food Hydrocolloids: Structures, Properties and Functions*. Springer, Boston, MA, USA, pp. 387-398. https://doi.org/10.1007/978-1-4615-2486-1_61
11. Dickinson, E. (2010). Flocculation of protein-stabilized oil-in-water emulsions. *Colloids and Surfaces B: Biointerfaces*, *81*(1), 130-140. https://doi.org/10.1016/j.colsurfb.2010.06.033
12. Frising, T., Noïk, Ch., Dalmazzone, Ch., Peysson, Y., Palermo, T. (2008). Contribution of the sedimentation and coalescence mechanism to the separation of concentrated water-in-oil emulsions. *Journal of Dispersion Science and Technology*, *29*(6), 827-834. https://doi.org/10.1080/01932690701781501
13. Gelin, J.L., Poyen, L., Courthaudon, J.L., Meste, M.Le., Lorient, D. (1994). Structural changes in oil-water emulsions during the manufacture of ice cream. *Food Hydrocolloids, 8*(3-4), 299-308. https://doi.org/10.1016/S0268-005X(09)80342-1
14. Goff, H.D. (1997). Colloidal aspects of ice cream – A review. *International Dairy Journal*, *7*(6-7), 363-373. https://doi.org/10.1016/S0958-6946(97)00040-X

15. Goff, H.D. (2002). Formation and stabilisation of structure in ice cream and related products. *Current Opinion in Colloid and Interface Science*, *7*(5-6), 432-437. https://doi.org/10.1016/S1359-0294(02)00076-6
16. Goff, H.D., Hartel, R.W. (2013). Composition and Formulations. In H.D. Goff, R.W. Hartel (Eds)., *Ice Cream*, 7th ed.; Springer: New York, pp. 19-44. https://doi.org/10.1007/978-1-4614-6096-1
17. Goff, H.D., Kinsella, J.E., Jordan, W.K. (1989). Influence of various milk protein isolates on ice cream emulsion stability. *Journal of Dairy Science*, *72*(2), 385-397. https://doi.org/10.3168/jds.S0022-0302(89)79120-7
18. Innocente, N., Biasutti, M., Venir, E., Spaziani, M., Marchesini, G. (2009). Effect of high-pressure homogenization on droplet size distribution and rheological properties of ice cream mixes. *Journal of Dairy Science, 92*(5), 1864-1875. https://doi.org/10.3168/jds.2008-1797
19. Kamińska-Dwórznicka, A., Antczak, A., Samborska, K., Lenart, A. (2015). Acid hydrolysis of kappa-carrageenan as a way of gaining new substances for freezing process modification and protection from excessive recrystallization of ice. *International Journal of Food Science and Technology, 50*(8), 1799-1806. https://doi.org/10.1111/ijfs.12820
20. Kiran-Yildrim, B., Hale, J., Wefers, D., Gaukel, V. (2021). Ice recrystallisation inhibition of commercial κ-, ι-, λ-carrageenans. *Journal of Food Engineering, 290*, art. no. 110269. https://doi.org/10.1016/j.jfoodeng.2020.110269
21. Konstantas, A., Stamford L., Azpagic, A. (2019). Environmental impacts of ice cream. *Journal of Cleaner Production, 209*, 259-272. https://doi.org/10.1016/j.jclepro.2018.10.237
22. Kot, A., Kamińska-Dwórznicka, A., Antczak, A., Jakubczyk, E., Matwijczuk, A. (2022). Effect of ι-carrageenan and its acidic and enzymatic hydrolysates on ice crystal structure changes in model sucrose solution. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 643*, art. no. 128744. https://doi.org/10.1016/j.colsurfa.2022.128744
23. Kot, A., Kamińska-Dwórznicka, A., Galus, S., Jakubczyk, E. (2021). Effects of different ingredients and stabilisers on properties of mixes based on almond drink for vegan ice cream production. *Sustainability, 13*(21), art. no. 12113. https://doi.org/10.3390/su132112113
24. Koxholt, M.M.R., Eisenmann, B., Hinrichs, J. (2001). Effect of the fat globule sizes on the meltdown of ice cream. *Journal of Dairy Sciences*, *84*(1), 31-37. https://doi.org/10.3168/jds.S0022-0302(01)74448-7
25. Liu, X., Sala, G., Scholten, E. (2022). Effect of fat aggregate and percentage of the melting properties of ice cream. *Food Research International,* 160, art. no. 111709. https://doi.org/10.1016/j.foodres.2022.111709
26. Lomolino, G., Zannoni, S., Zabara, A., Da Lio, M., De Iseppi, A. (2020). Ice recrystallisation and melting in ice cream with different proteins levels and subjected to thermal fluctuation. *International Dairy Journal, 100*, art. no. 104557. https://doi.org/10.1016/j.idairyj.2019.104557
27. Mendez-Velasco, C., Goff, H.D. (2012). Fat structures as affected by unsaturated or saturated monoglyceride and their effect on ice cream structure, texture and stability. *International Dairy Journal*, *24*(1), 33-39. https://doi.org/10.1016/j.idairyj.2011.11.009
28. Mostafavi, F.S., Tehrani, M.M., Mohebbi, M. (2017). Rheological and sensory properties of fat reduced vanilla ice creams containing milk protein concentrate (MPC). *Journal of Food Measurement and Characterization, 11*, 567-575. https://doi.org/10.1007/s11694-016-9424-y
29. Pal, R. (2019). Modeling of sedimentation and creaming in suspensions and pickering emulsions. *Fluids*, *4*(4), art. no. 186. https://doi.org/10.3390/fluids4040186
30. Pintor, A., Totosaus, A. (2012). Ice cream properties affected by lambda-carrageenan or iota-carrageenan interactions with locust bean gum/carboxymethylcellulose mixtures. *International Food Research Journal, 19*(4), 1409-1414.
31. Rao, M.A. (2007). Introduction: Food rheology and structure. In M.A. Rao (Ed.) *Rheology of Fluid and Semisolid Foods: Principles and Applications.* Springer Science & Business Media: New York, NY, USA, pp. 1-26. https://doi.org/10.1007/978-0-387-70930-7
32. Segall, K.I., Goff, H.D. (2002a). Secondary adsorption of milk proteins from the continuous phase to the oil-water interface in dairy emulsions. *International Dairy Journal*, *12*(11), 889-897. https://doi.org/10.1016/S0958-6946(02)00116-4
33. Segall, K.I., Goff, H.D. (2002b). A modified ice cream processing routine that promotes fat destabilization in the absence of added emulsifier. *International Dairy Journal*, *12*(12), 1013-1018. https://doi.org/10.1016/S0958-6946(02)00117-6
34. Seisun, D. (2010). Chapter 1 – Introduction. In A. Imeson (Edd.). *Food Stabilisers, Thickeners and Gelling Agents*. Blackwell Publishing Ltd, United Kingdom, pp.1-10. https://doi.org/10.1002/9781444314724.ch1
35. Seo, C.W., Oh, N.S. (2022). Functional application of Maillard conjugate derived from a κ-carrageenan/milk protein isolate mixture as a stabilizer in ice cream. *LWT – Food Science and Technology, 161*, art. no. 113406. https://doi.org/10.1016/j.lwt.2022.113406
36. Soukoulis, Ch., Chandrinos, I., Tzia, C. (2008). Study of the functionality of selected hydrocolloids and their blends with κ-carrageenan on storage quality of vanilla ice cream. *LWT – Food Science and Technology, 41*(10), 1816-1827. https://doi.org/10.1016/j.lwt.2007.12.009
37. Stanley, D.W., Goff, H.D., Smith, A.K. (1996). Texture-structure relationships in foamed dairy emulsions. *Food Research International*, *29*(1), 1-13. https://doi.org/10.1016/0963-9969(95)00063-1
38. Thrimawithana, T.R., Young, S., Dunstan, D.E., Alany, R.G. (2010). Texture and rheological characterization of kappa and iota carrageenan in the presence of counter ions. *Carbohydrate Polymers, 82*(1), 69-77. https://doi.org/10.1016/j.carbpol.2010.04.024
39. Voronin, G.L., Ning, G., Coupland, J.N., Roberts, R., Harte, F.M. (2020). Freezing kinetics and microstructure of ice cream from high-pressure-jet processing of ice cream mix. *Journal of Dairy Sciences*, *104*(3), 2843-2854. https://doi.org/10.3168/jds.2020-19011

applied sciences

MDPI

Article

The Effectiveness of Combination Stabilizers and Ultrasound Homogenization in Milk Ice Cream Production

Anna Kot *, Ewa Jakubczyk and Anna Kamińska-Dwórznicka *

Department of Food Engineering and Process Management, Institute of Food Sciences, Warsaw University of Life Sciences (WULS-SGGW), Nowoursynowska 159C, 02-776 Warsaw, Poland; ewa_jakubczyk@sggw.edu.pl
* Correspondence: anna_kot1@sggw.edu.pl (A.K.); anna_kaminska1@sggw.edu.pl (A.K.-D.)

Abstract: This study aims to contribute knowledge to the area of the ice cream industry by finding an effective way to prevent the recrystallization process in ice cream production. Stabilizers such as ι-carrageenan and its acid and enzymatic hydrolyzates were used with the combination of ultrasound homogenization (20 kHz and exposure time of 5 min) as a method to obtain the deliberate quality of ice cream. In this paper, a comprehensive analysis of the physical characteristic of milk ice creams was made, such as the cryoscopic temperature, osmotic pressure, overrun, and melting time. It was noted that cryoscopic temperature was lower in the samples after ultrasound treatment. Additionally, the osmotic pressure was changed in the case of the stabilizer used. The overrun of ice cream was less than 32% while the longest melting time was at the level of 27 min. The recrystallization process was analysed on the basis of images taken after 24 h, and 1 and 3 months of storage at −18 °C. Regarding the results, it was observed that ultrasound homogenization contributed to smaller ice crystals and had a positive influence on the ice crystals' structure.

Keywords: recrystallization; ice crystals; ultrasound; freezing; physical properties

check for updates

Citation: Kot, A.; Jakubczyk, E.; Kamińska-Dwórznicka, A. The Effectiveness of Combination Stabilizers and Ultrasound Homogenization in Milk Ice Cream Production. *Appl. Sci.* **2023**, *13*, 7561. https://doi.org/10.3390/app13137561

Academic Editors: Agnieszka Najda, Joanna Maria Klepacka and Marta Czarnowska-Kujawska

Received: 19 May 2023
Revised: 17 June 2023
Accepted: 23 June 2023
Published: 27 June 2023

Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Ice cream is defined as a complex colloidal system consisting of air bubbles, ice crystals, and fat droplets dispersed into the serum phase. Considered the category of dairy products, ice cream is one a palatable frozen dessert. Obtaining the desirable texture to meet the expectations of consumers is a requisite for manufacturers [1–4]. Perception of ice cream texture is connected with its creaminess. One of the factors attributed to this property is the crystal structure, especially the number of crystals and their size. Such a factor is inevitably connected with the components of ice cream and the production process [2,5]. The advisable size of ice crystals is between 10 to 20 µm, which guarantees achieving a favourable texture. Because larger crystals of more than 50 µm cause undeniable quality such as coarse or grainy [6–9], it is thus significant to understand the factors that affect it and how they are regulated.

Stabilizers have the ability to modify the water-binding capacity, freezing rates, rheological properties, or ice crystal formation. Polysaccharide stabilizers such as carrageenans, locust bean gum, or xanthan gum are usually used in the formulation of the ice crystal structure [10]. Carrageenan is a commonly used secondary stabilizer and, additionally, it helps prevent the process of wheying off. The fractions of carrageenan such as ι-carrageenan are able to react electrostatically with milk proteins and form a three-dimensional network. Such a structure contributes to the resistance separation of the suspended phase in ice cream mixes [10–12]. Moreover, interesting results were obtained using acid and hydrolyzates of ι-carrageenan in the model sucrose solutions with milk protein. It was proven that hydrolyzates more effectively inhibited the recrystallization process in the ice crystal structure than ι-carrageenan [13].

Ultrasound has been known in the food industry for many years. The mechanism of ultrasound is based on acoustic cavitation, which occurs due to the interaction between

ultrasonic waves, liquid, and dissolved gas. Considering the advantages of this technique, ultrasound is relatively cheap, simple, really fast, non-toxic, environmentally friendly, and also energy saving. Additionally, ultrasound can be used to minimize processing or increase quality and improve processing effectiveness and efficiency, as well as to provide food safety, while extending the shelf life of the product [14–16]. Currently, there is much research on which ultrasound can be used in ice cream production. For instance, during the freezing process, ultrasound may enhance the nucleation rate and the rate of crystal growth, which contribute to decreasing the ice crystal size and freezing time [15,17–19]. On the other hand, the influence of used ultrasound homogenization in milk ice cream and the changes during the creation of the ice crystal structure still require additional research. Moreover, it has been proven that using ultrasound during the pasteurization process can be used interchangeably with traditional pasteurization, with no undesirable changes in ice cream [20]. Furthermore, the study by Tüker and Dogan [21] shows that ultrasound homogenization improved the properties of ice cream such as the melting time. Ultrasound homogenization may generate positive results such as narrowing the particle size, which generates a more stable emulsion in ice cream. Based on this, considering the amount of fat and its structure in ice cream mixes might bring a beneficial effect on the ice crystal size, owing to the fact less space will be created for crystal formation and smaller ice crystals will be obtained [10]. Therefore, ultrasound may be a promising tool for inhibiting the recrystallization phenomena in ice cream, obtaining smaller crystals and improving the quality of the product.

