applied sciences

MDPI

Article

Physicochemical Properties of Protein Preparations and Their Use in the Formulation of Functional Beverages with Varying Osmolality

Anna Sadowska [1], Katarzyna Najman [1,*] and Katarzyna Starczewska [2]

[1] Department of Functional Foods, Organic Foods and Nutritional Sensory Science, Faculty of Health Sciences, Warsaw University of Life Sciences, Nowoursynowska 159c, 02-776 Warsaw, Poland; anna_sadowska@sggw.edu.pl

[2] Faculty of Human Nutrition, Warsaw University of Life Sciences, Nowoursynowska Str. 159c, 02-776 Warsaw, Poland

* Correspondence: katarzyna_najman@sggw.edu.pl

Abstract

Nine commercially available protein preparations, derived from milk, plants, or collagen, were compared in terms of selected physicochemical and technological properties, with particular emphasis on the relationship between the concentration of the preparation and the osmolality of aqueous systems. This study evaluated color, water activity, pH, water- and fat-binding capacities, ABTS radical scavenging activity, and selected sensory attributes. The osmolality of aqueous solutions of these preparations was measured across concentration ranges adopted to their specific technological properties. Significant differences were observed among the preparations tested. Within the ranges analyzed, osmolality increased almost linearly with concentration; this relationship was generally more pronounced for hydrolyzed preparations than for those consisting primarily of non-hydrolyzed proteins. ABTS radical scavenging activity ranged from 15.52 to 40.53 μmol TE/1 g, with the highest value recorded for calcium caseinate. Additionally, three sample formulations for powdered base beverages were developed, each containing 5% of a specific protein preparation; upon reconstitution, these beverages exhibited osmolality values of approximately 245, 302, and 555 mOsm/kg H_2O, respectively. The base beverages were designed to have varying osmolality levels, corresponding to hypotonic, isotonic, and hypertonic solutions. The results provide comparative data on individual ingredients used to develop a specific beverage type; this can be useful during the initial stage of formulating recipes for drinks intended for various purposes (e.g., before, during, or after physical activity). It should be noted, however, that estimating osmolality in complex beverage matrices requires further verification, given the variability in the osmolality of carbohydrate components (such as sweeteners or flavoring agents like fruit powders) and bioactive substances (such as electrolytes).

Keywords: physicochemical properties; protein preparations; osmolality; functional beverages formulation

Academic Editor: Antonios E. Koutelidakis

Received: 13 October 2026
Revised: 22 November 2026
Accepted: 24 November 2026
Published: 27 November 2026

Copyright: © 2026 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.

1. Introduction

In the literature, the term "functional beverage" refers to drinks made from ingredients intended to provide benefits beyond basic hydration or conventional nutrition. The composition of such beverages includes bioactive compounds (such as vitamins, miner-

als, proteins, amino acids, and components with high antioxidant potential), as well as natural additives, primarily of plant origin (e.g., dietary fiber). A wide range of innovative functional products, aimed at a diverse group of consumers, is available on the beverage market. These include probiotic, energy, isotonic, and "wellness" drinks, as well as beverages with high nutritional and energy value that can serve as meal replacements. Such products are enhanced with flavoring agents, including juices, purees, powdered fruits, or spices. An emerging trend in the production of functional beverages is the incorporation of protein preparations derived from animal, plant, or combined sources. The most frequently used protein sources in these products are milk proteins, collagen, and legume proteins [1–3]. As part of this evaluation, the following properties were examined: sensory characteristics, solubility, pH, color, water- and fat-binding capacities, thermal stability, antioxidant potential, and osmolality. The results show differences among these formulations, enabling the selection of those best suited to the development of new functional products. The most important characteristics of protein preparations used in functional beverages include solubility, water- and/or fat-binding capacity, thickening properties, stability at low pH—as well as during processing and storage—and their impact on taste and aroma [4]. The bioactive features of these ingredients are also significant, as they determine the special value of the developed products [2]. The market for beverages designed for physically active individuals, those that support hydration and provide nutrients, is developing particularly dynamically. Developing such beverages, especially "clean-label" ones, requires assessing the nutritional value of individual ingredients, their technological properties, and osmolality parameters, which is a major technological challenge. This process requires selecting an appropriate protein preparation and natural additives to shape the flavor profile. In the case of isotonic drinks, control of osmotic pressure is also crucial. Another technological challenge consists of achieving the stability of protein-based products. During storage, heat-treated beverages (e.g., those processed via UHT) often undergo undesirable changes, including increased viscosity, phase separation, and protein sedimentation at the bottom of the packaging. The thermal stability of whey proteins has been extensively documented in the literature [5–11]. These studies focus on undesirable phenomena that occur during the preparation of protein beverages and suggest ways to reduce them. Consequently, there is a lack of functional fruit-based beverages on the market that provide both high protein content and a range of osmolality levels, including those characteristic of isotonic drinks. Developing such a product in powder form provides a promising solution to this challenge. It will allow consumers to prepare the beverage themselves by adding water, milk, or other liquids to suit their preferences. Previous studies by members of the present research team investigated the osmolality of ingredients used in functional recovery drinks in powder form and the design of multicomponent beverages, including fruit-based formulations and systems containing electrolytes, peptides, carbohydrates, and prebiotic fiber [12,13]. The present study builds on this research by comparing nine commercial protein preparations from dairy, plant, and collagen sources with respect to selected physicochemical and technological properties, as well as concentration-dependent osmolality.

Taking the above into account, the objective of this study was to evaluate the sensory and physicochemical properties of selected protein preparations. In addition, the osmolality of their solutions at various concentrations was determined, enabling assessment of the relationship between the amount of preparation used and the system's osmotic properties. In the second stage of the study, formulations for functional beverages including these selected protein preparations were developed in powder form and were intended to be dissolved in an appropriate amount of water by the consumer.

