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Hydrożele białkowo-polisacharydowe w projektowaniu innowacyjnych układów strukturotwórczych i nośników substancji bioaktywnych

Protein-Polysaccharide Hydrogels in the Design of Innovative Structure-Forming Systems and Carriers for Bioactive Substances

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Summary

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In recent years, there has been a significant increase in consumer awareness regarding health issues and environmental sustainability. This has led to a growing need for the design of new functional foods, particularly plant-based products with enhanced nutritional value and bioavailability of bioactive compounds. Therefore, it is justified to continually expand the knowledge of plant-derived ingredients that can potentially aid in shaping the appearance, texture, and taste of innovative products.

The aim of this study was to determine the physicochemical properties of binary protein–polysaccharide hydrogels and to develop a model system in which the hydrogel would function as both a structure-forming component and a carrier of bioactive substances. The research was divided into four stages in accordance with the scope of the work. It began with a bibliometric analysis of the literature on protein–polysaccharide hydrogels using VOSviewer software, as well as the characterization of selected protein preparations (pea protein and wheat protein) and polysaccharide preparations (gellan gum, konjac gum, inulin, maltodextrin, psyllium husk, and tara gum). Based on the obtained results, two biopolymers were selected for further study: pea protein (added at 12.5 g/100 g of hydrogel) and psyllium husk (added at 0.5 g/100 g of hydrogel). The influence of pH levels (7, 4.5, and 3) and NaCl concentrations (0, 0.15, and 0.3 M) on the physical properties of these hydrogels was determined. In the final stage, the impact of the induction method on the properties of a system containing anthocyanins from black elderberry fruit extract (at 2 g/ 100 cm^3 of the hydrogel's aqueous phase) was examined. An innovative method of sequential induction was applied, combining thermal induction with secondary non-thermal induction—namely, ultrasonic homogenization (25 kHz, 70 W, 100% pulse, 100% amplitude, temperature of 20 ± 1 °C) and high hydrostatic pressure processing (500 MPa, set temperature of 20 ± 1 °C, maximum temperature during pressurization of 30 ± 1 °C). The obtained systems were analyzed in terms of physical stability, microrheological and textural parameters, color, efficiency of anthocyanin binding from the extract, FT-IR analysis, microstructure, total phenolic content, antioxidant activity (ABTS and DPPH assays), and thermal stability. Statistical analysis of the results was conducted using one-way and two-way ANOVA, along with Tukey's test. To effectively summarize the collected data, the results after each stage were subjected to principal component analysis (PCA) and hierarchical cluster analysis (HCA).

Based on the bibliometric analysis, it was demonstrated that research on food-grade binary hydrogels obtained by combining plant proteins and polysaccharides as building materials is a relatively new and interdisciplinary field. It was also emphasized that their innovativeness lies in the application of a "Bottom-Up Design" approach to develop new functional food matrices. This approach allows for controlling the interactions between proteins and polysaccharides to obtain complex structures with

customized properties, which can aid in the development of innovative and health-promoting food products.

Characterization of the physical properties of protein and polysaccharide preparations led to the identification of materials—pea protein, gellan gum, konjac gum, and psyllium husk—that exhibited the ability to form gel structures (solid–liquid balance coefficient SLB < 0.5). Systems prepared using these materials showed high physical stability (instability index < 0.06) due to the formation of the gel structure. In contrast, preparations of wheat protein, maltodextrin, and tara gum did not form gel structures (SLB > 0,5). Based on principal component analysis and cluster analysis, pea protein and psyllium husk were selected as hydrogel components for further studies. Analysis of the FT-IR spectra for the system containing pea protein, psyllium husk, and their binary hydrogel confirmed the presence of associative interactions (thermodynamic compatibility) between their chains in the form of intermolecular interactions, including van der Waals and electrostatic forces. It was demonstrated that the synergistic action of pea protein and psyllium husk enables the production of hydrogels with favorable physical properties while simultaneously reducing the concentration of biopolymers required to form the gel structure.

Based on the results from the next stage, it was concluded that modifying the pH values and adding NaCl resulted in hydrogels with varied physical properties. The studied hydrogels were classified as weak gels with properties similar to products like ketchup, yogurt, or pudding. Induction at lower pH levels (particularly pH 3 and 4.5) without the addition of NaCl led to the formation of weak, spreadable hydrogels, as indicated by a phase angle tangent ($\tan(\delta) \approx 0.26$) and a strain value in the linear viscoelastic range ($1.39\% < \gamma\ [\%] < 2.18\%$). The addition of NaCl (0.15 and 0.3 M) at the same pH values improved structural stability. At pH 3, increasing the NaCl concentration from 0 to 0.15 M resulted in an average increase in the storage (G′) and loss (G″) moduli by approximately 95 times, and increasing the NaCl concentration from 0 to 0.3 M led to an increase of up to 170 times. The use of lower pH and higher NaCl concentrations enhanced electrostatic interactions between pea protein and psyllium husk, leading to the formation of hydrogels with more favorable physical properties, including high structural stability. It was demonstrated that during induction, the effect of NaCl on the properties of the hydrogels depended on the pH of the system.

Upon analyzing the parameters in the final stage, it was determined that secondary induction using ultrasonic homogenization (U5 and U10) or high hydrostatic pressure (P5 and P10) led to the formation of hydrogel systems with significantly different physicochemical properties. The use of secondary ultrasonic homogenization (USH) resulted in systems with low physical stability and hardness (0.07 N), which adversely affected the binding capacity of black elderberry fruit extract ($EE_{U5} = 3\%$; $EE_{U10} = 9\%$) and the final antioxidant activity ($ABTS_{U5} = 3.6$ mg TE/100 g d.m.; $ABTS_{U10} = 3.1$ mg TE/100 g d.m.). Additionally, extending the induction time to 10 minutes (U10) caused the greatest degradation of polyphenols (9.31 g chlorogenic acid/100 g d.m.) compared to the control system ($TPC_C = 18.4$ g chlorogenic acid/100 g d.m.). In contrast, the

application of secondary high-pressure induction (HHP) resulted in systems with a compact microstructure (visible in scanning electron micrographs), leading to a high capacity for binding anthocyanins from the extract within the system ($EE_{P5} = 20\%$; $EE_{P10} = 33\%$). Model systems based on a pea protein and psyllium husk hydrogel containing black elderberry fruit extract, obtained through secondary HHP induction, exhibited high antioxidant activity. Notably, a ten-minute HHP induction achieved the highest total polyphenol content within the structure ($TPC_{P10} = 22.7$ g chlorogenic acid/100 g d.m.) and the highest antioxidant activity ($ABTS_{P10} = 5.8$ mg TE/100 g d.m.; $DPPH_{P10} = 1.08$ mg TE/100 g d.m.).

Based on the results, it was demonstrated that it is possible to develop a model system with health-promoting properties based on a binary hydrogel of pea protein and psyllium husk. In this system, the hydrogel serves both as a structure-forming component and as a carrier of bioactive substances. Furthermore, it was shown that the methods and parameters of induction significantly influenced the physicochemical properties of the hydrogel systems. Notably, the sequential technique involving the application of thermal induction and high hydrostatic pressure under appropriately selected conditions (pH = 3 and NaCl addition at 0.3 M) enabled the formation of a hydrogel system with optimal physicochemical properties, capable of serving both as a structure-forming component and as a carrier of bioactive substances.

Keywords – protein–polysaccharide hydrogels, pea protein, psyllium husk, physicochemical properties, black elderberry fruit extract, induction methods, ultrasonic homogenization, high hydrostatic pressure