Much of the available literature deals with the problem of recrystallization in ice cream; therefore, the results presented in this paper could be valuable in this field. The following two main points were studied: (1) the effect of using hydrocolloids namely ι-carrageenan and its acid and enzymatic hydrolyzates on the physical properties of milk ice cream, and (2) the effectiveness of ultrasound homogenization in the production of milk ice cream.

2. Materials and Methods

2.1. The Preparation of the Hydrolyzates of ι-Carrageenan

The materials and methods for the hydrolysis of the ι-carrageenan part were described in the paper by Kot et al. [13]. Briefly, ι-carrageenan (obtained from Sigma-Aldrich, St. Louis, MO, USA) was dissolved in distilled water heated up to 40 °C to obtain a 0.4 mg/mL solution. The enzymatic hydrolysis was carried out using β-galactosidase (1000 U/mg, from *Escherichia coli)* (Sigma-Aldrich, St. Louis, MO, USA) for 72 h, at 37 °C or using lactase (min. activity 5200 NLU/g) (Serowar s.c., Szczecin, Poland) for 24 h, at 5 °C. For both hydrolyses, the reaction was stopped by neutralization at 48 °C for 5 min. The acid hydrolysis of ι-carrageenan was performed by dissolving in 0.1 M hydrochloric acid (Chempur, Piekary Śląskie, Poland) solution (pH 3). Then, the solution was heated at 60 °C for 3 h and neutralized using 0.1 M sodium hydroxide (Chempur, Piekary Śląskie, Polska). All of the obtained hydrolyzates were stored frozen at –18° and thawed just before analysis. The molecular mass distribution was estimated through Size-exclusion chromatography analysis (SEC) using the Shimadzu high-performance liquid chromatography system consisting of a RID-10A detector (Shimadzu, Kyoto, Japan), and only hydrolyzates with the highest reduction in molecular mass were used for further analysis.

Moreover, to confirm that the IRI (ice recrystallization inhibition) activity of the obtained hydrolyzates of the ι-carrageenan depends on the functional group's position changes, Fourier transform infrared spectroscopy (FTIR) analysis was performed using a HATR Ge trough (45° cut, yielding 10 internal reflections) crystal plate at 20 °C, and recorded with a 670-IR spectrometer (Agilent, Santa Clara, CA, USA). Based on these results, only the samples with the highest molecular mass reduction and the longest time for hydrolysis were used for further research as a stabilizer in ice cream.

2.2. The Process Production of Ice Cream

The Materials for the Recipe for Ice Cream

The ingredients used to prepare the ice cream mixes were milk 0.5% (Mlekovita, Wysokie Mazowieckie, Poland), inulin (Orafti BENEO, Tienen, Belgium), skimmed milk in powder (Mlekovita, Wysokie Mazowieckie, Poland), white sugar (Diamant, Poznań, Poland), emulsifier E471 (Fooding Shanghai, Shanghai, China), LBG (Locust Bean Gum) (Fooding Shanghai, Shanghai, China), 0.02% xanthan gum (Fooding Shanghai, Shanghai, China), 0.01% ι-carrageenan (Fluka, Sigma-Aldrich, St. Louis, MO, USA), or 0.005% newly obtained acid hydrolyzates of ι-carrageenan and enzymatic hydrolysis by β-galactosidase and enzymatic hydrolysis by commercial lactase.

Based on the research by [22], an amount of ι-carrageenan of 0.01% is the minimal concentration that could influence the recrystallization process. While the amount of obtained hydrolyzates at 0.005% was connected with the fact that hydrolyzates of ι-carrageenan had the strongest effect compared with the ι-carrageenan. The percentage amount of each ingredient is presented in Table 1.

Table 1. The percentage composition of ice cream milk samples.

Ingredient	C, CH, CU	I, IH, IU	A, AH, AU	B, BH, BU	L, LH, LU
Milk 0.5	76.0	75.49	75.495	75.495	75.495
Inulin	10.0	10.0	10.0	10.0	10.0
Milk powder	7.0	7.0	7.0	7.0	7.0
White sugar	7.0	7.0	7.0	7.0	7.0
Emulsifier E471	0.4	0.4	0.4	0.4	0.4
Locust bean gum	-	0.08	0.08	0.08	0.08
Xanthan gum	-	0.02	0.02	0.02	0.02
ι-Carrageenan	-	0.01	-	-	-
Acid hydrolysate of ι-carrageenan	-	-	0.005	-	-
Enzymatic hydrolyzate of ι-carrageenan obtained by β-galactosidase treatment	-	-	-	0.005	
Enzymatic hydrolyzate of ι-carrageenan obtained by lactase treatment	-	-	-	-	0.005

The characteristics of the prepared samples of the ice cream mixes are presented in Table 2.

Table 2. The characteristic of the ice cream milk samples.

Sample	Stabilizers	Homogenization Treatment
C	Control sample without stabilizers	-
CH	Control sample without stabilizers	traditional homogenization treatment
CU	Control sample without stabilizers	ultrasound homogenization treatment
I	Sample with the combination of ι-carrageenan, LBG and xanthan gum	-
IH	Sample with the combination of ι-carrageenan, LBG and xanthan gum	traditional homogenization treatment
IU	Sample with the combination of ι-carrageenan, LBG and xanthan gum	ultrasound homogenization treatment

Table 2. *Cont.*

Sample	Stabilizers	Homogenization Treatment
A	Sample with the combination of acid hydrolyzates of ι-carrageenan, LBG and xanthan gum	-
AH	Sample with the combination of acid hydrolyzates of ι-carrageenan, LBG and xanthan gum	traditional homogenization treatment
AU	Sample with the combination of acid hydrolyzates of ι-carrageenan, LBG and xanthan gum	ultrasound homogenization treatment
B	Sample with the combination of enzymatic β-galactosidase hydrolyzates of ι-carrageenan, LBG and xanthan gum	-
BH	Sample with the combination of enzymatic β-galactosidase hydrolyzates of ι-carrageenan, LBG and xanthan gum	traditional homogenization treatment
BU	Sample with the combination of enzymatic β-galactosidase hydrolyzates ι-carrageenan, LBG and xanthan gum	ultrasound homogenization treatment
L	Sample with the combination of enzymatic commercial lactase hydrolyzates of ι-carrageenan, LBG and xanthan gum	-
LH	Sample with the combination of enzymatic commercial lactase hydrolyzates of ι-carrageenan, LBG and xanthan gum	traditional homogenization treatment
LU	Sample with the combination of enzymatic commercial lactase hydrolyzates of ι-carrageenan, LBG and xanthan gum	ultrasound homogenization treatment

2.3. The Production of Ice Cream

According to the recipe, the dry and liquid ingredients were weighed separately. After this, all components were mixed using a Bosch MaxoMixx 750W blender (Bosch, Gerlingen, Germany). Then, the pasteurization process was performed using a Vorwerk thermomixer (Vorwerk, Wuppertal, Germany) at a temperature of 85 °C within 1.5 min and then cooled to 25 °C. Two methods of homogenization were used:

- The traditional homogenization using the homogenizer IKA T 25 digital ULTRA-TURRAX 20 rpm (IKA®-Werke GmbH & Co. KG, Staufen, Germany) through 2.5 min.
- The ultrasound homogenization by using a homogenizer Ultrasonic Liquid Processor VCX 500 (Sonics & Materials, Inc., Newtown, CT, USA) with a diameter probe (Model CV334). 250 mL of ice cream mixes for each trial. The frequency of 20 kHz and exposure time of 5 min was used. The used frequency of ultrasound was also tested in accordance with other papers such as the paper by O'Sullivan et al. [23] for the ultrasound homogenization on soy and wheat protein isolates and the paper by de Silva et al. [24] during ultrasound homogenization of cupuaçu juice.

After the homogenization step, all of the prepared ice cream mixes were submitted to the maturation process for 24 h at 4 °C (fridge, Whirlpool, Warszawa, Poland).

2.4. The Freezing of Ice Cream

The freezing of ice cream mixes was performed in an ice cream maker, Neumaker Gelato 5K SC (Hemer, Germany), until the ice cream temperature was −7 °C for 15 min. Then, the samples were placed in plastic containers and stored at −18 °C for 24 h, 1 month, and 3 months (freezer, Whirlpool, Warszawa, Poland).

2.5. The Ice Cream's Physical Analysis

2.5.1. Cryoscopic Temperature and Osmolality

The cryoscopic temperature and the osmolality of the ice cream mixes were determined using an osmometer Marcel os3000 (Warszawa, Poland). The accuracy of measurement of the freezing temperature was 0.002 °C and for osmolality it was 1%. According to the instruction of the devices, 100 µL of ice cream mixes after the maturation process were taken into Eppendorf tubes and measured until the device was stabilized. The analysis was performed in duplicate.

2.5.2. Melting Time

The melting behaviour of the ice cream was determined using a cooled metal ring (11 cm in height and 2 cm in diameter, volume 35 mL) that was stored at −25 °C for 24 h before measurement. After this time, the ring was filled with ice cream directly after the freezing process and then stored for 24 h at −25 °C. After storage, the ring was placed on the funnel with two pins located at the ends of the ring at the controlled temperature of 25 °C. The first drop of melted ice cream was recorded as the melting time of the sample [25,26]. The analysis was performed in duplicate.

2.5.3. Determination of the Overrun

The overrun of ice cream was determined according to the following Formula (1) [25,26]:

$$\text{Overrun} = \frac{W_1 - W_2}{W_2} \times 100\% \quad (1)$$

where: W_1 is the mass of the unit volume of the mixture (g) and W_2 is the mass of the unit volume of ice cream (g).

2.5.4. Microstructural Analysis of Ice Crystals

The microstructure of the ice crystals was analysed based on the images taken after 24 h, 1 month, and 3 months of storage at −18 °C. To prepare the samples, a small amount of ice cream was taken from the centre of the plastic box (from at least three different locations, and a minimum of 3 cm away from the surface), then put on the cool slide using a spatula and covered with a cool slip glass on the top of the sample. All of the samples were prepared in a freezing chamber and transferred to a microscope with the cooling system Linkam Scientific PE 94.

The recrystallization process was analysed based on images taken using the Olympus model BX43F (Tokyo, Japan) microscope with the cooling system with liquid nitrogen-Linkam Scientific Instruments LTD model LNP96-S (Tokyo, Japan) and the Olympus model SC50 camera (Tokyo, Japan). The obtained images were analysed using the Olympus cellSens Dimension Desktop program. The around 300 ice crystals were marked for one sample, and then the area, equivalent diameter, and standard deviation were calculated.

2.5.5. The Statistical Analysis

For the melting time, overrun, cryoscopic temperature, and osmolality, a statistical analysis was performed. The data are expressed a mean with standard deviations (±SD) in Table 3. The results were analysed using the analysis of variance one-way ANOVA. Tukey's test was used to determine if the differences between the parameters of the ice cream samples were significant. The statistical appraisal was performed using the STATISTICA 13.3 software (Statsoft Polska, Kraków, Poland). The significance of the test was set at $\alpha = 0.05$.

Table 3. The physical analysis of the milk ice cream.

Sample	Cryoscopic Temperature, °C	Osmotic Pressure, mOsm/kg	Overrun, %	Melting Time, min.
C	−2.502 ± 0.006 ^{fg}	1347 ± 4 ^{ab}	15.35 ± 3.52 ^{bcde}	23.19 ± 2.23 ^{cde}
CH	−2.549 ± 0.005 ^{defg}	1372 ± 3 ^{d}	8.30 ± 0.49 ^{a}	22.25 ± 3.05 ^{bcde}
CU	−2.510 ± 0.018 ^{efg}	1355 ± 5 ^{ab}	17.53 ± 2.16 ^{de}	22.37 ± 3.01 ^{cde}
I	−2.486 ± 0.013 ^{g}	1358 ± 0 ^{bc}	24.40 ± 0.00 ^{f}	25.14 ± 0.97 ^{de}
IH	−2.531 ± 0.033 ^{defg}	1346 ± 7 ^{a}	10.90 ± 1.94 ^{abcd}	27.46 ± 1.34 ^{de}
IU	−2.519 ± 0.013 ^{defg}	1348 ± 4 ^{ab}	20.59 ± 0 ^{ef}	26.23± 2.09 ^{de}
A	−2.611 ± 0.044 ^{de}	1422 ± 0 ^{f}	18.86 ± 3.77 ^{ef}	28.26 ± 2.36 ^{de}
AH	−2.921 ± 0.008 ^{a}	1572 ± 4 ^{i}	9.05 ± 0.00 ^{ab}	27.33 ± 0.00 ^{de}
AU	−2.512 ± 0.066 ^{efg}	1377 ± 0 ^{de}	15.72 ± 0.05 ^{bcde}	30.34 ± 0.00 ^{e}
B	−2.721 ± 0.022 ^{bc}	1456 ± 0 ^{g}	31.79 ± 0.86 ^{g}	12.34 ± 0.00 ^{abc}
BH	−2.608 ± 0.025 ^{def}	1413 ± 0 ^{f}	16.06 ± 0.37 ^{cde}	12.17 ± 5.06 ^{ab}
BU	−2.754 ± 0.020 ^{b}	1490 ± 0 ^{h}	18.27 ± 0.00 ^{ef}	11.23 ± 0.45 ^{a}
L	−2.578 ± 0.000 ^{defg}	1388 ± 0 ^{e}	14.32 ± 0.99 ^{abcde}	18.15 ± 1.85 ^{abcd}
LH	−2.622 ± 0.026 ^{cd}	1421 ± 0 ^{f}	10.63 ± 2.33 ^{abc}	20.13 ± 1.85 ^{abcd}
LU	−2.535 ± 0.017 ^{defg}	1368 ± 4 ^{cd}	20.43 ± 1.10 ^{ef}	23.19 ± 5.89 ^{de}

Different superscript letters in columns represent significant differences in the means of the same parameter ($p < 0.05$). Values represent means ± standard deviations.