Subsequently, their osmolality was determined, and their sensory characteristics were evaluated. The study assumed that the results would serve as the basis for selecting protein preparations for the development of isotonic and regenerative drinks.

The innovation of this work consists of its detailed approach to evaluating the physicochemical, technological, and osmotic properties of protein preparations derived from dairy and plant sources, as well as collagen and whey hydrolyzates. Furthermore, these data were used to develop functional powdered beverages containing both protein preparations and fruit powders. Particular attention was paid to the relationship between the type and concentration of the protein preparation and the osmolality of its solutions, thereby enabling comparison of the osmotic properties of ingredients intended for use in products with diverse compositions and applications. A key element of this work is the integration of protein preparation characterization with the formulation of powdered beverages, in which bioactive ingredients are combined with fruit powders—sources of both water-soluble and water-insoluble components, as well as bioactive compounds. This method enables the development of products that serve two roles: they provide protein and fruit-derived components while also modulating the physicochemical and osmotic properties of the beverages once dissolved in water. An additional aspect of the work involves developing a formulation for an isotonic beverage containing sodium chloride. In this context, it is important to determine how the presence of protein preparations, fruit powders, and sodium chloride influences the final osmotic properties of a product. The innovative nature of this research also lies in the inclusion of various protein preparations—such as milk proteins, plant-based proteins, and collagen hydrolyzates—in a comparative assessment of their properties. This evaluation included sensory properties, solubility, pH, color, water- and fat-binding capacities, thermal stability, antioxidant potential, and osmolality. The results indicate differences among these formulations, enabling the selection of those best suited to the development of new functional products. Comparing these parameters with the osmolality of solutions containing the preparations in question at various concentrations, as well as with the osmolality of finished products, enables the selection of suitable ingredients to achieve products with specific technological and nutritional properties. Despite extensive research into the nutritional value, functional properties, and applications of protein preparations in food technology, knowledge regarding their impact on the osmolality of aqueous systems, in the context of designing functional beverages, remains relatively limited. Previous studies have focused primarily on the solubility, emulsifying properties, water- and fat-binding capacities, stability, and rheological properties of proteins. Much less attention has been devoted to the comparative assessment of the osmolality of protein preparations derived from various sources and to determining the relationship between their concentration and osmolality. Differences in molecular weight and the degree of protein hydrolysis are of particular importance here; both factors might considerably affect the number of osmotically active particles in a solution. Consequently, preparations with similar protein content may exhibit different osmotic properties, which is important when developing beverages with a targeted osmolality. The existing literature lacks sufficient data to enable a simultaneous comparison of the physicochemical and technological properties of various groups of protein preparations, as well as their influence on the osmolality of solutions and model functional beverages.

2. Materials and Methods

2.1. Materials

The study material consisted of commercially available protein preparations of animal and plant origin. The nutritional value of the tested preparations is presented in

Table 1. The products used in this study were obtained from the Polish market.

Table 1. Nutritional value of the tested preparations.

Tested Preparations	Energy Value (kcal/100 g)	Fat (Sat. Fatty Acids) (g/100 g)	Carbohydrates (Sugars) (g/100 g)	Protein (g/100 g)	Salt (g/100 g)
All milk proteins	351.5	1.5 (0.9)	4.5 (4.5)	80	0.30
Whey protein isolate	373	1.0 (0.3)	1.0 (0.5)	90	0.25
Hydrolyzed whey proteins	376	4.0 (1.8)	5.0 (3.5)	80	0.40
Hydrolyzed collagen proteins	360	0.0 (0.0)	0.0 (0.0)	90	0.50
Micellar casein	357.5	1.5 (0.9)	6.0 (4.5)	80	0.20
Calcium caseinate	358	2.0 (1.2)	5.0 (0.2)	80	0.30
Soy protein isolate	357.5	1.5 (0.3)	2.0 (0.1)	84	1.80
Pea protein concentrate	389.5	5.5 (1.2)	5.0 (0.2)	80	1.50
Rice protein concentrate	370	4.0 (1.0)	3.5 (0.5)	80	0.40

To design functional recovery beverages, in addition to the above-mentioned protein preparations, the following components were used: freeze-dried raspberry powder, cocoa powder (nonfat), sugar (sucrose), and stevia (steviol glycosides).

2.2. Methods

2.2.1. Sensory Evaluation of Protein Preparations and Beverages

The sensory evaluation encompassed both protein preparations and the developed beverages, which varied in osmolality. The protein preparations were assessed in powder form and as 2% (*w*/*v*) aqueous solutions at 22 °C ± 2 °C and 80 °C ± 2 °C (with the solutions held at the higher temperature for 10 min). An eight-member panel, trained in accordance with the EN ISO 8586:2012 standard [14], conducted the sensory evaluation using a simple descriptive method. This assessment covered appearance (including color), consistency, taste, and aroma, following the general methodological guidelines set out in the PN-ISO 6658:2017 [15] standard and utilizing the terminology defined in the PN-ISO 5492:1997 standard [16]. The samples were evaluated in two independent sessions. The samples were labeled with three-digit codes and presented to the evaluators in a random order. The evaluation took place in a sensory laboratory that met the requirements of the EN ISO 8589:2014 standard [17].

2.2.2. Color Parameters in the CIE Lab Color Space in Protein Preparations

Samples of protein preparations were placed in a specialized measuring vessel to ensure a uniform layer. Instrumental measurements included three chromatic coordinates within the CIE color space (L*, a*, b*). The L* value represents the lightness of the sample; +a* indicates a red hue, −a* represents a green hue, +b* a yellow hue, and −b* a blue hue. In the study, delta E values were also calculated [18] for all tested preparations in relation to the ideal whiteness standard (L* = 100, a* = 0; b* = 0). Four independent measurements were performed for each sample tested.