The frequency distribution of ice crystal size was calculated using Microsoft Excel 2011 macro data analysis. The relative frequency of any class interval was calculated as the number of crystals in that class (class frequency) divided by the total number of crystals, and expressed as a percentage (Figures 1–3). According to the method described by Regand and Goff [27], the parameter X_{50} was analysed as the mean diameter (D_A) for 50% of the crystals in the sample. The mean diameter (D_A) and standard deviations (SD) of each class were also calculated (Table 4). The method has been described previously [7,13].

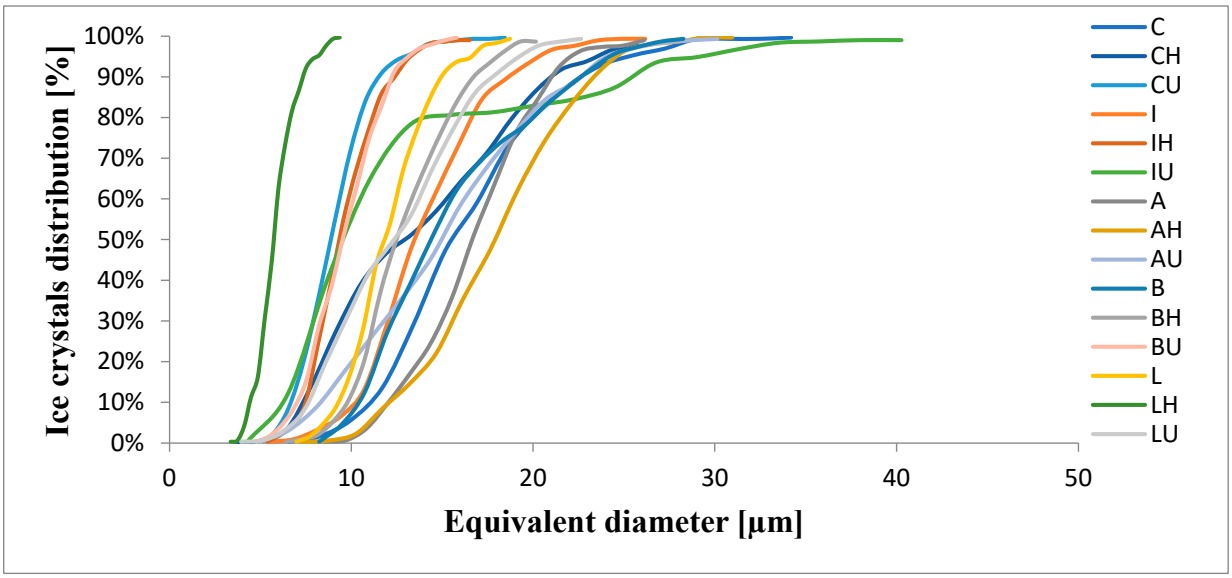


Figure 1. Ice crystal size distribution in ice cream after 24 h of storage at −18 °C.

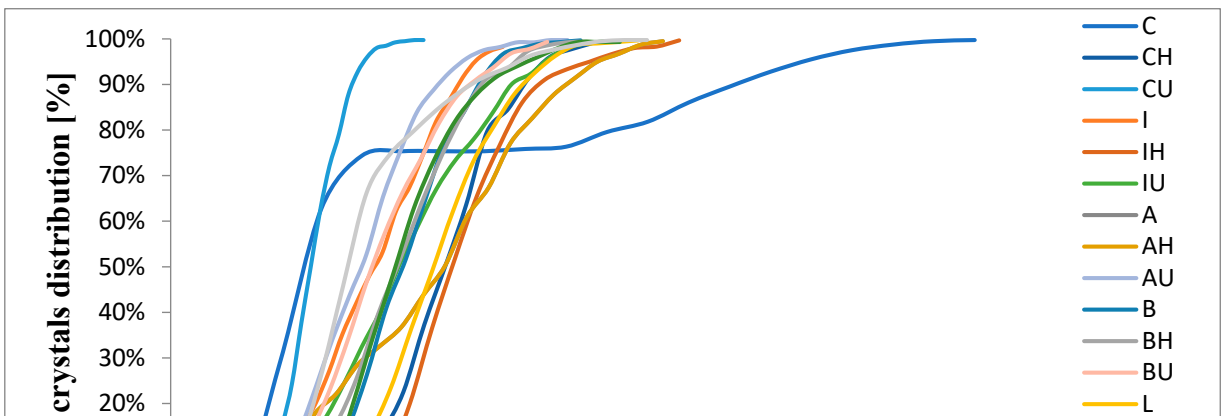


Table 4. Ice crystals size distribution in ice cream after 24 h, 1 and 3 months of storage at −18 °C.

Time of Storage and Variant of Ice Cream		Average Diameter D_A in the Class with the Highest Frequency [μm] ± SD	The Minimal Size of Ice Crystals [μm]	The Maximal Size of Ice Crystals [μm]
C	24 h	16.37 ± 4.71	8.12	32.61
	1 month	17.87 ± 13.16	3.91	27.26
	3 months	24.64 ± 7.45	15.18	30.91
CH	24 h	14.64 ± 5.45	7.10	24.82
	1 month	16.13 ± 4.00	7.58	28.57
	3 months	16.28 ± 3.34	9.01	25.47
CU	24 h	9.05 ± 2.08	5.37	18.53
	1 month	9.86 ± 2.07	5.79	16.40
	3 months	14.74 ± 3.37	8.33	22.69
I	24 h	13.9 ± 3.47	6.98	27.14
	1 month	15.57 ± 4.23	6.90	23.49
	3 months	18.52 ± 4.21	9.32	28.24
IH	24 h	9.64 ± 2.00	5.49	16.29
	1 month	19.9 ± 4.39	11.79	35.50
	3 months	21.74 ± 4.89	12.37	33.92
IU	24 h	13.23 ± 7.54	7.85	21.21
	1 month	18.00 ± 5.45	10.98	27.07
	3 months	18.40 ± 4.57	9.82	26.68
A	24 h	16.70 ± 3.46	9.44	27.52
	1 month	21.27 ± 6.93	8.33	33.86
	3 months	22.03 ± 5.27	11.58	32.33
AH	24 h	17.85 ± 4.20	9.46	28.51
	1 month	17.06 ± 6.93	9.20	26.16
	3 month	19.51 ± 5.27	11.92	29.13
AU	24 h	15.14 ± 5.35	5.00	28.69
	1 month	15.50 ± 3.93	8.01	25.53
	3 months	17.50 ± 8.48	6.30	34.27
B	24 h	16.40 ± 4.65	8.97	28.84
	1 month	16.09 ± 3.72	8.08	27.65
	3 months	16.87 ± 2.90	10.07	25.33
BH	24 h	12.92 ± 2.76	6.43	21.01
	1 month	15.91 ± 4.47	6.53	25.90
	3 months	15.14 ± 3.12	9.84	23.42
BU	24 h	9.45 ± 2.11	5.83	15.14
	1 month	14.28 ± 4.37	6.27	24.24
	3 months	15.13 ± 5.28	7.88	26.94
L	24 h	12.03 ± 2.31	6.97	19.46
	1 month	18.22 ± 4.29	8.59	33.45
	3 months	18.30 ± 4.25	10.64	28.57
LH	24 h	5.84 ± 1.12	4.48	9.17
	1 month	16.11 ± 4.14	8.24	33.21
	3 months	17.10 ± 4.94	9.14	26.38
LU	24 h	12.44 ± 3.92	6.58	20.95
	1 month	14.47 ± 4.83	7.32	22.80
	3 months	14.86 ± 3.38	9.17	24.68

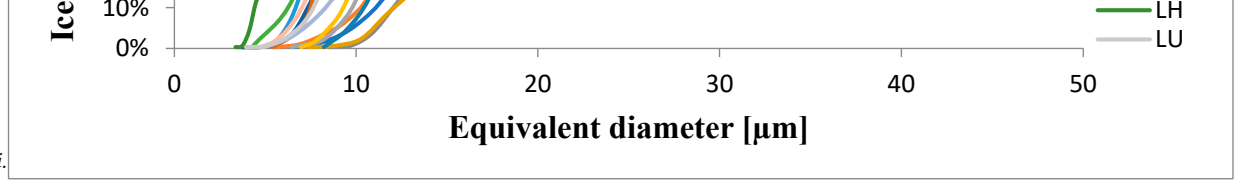


Figure 1. Ice crystal size distribution in ice cream after 24 h of storage at −18 °C.

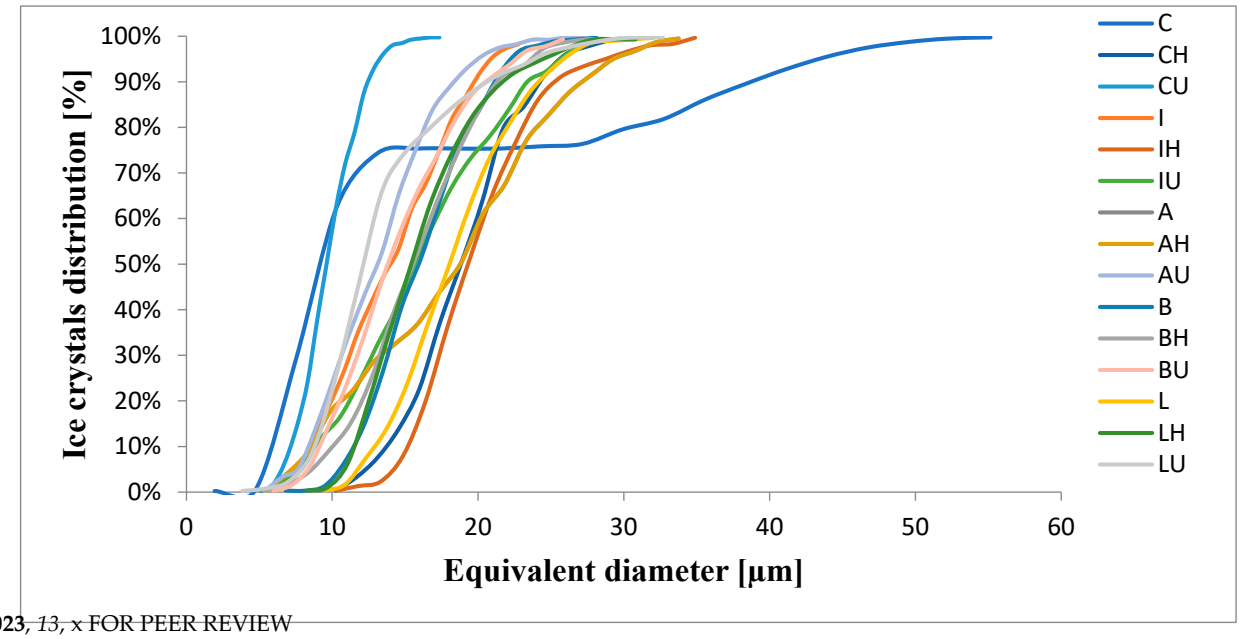


Figure 2. Ice crystal size distribution in ice cream after 1 month of storage at −18 °C.

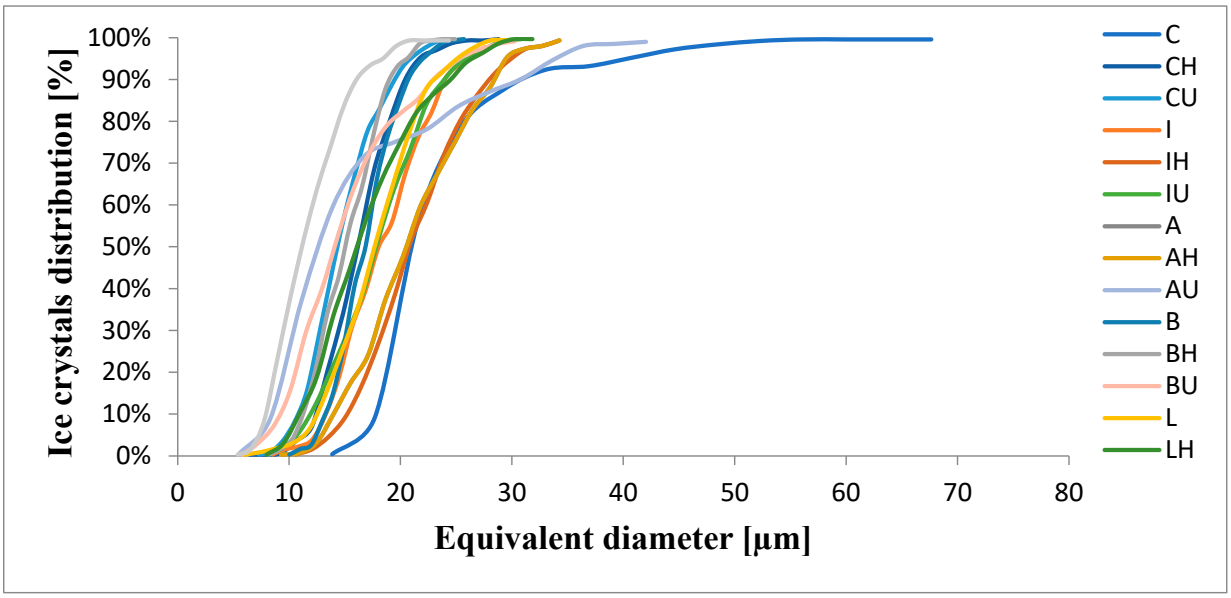


Figure 3. Ice crystal size distribution in ice cream after 3 months of storage at −18 °C.

3. Results and Discussion

3.1. The Physical Characteristic of Milk Ice Cream

The physical characteristic of milk ice cream was conducted based on the values of the cryoscopic temperature, osmotic pressure, overrun, and melting time. All of results are collected in Table 3.

The cryoscopic temperature of milk ice cream ranged from −2.921 to −2.486 °C (Tables 1–3). Based on the statistical analysis ($p < 0.05$), the differences between the stabilizers and homogenization types used were statistically significant. The lowest temperature was observed for sample AH (samples with the addition of acid hydrolyzates of ι-carrageenan and after traditional homogenization), while the higher one was for sample I (with the addition of ι-carrageenan). Overall, it can be seen that traditional homogenization contributed to the lower temperature, for instance, in samples CH (with the addition of ι-carrageenan), AH (with the addition of acid hydrolyzates of ι-carrageenan), or LH (with the addition of enzymatic commercial lactase hydrolyzates of ι-carrageenan). While ultrasound homogenization influenced the growth of the temperature. Moreover, it was observed that in the control sample, the differences between the sort of homoge-

(with the addition of enzymatic commercial lactase hydrolyzates of ι-carrageenan). While ultrasound homogenization influenced the growth of the temperature. Moreover, it was observed that in the control sample, the differences between the sort of homogenization used were not significant. Otherwise, the temperature for the samples after the ultrasound treatment was slightly lower. The reason for that may be improving in heat transfer during freezing [14]. Ultrasound homogenization might contribute to accelerating the freezing process, which resulted in higher temperatures. Nonetheless, the addition of stabilizers afforded changes in this parameter, with or without homogenization treatment. Hagiwara and Hartel [11] proved that the lower molecular mass of the sweeteners used in ice cream production resulted in greater depression of the freezing point. To follow these observations in our research, the ι-carrageenan and its hydrolyzates differed from each other by molecular mass and structure [13]. Therefore, it may be concluded that hydrolyzates of ι-carrageenan with a lower molecular weight and more flexible structure redounded on the higher cryoscopic temperature.

In the case of osmotic pressure, the lowest value was noted for sample IH (with the addition of ι-carrageenan and after traditional homogenization treatment) at the level of 1346 mOsm/kg and the highest for sample AH (samples with the addition of acid hydrolyzates of ι-carrageenan and after traditional homogenization), at a level of 1572 mOsm/kg. Additionally, the most striking observation was the dependence between cryoscopic temperature and osmotic pressure. In the control sample, the higher temperature of ice cream mixes displayed a lower pressure. For comparison, in samples with the addition of stabilizers, the lower temperature achieved a higher pressure. Moreover, Buniowska-Olejnik et al. [28] also reported that in low-fat milky ice cream with oat β-glucan, the higher osmolality of ice cream mixes resulted in a lower cryoscopic temperature. Such observations were connected with the fact that cryoscopic temperature depends on the type and concentration of dissolved substances. Consequently, the moisture-binding capacity of the stabilizers used may influence the method of creating the ice crystal structure during freezing. Additionally, the variations in the freezing point of ice cream mixes may alter the recrystallization rate during storage [29].

Overrun is defined as the increase in the volume of ice cream, affecting some properties such as melting time or hardness. Moreover, the properties of ice cream mixes (e.g., instance composition) or freezing (e.g., freezing time) may influence the overrun of ice cream [30]. In the present study, the overrun of the milk ice cream that was obtained was less than 32%. The lowest overrun was noted at the level of 8.3% for sample CH (the control sample after the traditional homogenization), while the highest value was in sample B (the sample with the addition of the enzymatic hydrolyzates by β-galactosidase of ι-carrageenan) (Table 3). Alvarez et al. [31] proved that the overrun of milk ice cream ranged from 65.04% to 72.54%. In our research, the reason for the lower overrun of prepared ice cream was presumably the small amount of fat in the recipe. The discrete and partially coalesced fat, as it is hydrophobic, is able to absorb at the air bubble surface. Otherwise, in ice cream with a low fat content, there is not enough fat to cover the whole surface of the air bubbles. Consequently, the air bubble structure may not be stabilized and the final product will have a lower overrun. As found by Sofijan and Hartel [30], the higher overrun would be expected to decrease the thermal diffusivity, providing the insulation effect and thus the ability to retard the melting time. Therefore, in our study, the lower overrun of ice cream may contribute to the lower value of melting time (Table 3). Moreover, the addition of fat replacers such as inulin may decrease the overrun values. In research by Mahdian and Karazhian [32] or Ismail et al. [33], the samples with the addition of inulin had a lower overrun than the samples without this ingredient. Inulin is able to absorb water, which may increase the viscosity of ice cream mixes. Therefore, the higher viscosity might be a primary reason for the decreased whipping abilities of ice cream [10]. According to the statistical appraisal ($p < 0.05$), the differences between samples could be explained by both the stabilizers and the homogenization process. Looking at the influence of stabilizers on the overrun of the obtained ice cream, it can be observed that ι-carrageenan, (I), acid

(A), and enzymatic hydrolyzates (B) of ι-carrageenan improved the air structure in ice cream (Table 3), and the obtained overrun for these samples was from 18.86 to 31.79%. However, the connection of stabilizers and traditional homogenization contributed to the lower overrun, for samples IH, BH, and AH, in which the value was from 9.05 to 16.06%. While the ultrasound homogenization increased this parameter, simultaneously rising the content of air bubbles in the structure of ice cream, the value of the overrun was from 15.72 to 20.43% (samples CU, BU, AU, and LU). Tüker and Dogan [21] also showed that the ultrasound homogenization resulted in increasing the value of the overrun of ice cream. Moreover, these findings proved that differences in such parameters may be a reason for the composition and the same for the ultrasound.

Melting time is a pivotal physical property of ice cream that may be affected by several factors such as ingredients, type and level of stabilizers or emulsifiers, or overrun [34]. Table 3 illustrates the measurement melting time in the presented research, which ranges from 11.23 to 30.34 min. The composition of ice cream and homogenization processes influenced the melting time of the ice cream, which is statistically significant at $p < 0.05$. However, the difference in samples such as I (with ι-carrageenan) (25.14 min), IH (with ι-carrageenan after traditional homogenization) (27.46 min), and IU (with ι-carrageenan after ultrasound treatment) (26.23 min) was not exactly visible to access the effect of ultrasound homogenization compared with the traditional one. The differences in all milk ice cream were connected with the sort of stabilizers used. For instance, it the advisable to use ι-carrageenan or its acid hydrolyzates with the combination of homogenization to prolong the melting time and at the same to raise the consumer acceptability. The addition of enzymatic hydrolyzates of ι-carrageenan, especially after β-galactosidase treatment (B), obtained the lowest value (11.23–12.34 min), even less than for the control sample (C), at 23.19 min. As mentioned in the discussion about overrun, fat may play a key role in properties such as overrun or melting time. The fact that during the freezing process, the clump of de-emulsified fat globules is able to form a protective layer around the air cells and tends to impart foam stability and yield better overrun. However, the samples with less fat clumps had a lower foam stability and overrun. Therefore, ice cream samples with less overrun with fewer air cells resulted in higher heat transfer and hence the faster meltdown of ice cream [34]. Additionally, the use of ultrasound homogenization contributed to the higher heat transfer by acoustic cavitation, hence the melting of ice cream was becoming faster. The next explanation for the short melting time in the prepared milk ice cream could be the addition of inulin. Mahdian and Karazhian [32] reported that with the increasing content of inulin in low-fat ice cream, the melting resistance decreased. Moreover, Góral et al. [26], in ice cream based on coconut milk, proved that the melting resistance was weaker in samples with a higher addition of inulin. Zambrano-Mayorga et al. [35] reported that the melting time in milk ice cream ranged from 19.5 to 31.5, with changes in the amount of whey powder and sweeteners.

3.2. The Microscopy Structure Analysis

Not only are the small ice crystals pivotal, but also the smooth and creamy mouthfeel of ice cream. Hence, controlling the crystallization process and the size or distribution of ice crystals are factors that determine the desired product and prolong the shelf life [15,32,36]. First and foremost, in the presented research, the size of the ice crystals was analysed based on the value of the average diameter (D_A) in Table 4. Moreover, the distribution of the obtained ice crystal structure was studied in accordance with distributions obtained from the image analysis, which were characterised by the values for the ice crystal equivalent diameter at 50% of the cumulative distribution (X_{50}) (Figures 1–3). To estimate the progress of recrystallization, the samples of milk ice cream were examined after 24 h, 1 month, and 3 months of storage at −18 °C.

After 24 h of storage, it was noted that the average diameter (D_A) ranged from 9.05 to 17.85 µm (Table 4). The lowest diameter of crystals was observed for sample CU (without the addition of stabilizers and after the ultrasound treatment), while the highest one was

for sample AH (with the acid hydrolyzates of ι-carrageenan and after the traditional homogenization treatment). Overall, the size of the ice crystals in the obtained milk ice cream did not exceed 18 μm. The ultrasound homogenization used contributed to the smaller ice crystals in milk ice cream, except for samples IU (sample with the ι-carrageenan, and after the ultrasound homogenization treatment) and LU (sample with the enzymatic commercial lactase hydrolyzates of ι-carrageenan and after the ultrasound homogenization treatment), in comparison with the traditional homogenization. The effect of ultrasound homogenization may be explained by the violent collapse of bubbles, which initiate the ice nucleation by creating local zones of high pressure in a very short time. Moreover, the force generated by the collapse of cavitation bubbles is able to fragment bigger ice crystals into smaller ones [15,18,37]. Therefore, ultrasound homogenization might be more effective in comparison with traditional homogenization. Based on the value of the ice crystal diameter at 50% of the cumulative distribution of the sample, the X_{50} diameter ranged from 6 to 17 μm (Figure 1). Kamińska-Dwórznicka et al. [38], in whey ice cream with the addition of ι-carrageenan as the main stabilizer, showed that the X_{50} was around 15 μm, which was similar to the presented research.

The analysis of the structure of ice crystals after 1 month of storage, for milk ice cream, concludes that the effect of the recrystallization process was visible (Table 4). The average diameter of ice crystals ranged from 9.86 to 21.27 μm. As mentioned in the description of results after 24 h of storage, sample CU (without the addition of stabilizers and after the ultrasound treatment) was characterized the smallest size of ice crystals. The highest progress of recrystallization was noticed for sample LH (sample with the enzymatic commercial lactase hydrolyzates of ι-carrageenan, and after the ultrasound treatment), owing to the fact that the crystals grew almost 11 μm. The most striking observation after this time in storage was the fact that the ultrasound treatment significantly contributed to the inhibition of the recrystallization process. In all of the prepared ice cream samples, the average diameter was smaller (from 9.86 to 18 μm) than in the samples after mechanical homogenization (from 16.11 to 19.90) or only with the addition of stabilizers (15.57 to 21.27 μm). Moreover, the value of ice crystal diameter at 50% of the cumulative distribution of the sample (X_{50} diameter) was observed, extending from 9 to 20 μm (Figure 2). In comparison with the results recorded after 24 h of storage, the recrystallization process could be proved based on parameter $X_{50.}$ The research by Kamińska-Dwórznicka et al. [19] also showed that using ultrasound (the frequency of 21.5 Hz) during freezing also contributed to the lower diameter of ice crystals for mango sorbet, of less than 10 μm. Moreover, in research by Mortazavi and Tabatabaie [18], the effect of ultrasound shortened the freezing time and at the same increased the overrun of ice cream, which improved the sensory of the final product. Additionally, Islam et al. [39] proved that ultrasound effectively triggered ice nucleation and minimized the size of the ice crystals in mushrooms. On the other hand, Dai et al. [40] indicated that the size of ice crystals was larger at higher nucleation temperatures and the sublimation time was reduced by almost 22 % compared with the control sample.

Looking at the results after 3 months of storage (Table 4 and Figure 3), it is seen that the average diameter of ice crystals was less than 25 μm. The highest ice crystals were noted for control sample C, while the smallest result was 14.74 μm for the control sample after ultrasound treatment (CU). For all samples with the addition of stabilizers but without homogenization treatment, the size of the ice crystals ranged from 16.87 to 22.03 μm. However, samples with the addition of stabilizers and after the traditional homogenization ranged from 15.14 to 21.74 μm, while after the ultrasound treatment, they ranged from 14.86 to 18.4 μm. Consequently, it was seen that the implementation of the homogenization process was necessary to obtain smaller ice crystals. However, the ultrasound was more beneficial than the traditional method. What should be highlighted is that the control sample (C) without stabilizers but after the ultrasound treatment achieved the smallest value for the average diameter at a level of 14.74 μm. Additionally, the range of obtained ice crystals was close to 20 or less than this value, which may guarantee the desirable texture of ice cream, especially for consumers. Based on the value of the X_{50} diameter, ranging

from 12 to 21 μm (Figure 3). Notable, to compare to results after 1 month of storage, the recrystallization process could be observed based on the parameter X_{50}.