2.2.3. Antioxidant Activity in Protein Preparations

Samples of each protein preparation (1.0 g) were weighed into sterile plastic Falcon tubes, mixed with 25 mL of deionized water, and incubated for 60 min at 30 °C with continuous shaking at 200 rpm in an incubator shaker (KS 4000 i Control, IKA, Staufen, Germany). The samples were then centrifuged (15 min, 5 °C, 10.000 rpm; 12,100× *g*) using a refrigerated centrifuge (MPW-380R, fixed-angle rotor no. 11776, MPW MED Instruments, Warsaw, Poland), and the supernatant was collected for further analysis. The antioxidant activity of the protein preparations was determined using the ABTS+• radical cation (2.2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) (Sigma-Aldrich, Poznań, Poland) following the method developed by Re et al. (1999) [19]. A mixture consisting of 1.5 mL of the diluted protein preparation supernatant and 3.0 mL of the ABTS+• radical cation solution in phosphate buffer was prepared using a vortex mixer (Wizard Advanced IR Vortex Mixer, VELP Scientifica Srl., Usmate (MB), Italy) and incubated for 6 min at 22 °C ± 2 °C. The absorbance of the samples was measured at a wavelength of λ = 734 nm using a spectrophotometer (UV/Vis UV-6100A, Metash Instruments Co. Ltd., Shanghai, China). The results, adjusted for the dilutions used and based on a calibration curve for the reference substance—Trolox ($y = -5.6017x + 0.7134$; $R^2 = 0.9998$)—were expressed in units of μmol TE/1 g of the preparation (TE—Trolox Equivalents). Each measurement was performed in triplicate for every sample tested.

2.2.4. pH Values of Protein Preparations

The pH value of nine protein preparations was determined using a potentiometric method based on the general guidelines of the PN-ISO 1842:1999 standard [20]. Due to the diverse physicochemical properties of the matrices analyzed, a standardized modification to the sample preparation procedure was applied. To ensure full comparability of the results, a 2% (*w*/*v*) aqueous solution of each preparation was prepared using distilled water. Measurements were performed at a controlled temperature of 20 °C ± 2 °C using a pH meter (Elmetron, Zabrze, Poland). Prior to analysis, the instrument was calibrated at two points using certified buffer solutions with pH values of 4.01 and 7.00. All measurements were conducted in three independent replicates.

2.2.5. Water Activity (a_w) of Protein Preparations

The water activity of protein preparations was measured using an AquaLab device (Decagon Devices, Pullman, WA, USA) at 20 ± 2 °C in accordance with the ISO 18787:2017 standard [21]. Three measurements were performed for each sample.

2.2.6. Water-Binding Capacity of Protein Preparations

The water-binding capacity of the preparations was determined using the modified method of Sosulski et al. [22]. After weighing empty Falcon tubes, approximately 1 g of protein preparation was measured into them, mixed with 30 mL of distilled water, and then centrifuged (MPW-380R fixed-angle rotor no. 11776, MPW MED Instruments, Warsaw, Poland) for 15 min at 9000 rpm (9780× *g*). Following centrifugation, the unbound water was removed. The tubes were left inverted for 10 min. The tubes, containing the sediment, were then weighed. Water-binding capacity was expressed in g of water per g of preparation. Three measurements were performed for each sample.

2.2.7. Fat-Binding Capacity of Protein Preparations

The fat-binding capacity of the preparations was determined using the modified method of Sosulski et al. [22]. After weighing empty Falcon tubes, approximately 5 g of the protein preparation and 25 mL of rapeseed oil were added to each tube and then

mixed. The tubes were left to stand for 5 min, after which their contents were mixed again and subjected to centrifugation (MPW-380R, fixed-angle rotor no. 11776, MPW MED Instruments, Warsaw, Poland) for 5 min at a speed of 5000 rpm (3019× *g*). Unbound oil was poured into a measuring cylinder. Fat binding capacity was expressed in mL oil/g of preparation. Three measurements were performed for each sample.

2.2.8. Osmolality of Protein Preparations and Designed Beverages

Osmolality was determined by freezing-point depression using a Cryo-scope 800CL device (Trident Med., Warsaw, Poland). Distilled water was used to establish the zero point (0 mOsm/kg H_2O). The osmolality of the preparations was evaluated in aqueous solutions at concentrations ranging from 0.5% to 14% (*w*/*w*) for milk proteins and collagen; from 1% to 10% (*w*/*w*) for pea and rice protein concentrates; and from 0.5% to 5% (*w*/*w*) for soy protein. Prior to osmolality measurement, samples containing visible suspensions (insoluble particles) were subjected to centrifugation (10,000 rpm; 12,100× *g*; for 10 min) using a refrigerated centrifuge (MPW-380R, fixed-angle rotor no. 11776; MPW MED Instruments, Warsaw, Poland) so that the clear supernatant could be used for analysis. A 100 µL aliquot of each sample was placed into a measurement tube, followed by supercooling and initiation of crystallization. The device converted the freezing-point temperature signal into an osmolality value, expressed in mOsm/kg H_2O. Three measurements were performed for each sample.

2.2.9. Preparation of Base Beverages

The base beverages were prepared using a gravimetric method. Dry ingredients were passed through a 0.8 mm mesh sieve and then weighed according to the recipe for each variant. To prepare the solutions for analysis, the weighed ingredients were dissolved in distilled water (20 °C) to achieve a final mass of 100 g. The solutions were mixed using a magnetic stirrer (500 rpm, 5 min) and then left to stand for 10 min to allow deaeration and stabilization prior to osmolality measurement. Each variant was prepared and analyzed in three independent batches.