Chow et al. [41] proved that existing big crystals might be broken up into smaller ice crystals by high-intensity ultrasound. Maybe, in this case, such an ability of ultrasound was observed. Therefore, this increases the chances of effectiveness to inhibit the recrystallization process in milk ice cream. Taking into consideration the influence of the stabilizers used on the recrystallization process, it may be seen that in samples with ι-carrageenan (I) and its enzymatic hydrolyzates (B and L), the size of ice crystals was similar (around 18 μm). For sample A, with the acid hydrolyzates of ι-carrageenan, the average diameter of ice crystals was higher (22.03 μm). The reason may be the difference in the molecular structure of these stabilizers. In our previous study [13], it was proven that the mentioned enzymatic hydrolyzates of ι-carrageenan were more flexible based on FTIR (Fourier Transform Infrared Spectroscopy) analysis, and the vibration intensity of the –OH groups in the model solutions was seen in comparison with acid hydrolyzates. Overall, for the obtained hydrolyzates of ι-carrageenan, smaller vibrations were observed at 1213 and 914 cm^{-1}, which was connected with the decreasing number of sulphate groups and determined the better flexibility of hydrolyzates. Hence, the distinctive influence of stabilizers on the ice crystal structure was noted. Moreover, the average diameter in samples B and L had a similar value in comparison with sample A after 3 months of storage. It is mentioned here because based on the FTIR analysis, it was confirmed that samples B and L after the enzymatic hydrolysis had a similar course on the FTIR spectra. It can be evidence that the enzymatic hydrolyzates of ι-carrageenan were more favourable in milk ice cream than ι-carrageenan and its acid hydrolyzates. In the research by Tecson et al. [42], the ultrasound-assisted depolymerization of κ-carrageenan was investigated. It was proven that the raw carrageenan average molecular mass was reduced by 89.32% while in the irradiated κ-carrageenan it was only 41.98%. Such observations were connected with the fact that larger molecules present more resistance to flow and the same accumulate greater sher force, which leads to more frequent ruptures as the cavitation bubbles collapse than in the shorter polymer. Therefore, in our research, presumably, the effect of ι-carrageenan was similar even if the initial molecular mass of this polymer was higher than the obtained hydrolyzates [13]. Moreover, in the same research, Tecson et al. [42] noticed that after ultrasound treatment, κ-carrageenan with a lower molecular mass revealed the retention of absorbance peaks with the decrease in the sulphate functional group and most of the peaks were retained, with one exception of a methylene peak. CIn our study, the chain of ι-carrageenan underwent changes by ultrasonic cavitations, which led to a similar effect on the IRI activity in ice cream as the hydrolyzates. Moreover, it generated information that hydrolyzates will be more stable and less vulnerable on the ultrasound treatment than pure ι-carrageenan. Nonetheless, further studies will be recommended to investigate the possible changes in the molecular structure of ι-carrageenan and its hydrolyzates after ultrasound treatment. Finally, the mechanism of ultrasound, such as inducing ice nucleation as well as increasing heat and mass transfer, is connected with the initial step of ice cream production. In our research, it was proven that ultrasound forces were so strong that they influenced the structure of ice cream even after a few months of storage and prevented excessive growth of ice crystals.

The observation of the progress of the recrystallization process was also analysed based on images (Figures 4 and 5). To access this we used the overview of the appearance of ice crystals during storage after 24 h and 3 months of storage at −18 °C (only representative photos were chosen to show in the paper, not including the photos after 1 month). Looking at the images, it may be noticed that the shape of the ice crystals was round and regular (Figure 4). The research by Sanchez-Garcia et al. [43] showed that in lactose solutions with the presence of whey proteins and κ-carrageenan, after 48 h of ultrasound-assisted crystallization, the shape of crystals was mostly irregular, even with the tomahawk-like shape. Nonetheless, it is known that high ultrasound energies may be responsible for breaking crystals already formed and generating a disruption of agglomerates or even

nuclei [44]. After 24 h of storage of ice cream, the changes between the samples treated with different sorts of homogenization were not visible. For example, in samples AH_24h (sample with acid hydrolyzates of ι-carrageenan and after traditional homogenization) and AU_24h (sample with acid hydrolyzates of ι-carrageenan and after ultrasound), the changes were connected with the size of ice crystals (which was confirmed in Table 4), but not in the appearance of crystals. A similar effect on the shape of the ice structure was noticed in frozen pork tissue [45] or in frozen mushrooms [39], that ultrasound treatment unified the shape and sizes of the ice crystals. Moreover, Xu et al. [46] indicated that differences in the structure of ice crystals may depend on the ultrasonic power intensities.

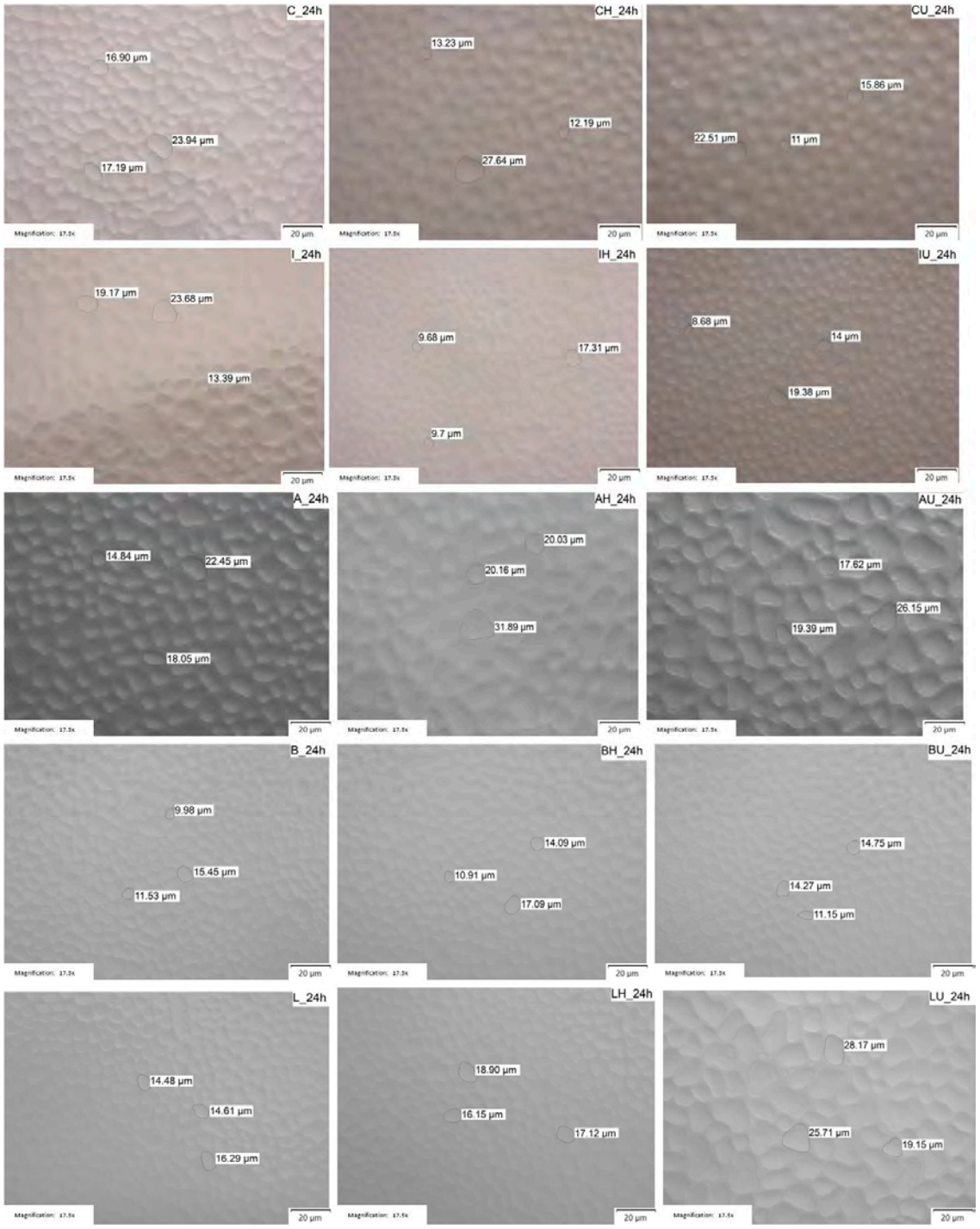


Figure 4. Ice crystal size distribution in ice cream after 24 h of storage at −18 °C.

Exploratory notes: C, control sample (without stabilizers); I, sample with ι-carrageenan, locust bean gum (LBG), and xanthan gum; A, B, and L, sample with hydrolyzates obtained by acid, β-galactosidase, and commercial lactase treatment of ι-carrageenan, respectively, LBG, and xanthan gum; H, samples after traditional homogenization treatment; U, samples after ultrasound homogenization treatment.

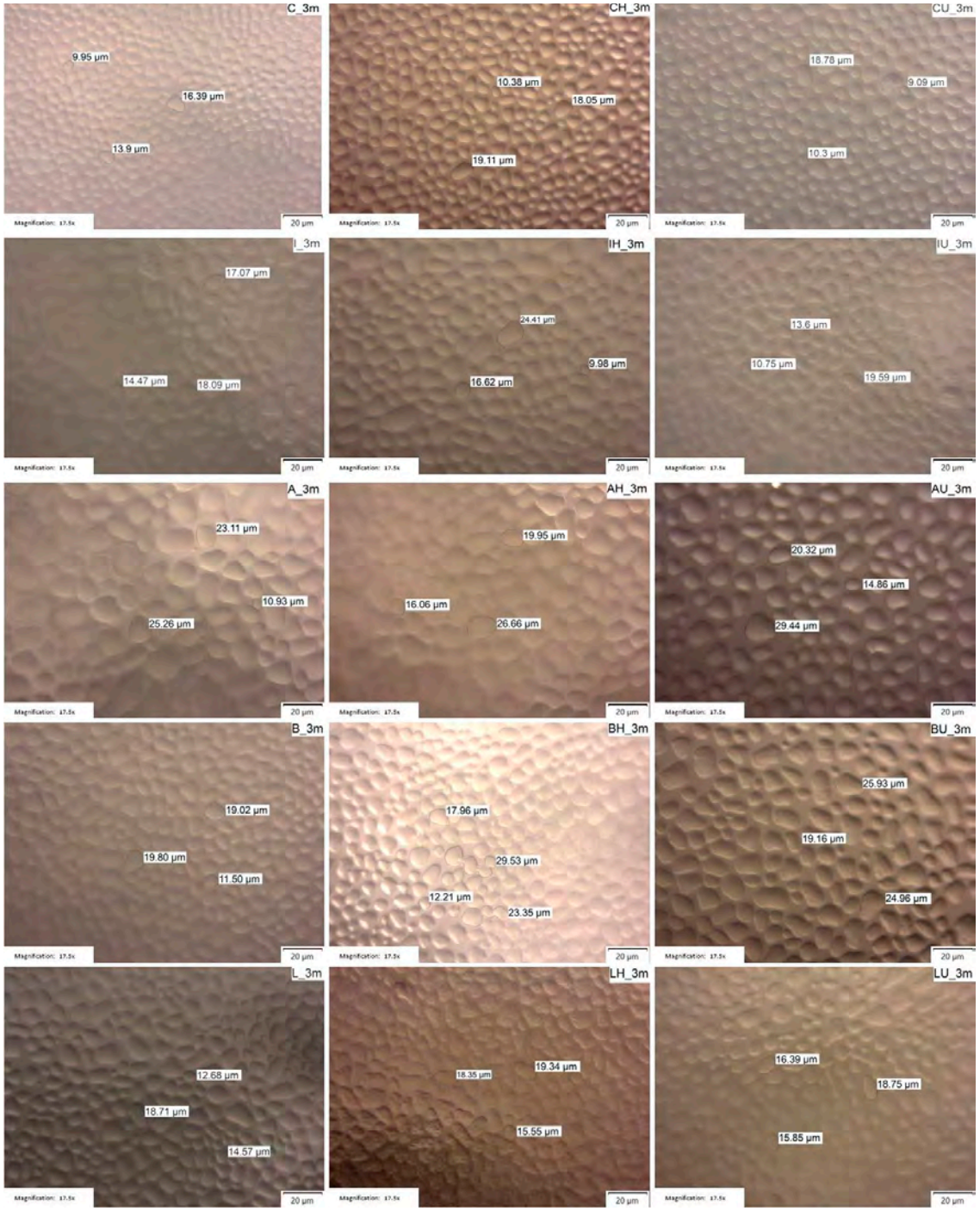


Figure 5. Ice crystal size distribution in ice cream after 3 months of storage at −18 °C.

Exploratory notes: C—control sample (without stabilizers); K—sample with ι-carrageenan, locust bean gum (LBG), and xanthan gum; A, B, and L—samples with hydrolyzates obtained by acid, β-galactosidase, and commercial lactase treatment of ι-carrageenan, respectively; LBG and xanthan gum; H—samples after traditional homogenization treatment; U—samples after ultrasound homogenization treatment.