2.2.10. Statistical Analysis

Statistical analysis was performed using the Statistica 13.1 software package (TIBCO Software Inc., Palo Alto, CA, USA). The assumption of normality for the distribution of dependent variables in the studied groups was verified using the Shapiro–Wilk test, which confirmed a normal distribution for all analyzed parameters ($p > 0.05$). Homogeneity of variance was assessed using Levene's test. For the results concerning antioxidant activity and color parameters (L*, a*, b*), Levene's test did not show statistical significance ($p > 0.05$), confirming homogeneity of variance. Consequently, a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was applied to evaluate these parameters, with a significance level of $p < 0.05$.

For the remaining parameters (water activity, pH, water-holding capacity, and fat binding capacity), Levene's test showed statistical significance ($p < 0.05$), indicating a lack of homogeneity of variances. Therefore, Welch's one-way analysis of variance (Welch's F-test) was used to assess the overall differences, while Welch's *t*-tests (*t*-tests for independent samples with separate variance estimation) were employed as post hoc tests, incorporating the Benjamini–Hochberg correction (to control the false discovery rate, FDR) for multiple comparisons at an adjusted significance level of $p < 0.05$. To evaluate the effect of the mass concentration of protein preparations on the osmolality of the solutions, a linear regression analysis was performed using the ordinary least squares method, described by the equation y = ax + b (where y represents osmolality, x is the mass concen-

tration of the preparation, a is the slope coefficient indicating the rate of change, and b is the intercept). To evaluate the statistical significance of the interaction effect (preparation × concentration) and to formally compare the slopes for all preparations, an analysis of covariance (ANCOVA) was performed using general regression models. Due to significant heterogeneity in the slopes ($p < 0.001$), as well as variations in the concentration ranges tested and the number of data points, individual regression models were developed independently for each of the nine protein sources.

The coefficient of determination (R^2) was used to assess the fitting of the linear models, while the statistical significance of the coefficients was verified using Student's *t*-test and analysis of variance (F-test) at a significance level of $p < 0.05$. Standard errors (SE) and 95-percent confidence intervals (95% CI) were calculated for all specified regression parameters (a and b). The correctness of the model fit was verified using residual analysis, including an examination of the normal distribution of the residuals and plots showing the relationship between the residuals and the predicted values to assess homoscedasticity. The obtained relationships are presented graphically in the form of a categorized (2D) scatter plot, showing empirical data points along with fitted linear regression lines for all nine preparations within a common coordinate system.

3. Results and Discussion

3.1. Technological and Physicochemical Features of the Tested Protein Powder Preparations

Table 2 and Figure 1 show the sensory characteristics of the protein preparations studied. The consistency of the preparations was powdery, with some tendency to form clumps (this applied to calcium caseinate, whey protein isolate, soy protein, and pea protein). Plant proteins formed larger clumps than animal proteins. All milk protein preparations had a similar taste: slightly milky and sweet. Micellar casein had a slightly sour aftertaste, while calcium caseinate had a bitter aftertaste. The taste of collagen hydrolyzate was neutral and resembled that of powdered gelatin. Plant proteins had an earthy, vegetal taste, although this note was slightly less noticeable in pea and soy preparations than in rice protein. The color of the tested preparations was visually assessed as white, cream, or yellow, depending on the product type. Animal-derived milk proteins appeared white; hydrolyzed collagen proteins appeared cream-colored; while the darkest yellow shade was characteristic of plant-based proteins—soy, pea, and rice (Figure 1).

Table 2. Organoleptic characteristics of selected protein preparations.

Preparation	Color	Aroma	Consistency	Taste
All milk proteins	white, milky	slightly milky, resembling powdered milk	loose, powdery	slightly milky, sweet
Collagen hydrolysate	cream	gelatin-like	iridescent	gelatin-like aftertaste
Whey hydrolysate	pale yellow, creamy	slightly milky	loose	bitter with a milky aftertaste
Calcium caseinate	white	neutral	loose, with a tendency to clump	neutral with a bitter aftertaste
Micellar casein	white, milky	milky	loose	neutral with a milky and slight sour aftertaste
Whey isolate	white	slightly milky, resembling powdered	free-flowing. may form small clumps	slightly milky

Preparation	Color	Aroma	Consistency	Taste
		milk		
Soy protein isolate	bright yellow	slightly earthy, barely perceptible	powder that tends to clump	slightly earthy, plant-like, soy-like
Pea protein concentrate	bright yellow	slightly earthy, barely perceptible	powder that tends to clump	slightly earthy, plant-like, pea-like
Rice protein	white, bright yellow	earthy, plant-like	loose	earthy, plant-like