4. Conclusions

Ultrasound treatment of ice cream resulted in the reduction in their sizes during storage and simultaneously increased the IRI (ice recrystallization inhibition) activity of the prepared samples. After 3 months of storage, in the control samples, after ultrasound

After 3 months of storage, milk ice cream was characterised by different sizes as a result of the progress of recrystallization (Figure 5 and Table 4). In addition, as mentioned earlier, the size of the ice crystals was regular and round. The changes were strictly connected with the size of the ice crystals, especially in samples after ultrasound treatment, where the average diameter was the smallest in comparison with the other samples of ice cream. However, in the image, BH_3m (the sample with enzymatic β-galactosidase hydrolyzates of ι-carrageenan and after the traditional homogenization); the coalescence between the crystals was marked, which proved the recrystallization phenomena during storage (Figure 5).

Exploratory notes: C, control sample (without stabilizers); I, sample with ι-carrageenan, locust bean gum (LBG), and xanthan gum; A, B, and L, sample with hydrolyzates obtained by acid, β-galactosidase, and commercial lactase treatment of ι-carrageenan, respectively, LBG, and xanthan gum; H, samples after traditional homogenization treatment; U, samples after ultrasound homogenization treatment.

Exploratory notes: C, control sample (without stabilizers); I, sample with ι-carrageenan, locust bean gum (LBG), and xanthan gum; A, B, and L, samples with hydrolyzates obtained by acid, β-galactosidase, and commercial lactase treatment of ι-carrageenan, respectively, LBG and xanthan gum; H, samples after traditional homogenization treatment; U, samples after ultrasound homogenization treatment.

4. Conclusions

Ultrasound treatment of ice cream resulted in the reduction in their sizes during storage and simultaneously increased the IRI (ice recrystallization inhibition) activity of the prepared samples. After 3 months of storage, in the control samples, after ultrasound treatment, the average diameter did not exceed 15 μm. In comparison, among the samples with the addition of stabilizers, this value was less than 19 μm. The beneficial influence of ultrasound is connected to acoustic cavitation and accelerating heat and mass transfer. This occurred to initiate the ice nucleation of ice crystals and improve the condition to build the ice structure during freezing. As a result, in cryoscopic temperature, osmotic pressure, overrun, and melting time such changes were observed. It was found that ultrasound influenced the increase in cryoscopic temperature in comparison with traditional homogenization. Additionally, the overrun of ice cream ranged from 8.3 to 31.79%. The lower overrun was created by the small amount of fat in ice cream and presumably the addition of inulin. Therefore, the influence of sort of homogenization was not significantly visible. The melting time of the prepared milk ice cream was less than 29 min. According to the results of the melting time, it was concluded that ultrasound homogenization in comparison with the traditional one did not differ, so they can be used interchangeably.

Detailed studies on the physical and presumably sensory properties, as well as the shelf stability of the ice cream from ultrasound mixes, will be recommended. Studies on the improvement in ice cream properties through the inclusion of stabilizers with the connection of ultrasound treatment can yield further optimization of the efficiency of the parameter. Finally, we investigated the possibilities of producing a milk ice cream with HIU (high-intensity ultrasound) that can confer desirable properties, which could possibly yield economically and environmentally attractive possibilities.

Author Contributions: Conceptualization, A.K. and A.K.-D.; methodology, A.K. and E.J.; software, A.K.; validation, A.K., A.K.-D. and E.J.; formal analysis, A.K. and A.K.-D.; investigation, A.K., A.K.-D. and E.J.; resources, E.J.; data curation, A.K., A.K.-D. and E.J.; writing—original draft preparation, A.K., A.K.-D. and E.J.; writing—review and editing, A.K., A.K.-D. and E.J.; visualization, A.K.; supervision, E.J.; project administration, A.K.-D. All authors have read and agreed to the published version of the manuscript.

Funding: Research equipment was purchased as part of the "Food and Nutrition Centre-modernisation of the WULS campus to create a Food and Nutrition Research and Development Centre (CŻiŻ)" co-financed by the European Union from the European Regional Development Fund under the Regional Operational Programme of the Mazowieckie Voivodeship for 2014-2020 (Project No. RPMA.01.01.00-14-8276/17).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Goff, H.D. Colloidal aspects of ice cream—A review. *Int. Dairy J.* **1997**, *7*, 363–373. [CrossRef]
2. Soukoulis, C.; Chandrinos, I.; Tzia, C. Study of the functionality of selected hydrocolloids and their blends with κ-carrageenan on storage quality of vanilla ice cream. *LWT-Food Sci. Technol.* **2008**, *41*, 1816–1827. [CrossRef]
3. Inoue, K.; Ochi, H.; Habara, K.; Taketsuka, M.; Saito, H.; Ichihashi, N.; Iwatsuki, K. Modeling of the effect of freezer conditions on the hardness of ice cream using response surface methodology. *J. Dairy Sci.* **2009**, *92*, 5834–5842. [CrossRef] [PubMed]
4. Clarke, C. *The Science of Ice Cream*; The Royal Society of Chemistry: Cambridge, UK, 2004.
5. Wildmoser, H.; Scheiwiller, J.; Windhab, E.J. Impact of disperse microstructure on rheology and quality aspects of ice cream. *LWT-Food Sci. Technol.* **2004**, *37*, 881–891. [CrossRef]
6. Gaukel, V.; Leiter, A.; Spiess, W.E.L. Synergism of different fish antifreeze proteins and hydrocolloids on recrystallization inhibition of ice in sucrose solutions. *J. Food Eng.* **2014**, *141*, 44–50. [CrossRef]
7. Kamińska-Dwórznicka, A.; Gondek, E.; Łaba, S.; Jakubczyk, E.; Samborska, K. Characteristics of instrumental methods to describe and assess the recrystallization process in ice cream systems. *Foods* **2019**, *8*, 117. [CrossRef]
8. Smith, A.K.; Goff, H.D.; Kakuda, Y. Microstructure and rheological properties of whipped cream as affected by heat treatment and addition of stabilizer. *Int. Dairy J.* **2000**, *10*, 295–301. [CrossRef]
9. Fiol, C.; Prado, D.; Romero, C.; Laburu, N.; Mora, M.; Alava, I.J. Introduction of a new family of ice creams. *Int. J. Gastron. Food Sci.* **2017**, *7*, 5–10. [CrossRef]
10. Adapa, S.; Schmidt, K.A.; Jeon, I.J.; Herald, T.J.; Flores, R.A. Mechanisms of ice crystallization and recrystallization in ice cream: A review. *Food Rev. Int.* **2000**, *16*, 259–271. [CrossRef]
11. Hagiwara, T.; Hartel, R.W. Effect of sweetener, stabilizer, and storage temperature on ice recrystallization in ice cream. *J. Dairy Sci.* **1996**, *79*, 735–744. [CrossRef]
12. Lomolino, G.; Zannoni, S.; Zabara, A.; Da Lio, M.; De Iseppi, A. Ice recrystallisation and melting in ice cream with different proteins levels and subjected to thermal fluctuation. *Int. Dairy J.* **2020**, *100*, 104557. [CrossRef]
13. Kot, A.; Kamińska-Dwórznicka, A.; Antczak, A.; Jakubczyk, E.; Matwijczuk, A. Effect of ι-carrageenan and its acidic and enzymatic hydrolysates on ice crystal structure changes in model sucrose solution. *Colloids Surf. A Physicochem. Eng. Asp.* **2022**, *643*, 128744. [CrossRef]
14. Chemat, F.; Zill-e-Huma; Khan, M.K. Application of ultrasound in food technology: Processing, preservation and extraction. *Ultrason. Sonochem.* **2011**, *18*, 813–835.
15. Akdeniz, V.; Akalin Sibel, A. New approach for yoghurt and ice cream production: High-intensity ultrasound. *Trends Food Sci. Technol.* **2019**, *86*, 392–398. [CrossRef]
16. Carrillo-Lopez, L.M.; Garcia-Galicia, I.A.; Tirado-Gallegos, J.M.; Sanchez-Vega, R.; Huerta-Jimenez, M.; Ashokkumar, M.; Alarcon-Rojo, A.D. Recent advances in the application of ultrasound in dairy products: Effect on functional, physical, chemical, microbiological and sensory properties. *Ultrason. Sonochem.* **2021**, *73*, 105467. [CrossRef] [PubMed]
17. Firouz, M.S. Application of high-intensity ultrasound in food processing for improvement of food quality. In *Design and Optimization of Innovative Food Processing Techniques Assisted by Ultrasound*; Developing Healthier and Sustainable Food Products; Barba, F., Cravotto, G., Chemat, F., Lorenzo Rodriguez, J.M., Sichetti Munekata, P.E., Eds.; Elsevier Inc.: Amsterdam, The Netherlands, 2021; pp. 155–159.
18. Mortazavi, A.; Tabatabaie, F. Study of ice cream freezing process after treatment with ultrasound. *World Appl. Sci. J.* **2008**, *4*, 188–190.
19. Kamińska-Dwórznicka, A.; Kot, A.; Jakubczyk, E.; Buniowska-Olejnik, M.; Nowacka, M. Effect of ultrasound-assisted freezing on the crystal structure of mango sorbet. *Crystals* **2023**, *13*, 396. [CrossRef]
20. Nazarewicz, S.; Kozłowicz, K.; Kobus, Z.; Gładyszewska, B.; Matwijczuk, A.; Slusarczyk, L.; Skrzypek, T.; Sujka, M.; Kozłowicz, N. The Use of Ultrasound in Shaping the Properties of Ice Cream with Oleogel Based on Oil Extracted from Tomato Seeds. *Appl. Sci.* **2022**, *12*, 9165. [CrossRef]
21. Tüker, D.A.; Dogan, M. Effect of ultrasound homogenization on the structural and sensorial attributes of ice cream: Optimization with Taguchi and data envelopment analysis. *J. Food Meas. Charact.* **2021**, *15*, 4888–4898.
22. Kamińska-Dwórznicka, A.; Skrzypczak, P.; Gondek, E. Modification of kappa carrageenan by β-galactosidase as a new method to inhibit recrystallization of ice. *Food Hydrocoll.* **2016**, *61*, 31–35. [CrossRef]
23. O'Sullivan, J.; Park, M.; Beevers, J. The effect of ultrasound upon the physicochemical and emulsifying properties of wheat and soy protein isolates. *J. Cereal Sci.* **2016**, *69*, 77–84. [CrossRef]
24. da Silva, L.F.R.; da Silva Gomes, A.; Castro, D.R.G.; das Chagas do Amaral Souza, F.; Mar, J.M.; Souza da Silva, L.; Sanches, E.A.; de Araújo Bezerra, J.; Bakry, A.M.; Campelo, P.H. Ultrasound-assisted homogenisation and gum Arabic combined to physicochemical quality of cupuaçu juice. *J. Food Process. Preserv.* **2019**, *43*, e14072. [CrossRef]
25. Dłużewska, E.; Gazda, B.; Leszczyński, K. Wpływ wybranych hydrokoloidów polisacharydowych na jakość koncentratów lodów owocowych. *Acta Sci. Pol. Technol. Aliment.* **2003**, *2*, 97–107.
26. Góral, M.; Kozłowicz, K.; Pankiewicz, U.; Góral, D.; Kluza, F.; Wójtowicz, A. Impact of stabilizers on the freezing process, and physicochemical and organoleptic properties of coconut milk-based ice cream. *LWT-Food Sci. Technol.* **2018**, *92*, 516–522. [CrossRef]