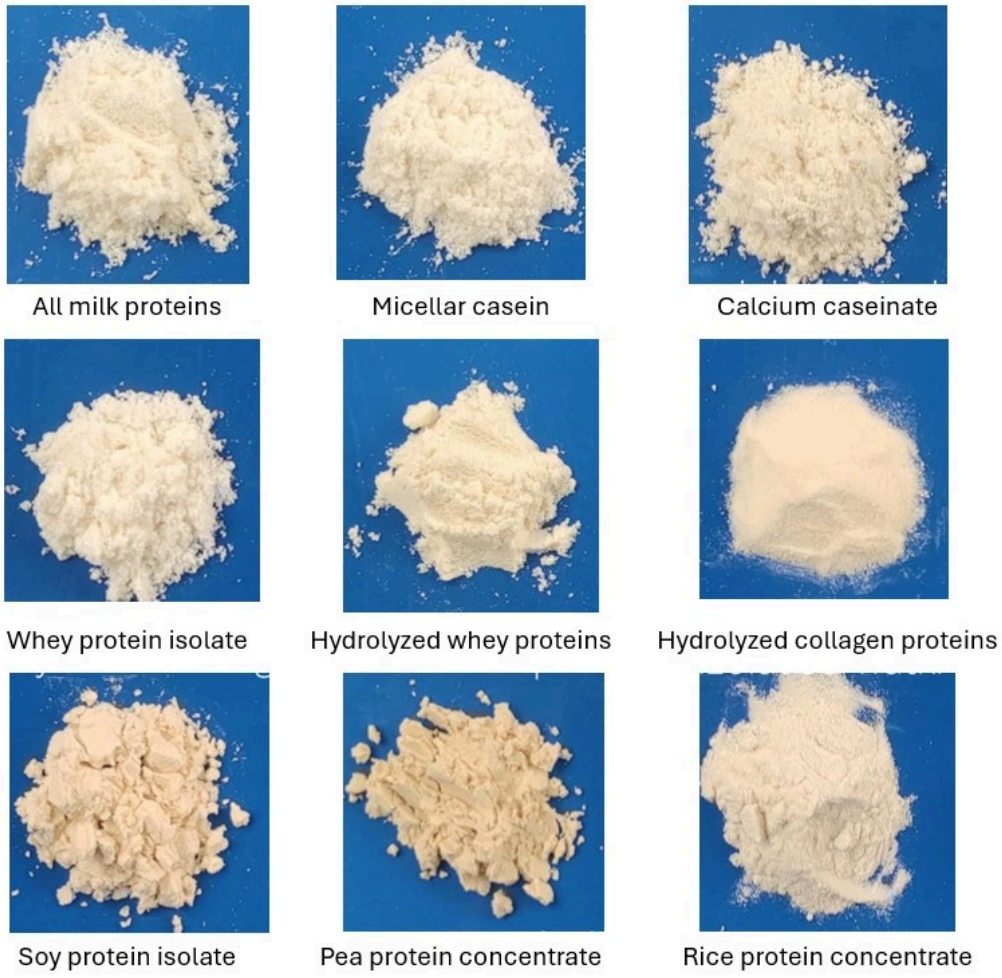


Figure 1. The appearance of the tested protein preparations.

Instrumental color evaluation in the CIE L*a*b* color space showed significant differences in the chromatic coordinates L*, a* and b* (Table 3). A comparison of the L* parameter, which defines lightness, showed that all protein preparations were characterized by very high lightness values, ranging from approximately 84 to 96. Significantly ($p < 0.05$), the highest lightness was recorded for calcium caseinate, whey protein hydrolyzate, milk proteins, and micellar casein, whereas significantly ($p < 0.05$) lower values were observed for whey protein isolate and collagen hydrolyzate. Plant-based protein preparations had a significantly ($p < 0.05$) darker color; among them, rice and pea protein concentrates had the lowest L* value, meaning they had the darkest color of all the preparations analyzed in this study. The a* parameter, which describes color devia-

tions ranging from green (−a*) to red (+a*), ranged from −1.80 to 3.80. The lowest values were found in the majority of animal-derived protein preparations, showing a higher saturation of green tones. In contrast, plant-based protein preparations exhibited positive a* values, showing a higher saturation of red tones in these samples. All analyzed protein preparations exhibited positive b* values, denoting a color turn towards yellow. Among the animal-derived preparations, the highest yellow color intensity was observed for whey protein isolate, whereas among plant-derived preparations, it was found in pea protein concentrate. There is limited data in the available literature regarding color parameters in the CIE L*a*b* color space for various powdered protein preparations. Table 3 also presents the ΔE values calculated for all tested preparations relative to an ideal white reference (L* = 100; a* = 0; b* = 0). Lower ΔE values indicate a smaller color deviation from the ideal white reference, whereas higher values indicate a greater departure from ideal white. The smallest deviations were observed in calcium caseinate, hydrolyzed whey protein, and micellar casein preparations. All these preparations exhibited a milky-white appearance with a slight greenish tint. Furthermore, calcium caseinate showed the highest lightness (L* = 96.28) and a low degree of yellowness. A greater difference from the ideal white reference was observed in all-milk proteins preparation (ΔE = 14.93) and in hydrolyzed collagen proteins (ΔE = 18.55). Despite their relatively high lightness, their color coordinates indicated shifts towards creamy or slightly yellowish tones. The biggest differences among the tested preparations were observed for soy protein (ΔE = 20.44) and whey protein isolate (ΔE = 22.45). In the case of soy protein isolate, this difference was connected with lower lightness, while whey protein isolate was characterized by a distinct yellow hue (b* = 21.44). Rice protein (ΔE = 24.71) and pea protein concentrate (ΔE = 29.54) showed the largest deviations from the ideal white reference. These preparations also had the lowest lightness values (L* < 85) and distinct natural color characteristics, described as yellow-brown for rice protein concentrate and yellow-green for pea protein concentrate.

Table 3. Color parameters in the CIE Lab color space in tested protein preparations.

Type of Protein Preparation	L*	a*	b*	ΔE (Relative to White)
All milk proteins	95.41 ± 0.19 ^{e}	−1.80 ± 0.04 ^{a}	14.09 ± 0.42 ^{c}	14.93 ± 0.40 ^{c}
Micellar casein	95.73 ± 0.35 ^{e}	−1.23 ± 0.06 ^{b}	10.01 ± 0.25 ^{b}	10.95 ± 0.27 ^{b}
Calcium caseinate	96.28 ± 0.64 ^{e}	−1.29 ± 0.04 ^{b}	8.68 ± 0.55 ^{a}	9.53 ± 0.56 ^{a}
Whey protein isolate	93.33 ± 0.54 ^{d}	−0.19 ± 0.08 ^{d}	21.44 ± 0.20 ^{f}	22.45 ± 0.25 ^{f}
Hydrolyzed whey proteins	95.97 ± 0.23 ^{e}	−0.73 ± 0.04 ^{c}	9.37 ± 0.026 ^{a,b}	10.23 ± 0.09 ^{a}
Hydrolyzed collagen proteins	90.44 ± 0.92 ^{c}	0.86 ± 0.11 ^{e}	15.87 ± 0.54 ^{d}	18.55 ± 0.66 ^{d}
Soy protein isolate	88.19 ± 0.34 ^{b}	0.74 ± 0.05 ^{e}	16.67 ± 0.32 ^{d}	20.44 ± 0.33 ^{e}
Pea protein concentrate	84.89 ± 0.18 ^{a}	3.80 ± 0.06 ^{g}	25.10 ± 0.28 ^{g}	29.54 ± 0.26 ^{h}
Rice protein concentrate	84.16 ± 0.23 ^{a}	1.14 ± 0.05 ^{f}	18.93 ± 0.15 ^{e}	24.71 ± 0.19 ^{g}

The table presents mean values along with standard deviations. The letters a–h in the columns indicate statistically significant differences between the obtained results ($p < 0.05$).

Table 4 presents the results of the analyses concerning water activity, as well as the water- and fat-binding capacities of the tested preparations. Water activity ranged from approximately 0.199 for whey protein isolate to approximately 0.374 for pea protein con-

centrate and 0.375 for calcium caseinate (Table 4). Hydrolyzed collagen, whey protein, and soy protein isolate exhibited similar values ($p > 0.05$) for this parameter (0.235, 0.236, and 0.239, respectively). A similar trend was found for all-milk proteins preparation, micellar casein, and rice protein concentrate ($p < 0.05$). The differences in water activity between whey protein isolate and calcium caseinate may stem from variations in their macromolecular structure. Caseinates, characterized by an irregular structure, absorb moisture into the micropores of the powder more readily than whey proteins. Even after the spray-drying process, calcium caseinate molecules retain a strong affinity for water, resulting in a higher moisture content in the final product [23,24]. In contrast, the globular structure of whey proteins in a dry state limits water access to the interior of the molecule [25]. Moreover, this preparation undergoes microfiltration combined with ultrafiltration/diafiltration, a process that almost entirely removes other hygroscopic components, such as mineral salts or lactose [26]. According to the literature, food products with a water activity not exceeding 0.6 do not support the growth of microorganisms, including the most osmotolerant yeasts and xerophilic molds [27]. On this basis, it can be concluded that all the analyzed protein preparations can be considered stable with respect to microbial growth under appropriate storage conditions.

Table 4. Water activity and absorption of water of the insoluble protein fraction and fat holding capacity in tested protein preparations.

Type of Protein Preparation	Water Activity (aw)	Fat Holding Capacity (mL/g)	Water Holding Capacity (g/g)
All milk proteins	0.343 ± 0.003 ^{c}	2.89 ± 0.05 ^{f}	1.0± 0.12 ^{a}
Micellar casein	0.306 ± 0.003 ^{c}	1.49 ± 0.06 ^{c,d}	0.63 ± 0.30 ^{a}
Calcium caseinate	0.375 ± 0.005 ^{d}	1.35 ± 0.10 ^{b,c}	3.2± 0.24 ^{d}
Whey protein isolate	0.199 ± 0.000 ^{a}	1.84 ± 0.15 ^{d,e}	<1.0
Hydrolyzed whey proteins	0.236 ± 0.004 ^{b}	1.31 ± 0.02 ^{c}	<0.2
Hydrolyzed collagen proteins	0.235 ± 0.001 ^{b}	1.84 ± 0.06 ^{e}	<0.2
Soy protein isolate	0.239 ± 0.003 ^{b}	4.04 ± 0.07 ^{g}	6.02 ± 0.32 ^{e}
Pea protein concentrate	0.374 ± 0.000 ^{d}	0.90 ± 0.03 ^{a}	1.61 ± 0.08 ^{c}
Rice protein concentrate	0.346 ± 0.002 ^{c}	1.08 ± 0.02 ^{b}	1.29 ± 0.41 ^{b,c}

The table presents mean values along with standard deviations. The letters a–g in the columns indicate statistically significant differences between the obtained results ($p < 0.05$).

The tested preparations differed in water- and fat-absorption capacities (Table 4). It is worth noting that the water-binding values obtained for the preparations with the highest solubility (whey protein and collagen hydrolyzates, whey protein isolate) were close to zero. This result points to a methodological limitation of the experimental procedure used when applied to samples with high solubility and the presence of short-chain peptides; these components dissolve completely in the aqueous phase and do not form a sediment during centrifugation (even at a force of 9780× g). Consequently, the dissolved components are removed during the decantation of the supernatant. This phenomenon is well-documented in the scientific literature, which emphasizes that the centrifugation method effectively measures only the water retained by the insoluble fraction of a preparation [28,29]. This is reflected in the results, which show a decrease in measured bound water with increasing protein solubility (Table 4). Therefore, the low bound-water values obtained for the whey protein and collagen hydrolyzates, as well as the whey protein isolate, do not indicate a lack of affinity for water; rather, they are a consequence of the specific analytical technique employed. In this context, a more accurate description of

the phenomena detected in this study would be "water-retention capacity of the insoluble fraction" rather than "water-binding capacity." Among the other preparations tested, soy protein isolate exhibited the highest values for both the water-holding capacity of the insoluble fraction (6.02 ± 0.32 g/g) and fat absorption (4.04 ± 0.07 mL/g). All milk proteins showed relatively high fat-binding properties (2.89 ± 0.05 mL/g), whereas pea and rice protein concentrates displayed the lowest values for this parameter (0.90 ± 0.03 and 1.08 ± 0.02 mL/g, respectively). Differences in absorption capacity may reflect multiple properties of the commercial preparations, including composition, processing history, particle attributes, and protein structure.