27. Regand, A.; Goff, H.D. Structure and ice recrystallization in frozen stabilized ice cream model systems. *Food Hydrocoll.* **2003**, *17*, 95–102. [CrossRef]
28. Buniowska-Olejnik, M.; Mykhalevych, A.; Polishchuk, G.; Sapiga, V.; Znamirowska-Piotrowska, A.; Kot, A.; Kamińska-Dwórznicka, A. Study of Water Freezing in Low-Fat Milky Ice Cream with Oat β-Glucan and Its Influence on Quality Indicators. *Molecules* **2023**, *28*, 2924. [CrossRef]
29. Patel, M.R.; Baer, R.J.; Acharaya, M.R. Increasing the protein content of ice cream. *J. Dairy Sci.* **2006**, *89*, 1400–1406. [CrossRef]
30. Sofijan, P.S.; Hartel, R.W. Effect of overrun on structural and physical characteristic of ice cream. *Int. Dairy J.* **2004**, *14*, 255–262. [CrossRef]
31. Alvarez, V.B.; Wolters, C.L.; Vodovotz, Y.; Ji, T. Physical properties of ice cream containing milk protein concentrates. *J. Dairy Sci.* **2005**, *88*, 862–871. [CrossRef]
32. Mahdian, E.; Karazhian, R. Effect of fat replacers and stabilizers on rheological, physicochemical and sensory properties of reduced-fat ice cream. *J. Agric. Sci. Technol.* **2013**, *15*, 1163–1174.
33. Ismail, E.A.; Al-Saleh, A.A.; Metwalli, A.A.M. Effect of Inulin Supplementation on Rheological Properties of Low-Fat Ice Cream. *Life Sci.* **2013**, *10*, 1742–1746.
34. Roy, S.; Hussain, S.A.; Prasad, W.G.; Khetra, Y. Quality attributes of high protein ice cream prepared by incorporation of whey protein isolate. *Appl. Food Res.* **2022**, *2*, 100029. [CrossRef]
35. Zambrano-Mayorga, L.; Ramirez-Navas, J.; Ochoa-Martinez, C.I. Influence of the formulation on the thermophysical properties and the quality parameters of diary ice cream. *Dyna* **2019**, *86*, 117–125. [CrossRef]
36. Regand, A.; Goff, H.D. Effect of biopolymers on structure and ice recrystallization in dynamically frozen ice cream model systems. *J. Dairy Sci.* **2002**, *85*, 2722–2732. [CrossRef]
37. Cheng, L.N.; Sun, D.W.; Zhu, Z.W.; Zhang, Z. Emerging techniques for assisting and accelerating food freezing processes: A of recent research progresses. *Crit. Rev. Food Sci. Nutr.* **2017**, *57*, 769–781. [CrossRef]
38. Kamińska-Dwórznicka, A.; Łaba, S.; Jakubczyk, E. The effects of selected stabilizers addition on physical properties and change in crystal structure of whey ice cream. *LWT-Food Sci. Technol.* **2022**, *154*, 112841. [CrossRef]
39. Islam, M.N.; Zhang, M.; Fang, Z.; Sun, J. Direct contact ultrasound assisted freezing of mushroom (*Agaricus bisporus*): Growth and size distribution of ice crystals. *Int. J. Refrig.* **2015**, *57*, 46–53. [CrossRef]
40. Dai, C.; Zhou, X.; Zhang, S.; Zhou, N. Influence of ultrasound-assisted nucleation on freeze-drying of carrots. *Dry Technol.* **2016**, *34*, 1196–1203. [CrossRef]
41. Chow, R.; Blindt, R.; Chivers, R.; Povey, M. A study on the primary and secondary nucleation of ice by power ultrasound. *Ultrasonics* **2005**, *43*, 227–230. [CrossRef]
42. Tecson, M.G.; Abad, L.V.; Ebajo, V.D., Jr.; Camacho, D.H. Ultrasound-assisted depolymerization of kappa-carrageenan and characterization of a degradation product. *Ultrason. Sonoch.* **2021**, *73*, 105540. [CrossRef]
43. Sánchez-Garcia, Y.; García-Vega, K.S.; Leal-Ramos, M.Y.; Salmeron, I.; Gutièrrez-Mèndez, N. Ultrasound-assisted crystallization if lactose in the presence of whey proteins and κ-carrageenan. *Ultrason. Sonochem.* **2018**, *42*, 714–722. [CrossRef]
44. de Castro, M.D.; Priego-Capote, F. Ultrasound-assisted crystallization (sonocrystallization). *Ultrason. Sonochem.* **2007**, *14*, 717–724. [CrossRef] [PubMed]
45. Zhang, P.Z.; Zhu, Z.W.; Sun, D.W. Using power ultrasound to accelerate food freezing processes: Effects on freezing efficiency and food microstructure. *Crit. Rev. Food Sci. Nutr.* **2018**, *58*, 2842–2853. [CrossRef] [PubMed]
46. Xu, B.-G.; Zhang, M.; Bhandari, B.; Cheng, X.-F.; Islam, M.N. Effect of ultrasound-assisted freezing on the physic-chemical properties and volatile compounds of red radish. *Ultrason. Sonochem.* **2015**, *27*, 316–324. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

OŚWIADCZENIA WSPÓŁAUTORÓW PUBLIKACJI

Warszawa, 30.08.2023r.

mgr inż. Anna Kot
anna_kot1@sggw.edu.pl

Rada Dyscypliny Technologia Żywności i Żywienia

lub Senat

Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy: Anna Kot, Anna Kamińska-Dwórznicka, Andrzej Antczak, Ewa Jakubczyk, Arkadiusz Matwijczuk, 2022, *„Effect of ι-carrageenan and its acidic and enzymatic hydrolysates on ice crystal structure changes in model sucrose solution"*, Colloids and Surfaces A: Physicochemical and Engineering Aspects, 643, 128744, mój indywidualny udział w jej powstaniu polegał na przeprowadzeniu części doświadczalnej, opracowaniu i analizie otrzymanych wyników oraz pisaniu, edycji i recenzji manuskryptu.

Jednocześnie wyrażam zgodę, aby prace zostały wykorzystane w rozprawie doktorskiej mgr inż. Anny Kot.

Podpis

Warszawa, 30.08.2023r.

dr hab. Anna Kamińska-Dwórznicka, prof. SGGW
anna_kaminska1@sggw.edu.pl

Rada Dyscypliny Technologia Żywności i Żywienia

lub Senat

Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy: Anna Kot, Anna Kamińska-Dwórznicka, Andrzej Antczak, Ewa Jakubczyk, Arkadiusz Matwijczuk, 2022, *„Effect of ι-carrageenan and its acidic and enzymatic hydrolysates on ice crystal structure changes in model sucrose solution"*, Colloids and Surfaces A: Physicochemical and Engineering Aspects, 643, 128744, mój indywidualny udział w jej powstaniu polegał na utworzeniu koncepcji badań, nadzorze prowadzonych prac laboratoryjnych, przygotowywaniu manuskryptu oraz jego edycji i recenzji.

Jednocześnie wyrażam zgodę, aby prace zostały wykorzystane w rozprawie doktorskiej mgr inż. Anny Kot.

Podpis

Anna Kamińska-Dwórznicka

dr hab. Andrzej Antczak, prof. SGGW
andrzej_antczak@sggw.edu.pl

Warszawa, 01.09.2023r.

Rada Dyscypliny Technologia Żywności i Żywienia

lub Senat

Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy: Anna Kot, Anna Kamińska-Dwórznicka, Andrzej Antczak, Ewa Jakubczyk, Arkadiusz Matwijczuk, 2022, *„Effect of ι-carrageenan and its acidic and enzymatic hydrolysates on ice crystal structure changes in model sucrose solution"*, Colloids and Surfaces A: Physicochemical and Engineering Aspects, 643, 128744, mój indywidualny udział w jej powstaniu polegał na udziale w części doświadczalnej manuskryptu.

Jednocześnie wyrażam zgodę, aby prace zostały wykorzystane w rozprawie doktorskiej mgr inż. Anny Kot.

Podpis

A. Antczak

Warszawa, 30.08.2023r.

dr hab. Ewa Jakubczyk, prof. SGGW
ewa_jakubczyk@sggw.edu.pl

Rada Dyscypliny Technologia Żywności i Żywienia

lub Senat

Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy: Anna Kot, Anna Kamińska-Dwórznicka, Andrzej Antczak, Ewa Jakubczyk, Arkadiusz Matwijczuk, 2022, *„Effect of ι-carrageenan and its acidic and enzymatic hydrolysates on ice crystal structure changes in model sucrose solution"*, Colloids and Surfaces A: Physicochemical and Engineering Aspects, 643, 128744, mój indywidualny udział w jej powstaniu polegał na udziale w części doświadczalnej manuskryptu.

Jednocześnie wyrażam zgodę, aby prace zostały wykorzystane w rozprawie doktorskiej mgr inż. Anny Kot.

Podpis

Ewa Jakubczyk

[Lublin], [1-09-2023]

dr hab. Arkadiusz Matwijczuk, prof. uczelni
arkadiusz.matwijczuk@up.lublin.pl

Rada Dyscypliny Technologia Żywności i Żywienia

lub Senat

Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy: Anna Kot, Anna Kamińska-Dwórznicka, Andrzej Antczak, Ewa Jakubczyk, Arkadiusz Matwijczuk, 2022, *„Effect of ι-carrageenan and its acidic and enzymatic hydrolysates on ice crystal structure changes in model sucrose solution"*, Colloids and Surfaces A: Physicochemical and Engineering Aspects, 643, 128744, mój indywidualny udział w jej powstaniu polegał na udziale w części doświadczalnej oraz analizie otrzymanych wyników.

Jednocześnie wyrażam zgodę, aby prace zostały wykorzystane w rozprawie doktorskiej mgr inż. Anny Kot.

Arkadiusz Matwijczuk

Podpis

Warszawa, 29.08.2023r.

mgr inż. Anna Kot
anna_kot1@sggw.edu.pl

Rada Dyscypliny Technologia Żywności i Żywienia

lub Senat

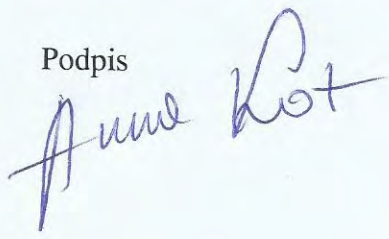
Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy: Anna Kot, Anna Kamińska-Dwórznicka, 2022, *„Vegan Ice cream – A review”*, Przemysł Spożywczy, 76 (12), 28-32, mój indywidualny udział w jej powstaniu polegał na opracowaniu koncepcji artykułu, zestawieniu danych oraz interpretacja wyników, przygotowanie przeglądu literatury, pisaniu oraz edycji manuskryptu.

Jednocześnie wyrażam zgodę, aby prace zostały wykorzystane w rozprawie doktorskiej mgr inż. Anny Kot.

Podpis



Warszawa, 30.08.2023r.

dr hab. Anna Kamińska-Dwórznicka, prof. SGGW
anna_kaminska1@sggw.edu.pl

Rada Dyscypliny Technologia Żywności i Żywienia

lub Senat

Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy: Anna Kot, Anna Kamińska-Dwórznicka, 2022, *„Vegan Ice cream – A review"*, Przemysł Spożywczy, 76 (12), 28-32, mój indywidualny udział w jej powstaniu polegał na przygotowaniu przeglądu literatury, zestawieniu danych, recenzji oraz edycji manuskryptu.

Jednocześnie wyrażam zgodę, aby prace zostały wykorzystane w rozprawie doktorskiej mgr inż. Anny Kot.

Podpis

Anna Kamińska-Dwórznicka

Warszawa, 29.08.2023r.

mgr inż. Anna Kot
anna_kot1@sggw.edu.pl

Rada Dyscypliny Technologia Żywności i Żywienia

lub Senat

Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy: Anna Kot, Anna Kamińska-Dwórznicka, Alicja Barańska, 2020, „*Study of the properties of vegan ice cream based on almond drink*", Zeszyty Problemowe Postępów Nauk Rolniczych, nr 600, 21-30 , mój indywidualny udział w jej powstaniu polegał na przeprowadzeniu części doświadczalnej, opracowaniu i analizie otrzymanych wyników, pisaniu oraz edytowaniu manuskryptu.

Jednocześnie wyrażam zgodę, aby prace zostały wykorzystane w rozprawie doktorskiej mgr inż. Anny Kot.

Podpis

Warszawa, 30.08.2023r.

dr hab. Anna Kamińska-Dwórznicka, prof. SGGW
anna_kaminska1@sggw.edu.pl

Rada Dyscypliny Technologia Żywności i Żywienia

lub Senat

Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy: Anna Kot, Anna Kamińska-Dwórznicka, Alicja Barańska, 2020, „*Study of the properties of vegan ice cream based on almond drink*", Zeszyty Problemowe Postępów Nauk Rolniczych, nr 600, 21-30, mój indywidualny udział w jej powstaniu polegał na utworzeniu koncepcji badań, nadzorze prowadzonych prac laboratoryjnych, przygotowywaniu manuskryptu oraz jego recenzji.

Jednocześnie wyrażam zgodę, aby prace zostały wykorzystane w rozprawie doktorskiej mgr inż. Anny Kot.

Podpis

Anna Kamińska-Dwórznicka

Warszawa, 04.09.2023

mgr inż. Alicja Barańska
alicja_baranska@sggw.edu.pl

Rada Dyscypliny Technologia Żywności i Żywienia

lub Senat

Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie

Oświadczenie.o współautorstwie

Niniejszym oświadczam, że w pracy: Anna Kot, Anna Kamińska-Dwórznicka, Alicja Barańska, 2020, „*Study of the properties of vegan ice cream based on almond drink*", Zeszyty Problemowe Postępów Nauk Rolniczych, nr 600, 21-30, mój indywidualny udział w jej powstaniu polegał na udziale części doświadczalnej oraz w edycji manuskryptu.

Jednocześnie wyrażam zgodę, aby prace zostały wykorzystane w rozprawie doktorskiej mgr inż. Anny Kot.

Podpis

A. Barańska

Warszawa, 30.08.2023r.

mgr inż. Anna Kot
anna_kot1@sggw.edu.pl

Rada Dyscypliny Technologia Żywności i Żywienia

lub Senat

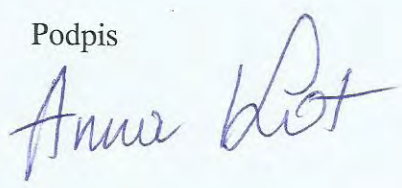
Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy: Anna Kot, Anna Kamińska-Dwórznicka, Sabina Galus, Ewa Jakubczyk, 2021, *„Effects of different ingredients and stabilisers on properties of mixes based on almond drink for vegan ice cream production”,* Sustainability, 13 (21), 121113, mój indywidualny udział w jej powstaniu polegał na polegał na udziale w tworzeniu koncepcji badań, przeprowadzeniu części doświadczalnej, opracowaniu i analizie otrzymanych wyników oraz pisaniu, recenzji i edycji manuskryptu.

Jednocześnie wyrażam zgodę, aby prace zostały wykorzystane w rozprawie doktorskiej mgr inż. Anny Kot.

Podpis



Warszawa, 30.08.2023r.

dr hab. Anna Kamińska-Dwórznicka, prof. SGGW
anna_kaminska1@sggw.edu.pl

Rada Dyscypliny Technologia Żywności i Żywienia

lub Senat

Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy: Anna Kot, Anna Kamińska-Dwórznicka, Sabina Galus, Ewa Jakubczyk, 2021, *„Effects of different ingredients and stabilisers on properties of mixes based on almond drink for vegan ice cream production"*, Sustainability, 13 (21), 121113, mój indywidualny udział w jej powstaniu polegał na utworzeniu koncepcji badań, nadzorze prowadzonych prac laboratoryjnych, przygotowywaniu manuskryptu oraz jego edycji i recenzji.