3.2. Technological and Physicochemical Characteristics of Tested Protein Preparations in 2% Aqueous Solutions

This study examined the technological and physicochemical properties of protein preparations in 2% aqueous dispersions. The appearance of 2% aqueous dispersions was assessed at 22 ± 2 °C (Table 5 and Figure 2a). Collagen hydrolyzate formed a clear solution, whereas whey protein isolate and whey protein hydrolyzate showed good visual dispersion characteristics. Pea and rice protein concentrates exhibited visible undissolved material. Table 5 also presents the pH values of the prepared solutions, which ranged from 5.87 to 7.23. Among all the solutions analyzed, the lowest pH value (5.87) was recorded for collagen hydrolyzates, while the highest (7.23) was observed for soy protein isolate. These differences may result from the production methods used for each preparation. In the case of hydrolyzates, the production process most often includes stages such as protein precipitation, control of molecular aggregation, and enzymatic hydrolysis. Although the final product is neutralized, these processes can affect its final pH. Whey protein concentrates, by contrast, are produced using milder methods (primarily, mechanical ultrafiltration), which contribute to obtaining a pH close to that of the raw material. Proteins exhibit their lowest solubility at their isoelectric point (the pH value at which the net charge of the protein is zero), which leads to their aggregation [30]. This relationship was confirmed in a study by Boukid et al. (2021) [31], which showed that pea protein has poor solubility at its isoelectric point of approximately 4.6. Protein manufacturers intentionally modify the pH of the final product (very often using buffers) to shift it away from the isoelectric point. As a result, protein molecules repel each other more strongly in an aqueous environment, which prevents them from clumping together and facilitates their dissolution.

Table 5. pH and solubility of the tested 2% protein preparation solutions.

Type of Protein Preparation	Solubility	pH
All milk proteins	Moderate; small amount of sediment	7.09 ± 0.00 ^{e,f}
Micellar casein	Moderate; small amount of sediment	7.03 ± 0.01 ^{e}
Calcium caseinate	Moderate; sediment	7.08 ± 0.03 ^{e,f}
Whey protein isolate	Good	6.45 ± 0.03 ^{b,c}
Hydrolyzed whey proteins	Very good	6.56 ± 0.02 ^{c,d}
Hydrolyzed collagen proteins	Total	5.87 ± 0.05 ^{a}
Soy protein isolate	Moderate; small amount of sediment	7.23 ± 0.02 ^{f}
Pea protein concentrate	Low	6.23 ± 0.05 ^{b}
Rice protein concentrate	Low	6.64 ± 0.03 ^{d}

The table presents mean values along with standard deviations. The letters a–f in the columns indicate statistically significant differences between the obtained results ($p < 0.05$).

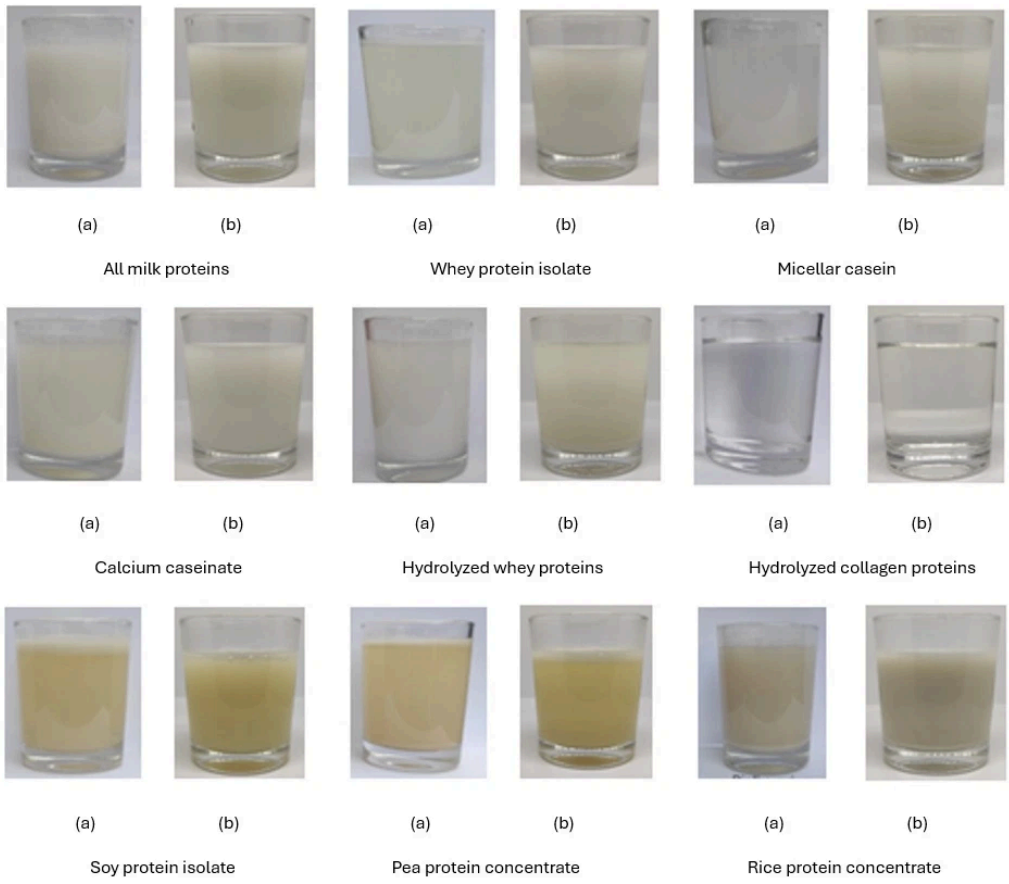


Figure 2. Appearance of 2% aqueous solutions of the protein preparations tested, prepared at about 20 °C (**a**) and heated to about 80 °C (**b**).