Jednocześnie wyrażam zgodę, aby prace zostały wykorzystane w rozprawie doktorskiej mgr inż. Anny Kot.

Podpis

Anna Kamińska-Dwórznicka

Warszawa, 30.08.2023r.

dr hab. Sabina Galus, prof. SGGW
sabina_galus@sggw.edu.pl

Rada Dyscypliny Technologia Żywności i Żywienia

lub Senat

Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy: Anna Kot, Anna Kamińska-Dwórznicka, Sabina Galus, Ewa Jakubczyk, 2021, *„Effects of different ingredients and stabilisers on properties of mixes based on almond drink for vegan ice cream production"*, Sustainability, 13 (21), 121113, mój indywidualny udział w jej powstaniu polegał na przeglądzie literatury oraz udziale w edycji i recenzji manuskryptu.

Jednocześnie wyrażam zgodę, aby prace zostały wykorzystane w rozprawie doktorskiej mgr inż. Anny Kot.

Podpis

Warszawa, 30.08.2023r.

dr hab. Ewa Jakubczyk, prof. SGGW
ewa_jakubczyk@sggw.edu.pl

Rada Dyscypliny Technologia Żywności i Żywienia

lub Senat

Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie

Oświadczenie o.współautorstwie

Niniejszym oświadczam, że w pracy: Anna Kot, Anna Kamińska-Dwórznicka, Sabina Galus, Ewa Jakubczyk, 2021, *„Effects of different ingredients and stabilisers on properties of mixes based on almond drink for vegan ice cream production”,* Sustainability, 13 (21), 121113, mój indywidualny udział w jej powstaniu polegał na analizie otrzymanych wyników oraz pisaniu i edycji manuskryptu.

Jednocześnie wyrażam zgodę, aby prace zostały wykorzystane w rozprawie doktorskiej mgr inż. Anny Kot.

Podpis

Ewa Jakubczyk

Warszawa, 29.08.2023r.

mgr inż. Anna Kot
anna_kot1@sggw.edu.pl

Rada Dyscypliny Technologia Żywności i Żywienia

lub Senat

Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy: Anna Kot, Anna Kamińska-Dwórznicka, Ewa Jakubczyk, 2022, *"Study on the influence of ultrasound homogenisation on the physical properties of vegan ice cream mixes"*, Applied Sciences, 12 (17), 8492, mój indywidualny udział w jej powstaniu polegał na polegał na udziale w tworzeniu koncepcji badań, przeprowadzeniu części doświadczalnej, opracowaniu i analizie otrzymanych wyników oraz pisaniu, recenzji i edycji manuskryptu.

Jednocześnie wyrażam zgodę, aby prace zostały wykorzystane w rozprawie doktorskiej mgr inż. Anny Kot.

Podpis

Anna Kot

Warszawa, 30.08.2023r.

dr hab. Anna Kamińska-Dwórznicka, prof. SGGW
anna_kaminska1@sggw.edu.pl

Rada Dyscypliny Technologia Żywności i Żywienia

lub Senat

Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy: Anna Kot, Anna Kamińska-Dwórznicka, Ewa Jakubczyk, 2022, *"Study on the influence of ultrasound homogenisation on the physical properties of vegan ice cream mixes"*, Applied Sciences, 12 (17), 8492, mój indywidualny udział w jej powstaniu polegał na utworzeniu koncepcji badań, nadzorze prowadzonych prac laboratoryjnych, przygotowywaniu manuskryptu oraz jego recenzji edycji.

Jednocześnie wyrażam zgodę, aby prace zostały wykorzystane w rozprawie doktorskiej mgr inż. Anny Kot.

Podpis

Anna Kamińska-Dwórznicka

Warszawa, 30.08.2023r.

dr hab. Ewa Jakubczyk, prof. SGGW
ewa_jakubczyk@sggw.edu.pl

Rada Dyscypliny Technologia Żywności i Żywienia

lub Senat

Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy: Anna Kot, Anna Kamińska-Dwórznicka, Ewa Jakubczyk, *"Study on the influence of ultrasound homogenisation on the physical properties of vegan ice cream mixes"*, Applied Sciences, 12, 8492, mój indywidualny udział w jej powstaniu polegał na analizie otrzymanych wyników oraz pisaniu, edycji i recenzji manuskryptu.

Jednocześnie wyrażam zgodę, aby prace zostały wykorzystane w rozprawie doktorskiej mgr inż. Anny Kot.

Podpis

Ewa Jakubczyk

Warszawa, 08.10.2023r.

mgr inż. Anna Kot
anna_kot1@sggw.edu.pl

Rada Dyscypliny Technologia Żywności i Żywienia

lub Senat

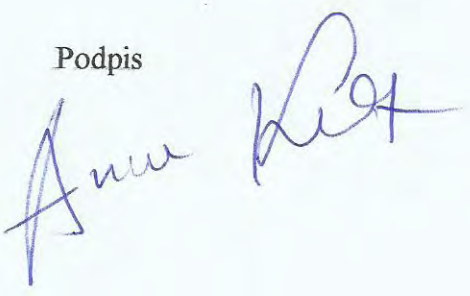
Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy: Anna Kot, Anna Kamińska-Dwórznicka, 2023, *„The influence of ultrasound homogenization on recrystallization during the storage of vegan ice cream"*, Journal of Food Process Engineering, e14472, mój indywidualny udział w jej powstaniu polegał na udziale w tworzeniu koncepcji badań, przeprowadzeniu części doświadczalnej, opracowaniu i analizie otrzymanych wyników oraz pisaniu, recenzji i edycji manuskryptu.

Jednocześnie wyrażam zgodę, aby prace zostały wykorzystane w rozprawie doktorskiej mgr inż. Anny Kot.

Podpis



Warszawa, 08.10.2023r.

dr hab. Anna Kamińska-Dwórznicka, prof. SGGW
anna_kaminska1@sggw.edu.pl

Rada Dyscypliny Technologia Żywności i Żywienia

lub Senat

Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy: Anna Kot, Anna Kamińska-Dwórznicka, 2023, *„The influence of ultrasound homogenization on recrystallization during the storage of vegan ice cream"*, Journal of Food Process Engineering, e14472, mój indywidualny udział w jej powstaniu polegał na utworzeniu koncepcji badań, nadzorze prowadzonych prac laboratoryjnych, przygotowywaniu manuskryptu oraz jego edycji i recenzji.

Jednocześnie wyrażam zgodę, aby prace zostały wykorzystane w rozprawie doktorskiej mgr inż. Anny Kot.

Podpis

Anna Kamińska-Dwórznicka

Warszawa, 29.08.2023r.

mgr inż. Anna Kot
anna_kot1@sggw.edu.pl

Rada Dyscypliny Technologia Żywności i Żywienia

lub Senat

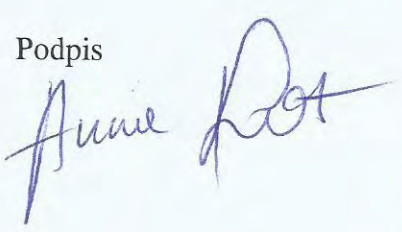
Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy: Anna Kot, Ewa Jakubczyk, Magdalena Buniowska-Olejnik, Anna Kamińska-Dwórznicka, 2023, *„Effect of ι-carrageenan and its hydrolysates on the stability of milk ice cream mixes"*, Polish Journal of Food and Nutrition Sciences, 73(2), 196-204, mój indywidualny udział w jej powstaniu polegał na udziale w tworzeniu koncepcji badań, przeprowadzeniu części doświadczalnej, opracowaniu i analizie otrzymanych wyników oraz pisaniu, recenzji i edycji manuskryptu.

Jednocześnie wyrażam zgodę, aby prace zostały wykorzystane w rozprawie doktorskiej mgr inż. Anny Kot.

Podpis



Warszawa, 30.08.2023r.

dr hab. Ewa Jakubczyk, prof. SGGW
ewa_jakubczyk@sggw.edu.pl

Rada Dyscypliny Technologia Żywności i Żywienia

lub Senat

Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy: Anna Kot, Ewa Jakubczyk, Magdalena Buniowska-Olejnik, Anna Kamińska-Dwórznicka, 2023, *„Effect of ι-carrageenan and its hydrolysates on the stability of milk ice cream mixes"*, Polish Journal of Food and Nutrition Sciences, 73(2), 196-204, mój indywidualny udział w jej powstaniu polegał na recenzji oraz edycji manuskryptu.

Jednocześnie wyrażam zgodę, aby prace zostały wykorzystane w rozprawie doktorskiej mgr inż. Anny Kot.

Podpis

Rzeszów, 30.08.23

dr inż. Magdalena Buniowska-Olejnik
mbuniowska@ur.edu.pl

Rada Dyscypliny Technologia Żywności i Żywienia

lub Senat

Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy: Anna Kot, Ewa Jakubczyk, Magdalena Buniowska-Olejnik, Anna Kamińska-Dwórznicka, 2023, *„Effect of ι-carrageenan and its hydrolysates on the stability of milk ice cream mixes"*, Polish Journal of Food and Nutrition Sciences, 73(2), 196-204, mój indywidualny udział w jej powstaniu polegał na przeglądzie literatury oraz udziale w edycji manuskryptu.

Jednocześnie wyrażam zgodę, aby prace zostały wykorzystane w rozprawie doktorskiej mgr inż. Anny Kot.

Warszawa, 30.08.2023r.

dr hab. Anna Kamińska-Dwórznicka, prof. SGGW
anna_kaminska1@sggw.edu.pl

Rada Dyscypliny Technologia Żywności i Żywienia

lub Senat

Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy: Anna Kot, Ewa Jakubczyk, Magdalena Buniowska-Olejnik, Anna Kamińska-Dwórznicka, 2023, *„Effect of ι-carrageenan and its hydrolysates on the stability of milk ice cream mixes"*, Polish Journal of Food and Nutrition Sciences, 73(2), 196-204, mój indywidualny udział w jej powstaniu polegał na utworzeniu koncepcji badań, nadzorze prowadzonych prac laboratoryjnych, przygotowywaniu manuskryptu oraz jego edycji i recenzji.

Jednocześnie wyrażam zgodę, aby prace zostały wykorzystane w rozprawie doktorskiej mgr inż. Anny Kot.

Podpis

Anna Kamińska-Dwórznicka

Warszawa, 29.08.2023r.

mgr inż. Anna Kot
anna_kot1@sggw.edu.pl

Rada Dyscypliny Technologia Żywności i Żywienia

lub Senat

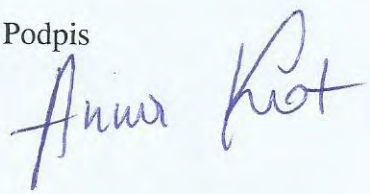
Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy: Anna Kot, Ewa Jakubczyk, Anna Kamińska-Dwórznicka, 2023, *„The effectiveness of combination stabilizers and ultrasound homogenization in milk ice cream production"*, Applied Sciences, 13 (13), 7561, mój indywidualny udział w jej powstaniu polegał na udziale w tworzeniu koncepcji badań, przeprowadzeniu części doświadczalnej, opracowaniu i analizie otrzymanych wyników oraz pisaniu, recenzji i edycji manuskryptu.

Jednocześnie wyrażam zgodę, aby prace zostały wykorzystane w rozprawie doktorskiej mgr inż. Anny Kot.

Podpis



Warszawa, 30.08.2023r.

dr hab. Ewa Jakubczyk, prof. SGGW
ewa_jakubczyk@sggw.edu.pl

Rada Dyscypliny Technologia Żywności i Żywienia

lub Senat

Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy: Anna Kot, Ewa Jakubczyk, Anna Kamińska-Dwórznicka, 2023, *„The effectiveness of combination stabilizers and ultrasound homogenization in milk ice cream production”*, Applied Sciences, 13, (13), 7561, mój indywidualny w jej powstaniu polegał na przeglądzie literatury oraz udziale w edycji i recenzji manuskryptu.

Jednocześnie wyrażam zgodę, aby prace zostały wykorzystane w rozprawie doktorskiej mgr inż. Anny Kot.

Podpis

Ewa Jakubczyk

Warszawa, 30.08.2023r.

dr hab. Anna Kamińska-Dwórznicka, prof. SGGW
anna_kaminska1@sggw.edu.pl

Rada Dyscypliny Technologia Żywności i Żywienia

lub Senat

Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy: Anna Kot, Ewa Jakubczyk, Anna Kamińska-Dwórznicka, 2023, *„The effectiveness of combination stabilizers and ultrasound homogenization in milk ice cream production"*, Applied Sciences, 13, (13), 7561, mój indywidualny udział w jej powstaniu polegał na utworzeniu koncepcji badań, nadzorze prowadzonych prac laboratoryjnych, przygotowywaniu manuskryptu oraz jego edycji i recenzji.

Jednocześnie wyrażam zgodę, aby prace zostały wykorzystane w rozprawie doktorskiej mgr inż. Anny Kot.

Podpis

Anna Kamińska-Dwórznicka

Wyrażam zgodę na udostępnianie mojej pracy w czytelniach Biblioteki SGGW.

Anna Kot

.....

(czytelny podpis autora)