Upon heating to 80 ± 2 °C, visible changes were observed in some preparations, including increased turbidity and the formation of a precipitate (Figure 2b). These observations make it possible to determine how the preparations behave under elevated temperature conditions, such as those encountered during the pasteurization process. For dairy-based preparations (whey protein hydrolyzate, whey protein isolate, and all milk proteins) heated to approximately 80 °C, the appearance of a precipitate or a reduction in solution clarity was noted. In the case of plant-based proteins, it was more difficult to observe changes related to protein precipitation, as the solutions became turbid and a substantial precipitate formed as soon as they were mixed with water at a temperature of approximately 20 °C. The only preparation whose appearance remained unchanged upon heating was collagen protein hydrolyzate.

The osmolality of aqueous solutions of the investigated protein preparations varied considerably depending on both the type of preparation and its concentration (Figures 3 and 4 and Table 6). The osmolality of the tested protein preparations was evaluated in aqueous solutions with concentrations ranging from 0.5% to 14% for milk proteins and collagen; for plant proteins, the concentrations ranged from 1% to 10% for pea and rice protein concentrates and from 0.5% to 5% for soy protein. The differences in concentration ranges stemmed from the technological characteristics of the individual preparations, as well as limitations related to the preparation of homogeneous samples with appropriate fluidity. The results revealed a clear variation in the osmolality values of the tested preparations. Among the animal-based proteins, the lowest values were recorded for calcium caseinate and whey protein isolate. Higher values were observed for micellar

casein and all milk proteins, while the highest values were found for whey protein hydrolyzates and collagen. Among plant-based proteins, rice and pea proteins exhibited low osmolality, comparable to the values obtained for calcium caseinate and whey protein isolate. Soy protein showed significantly higher osmolality than the other plant-based preparations tested; these values were similar to those obtained for micellar casein and all milk proteins. The osmolality of these solutions was caused by the presence of low-molecular-weight components, such as mineral salts, lactose, and other substances, rather than the main ingredients alone. The osmolality of an aqueous system is governed by the number of particles (ions or molecules) present rather than their molecular mass [32]. The most important osmotic effect is caused by dissociated mineral salts, whether they occur naturally in the raw material or are introduced during processing (e.g., through alkaline extraction, isoelectric precipitation, or neutralization) [33,34]. In dairy-based preparations, soluble lactose also contributes to osmolality, whereas its impact is far less pronounced in highly purified isolates and caseinates [32,35]. In plant-based preparations, especially in less-purified concentrates such as pea or rice concentrates, osmolality is influenced by residual salts, soluble sugars, and oligosaccharides, whereas starch fractions and insoluble fiber do not affect osmolality [34].

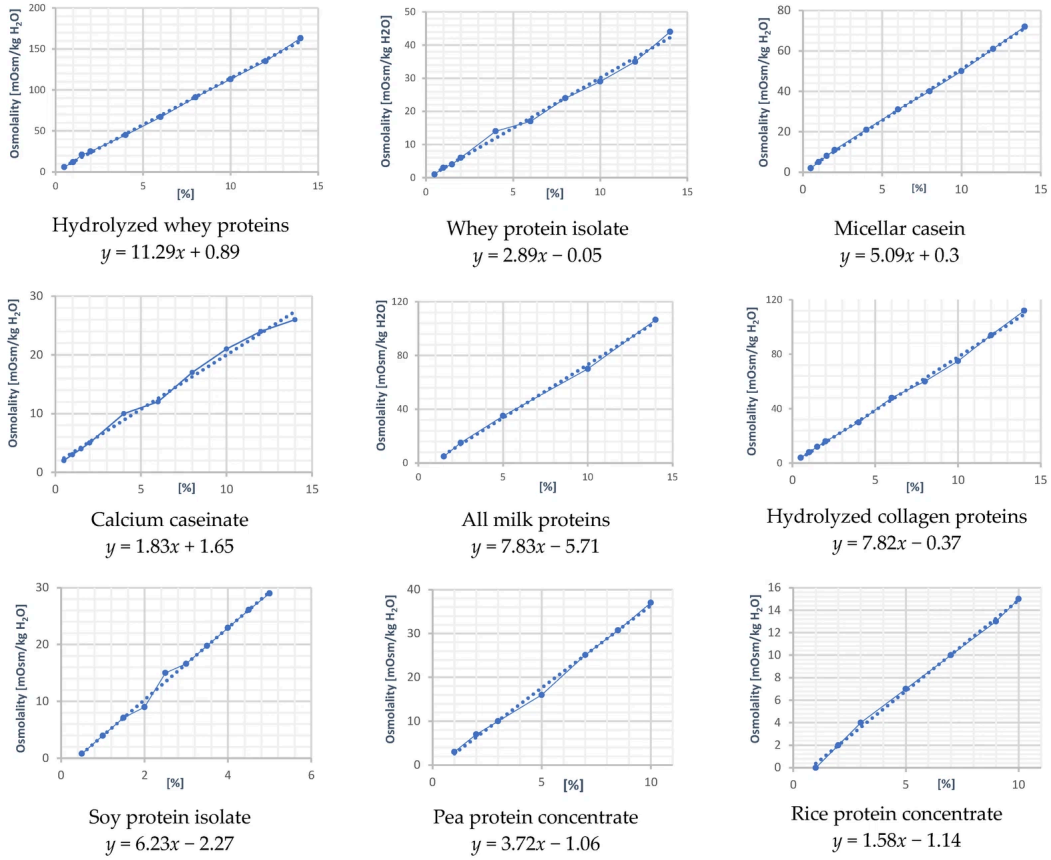


Figure 3. The relationship between osmolality and the percentage concentration of various protein preparations in aqueous solutions.

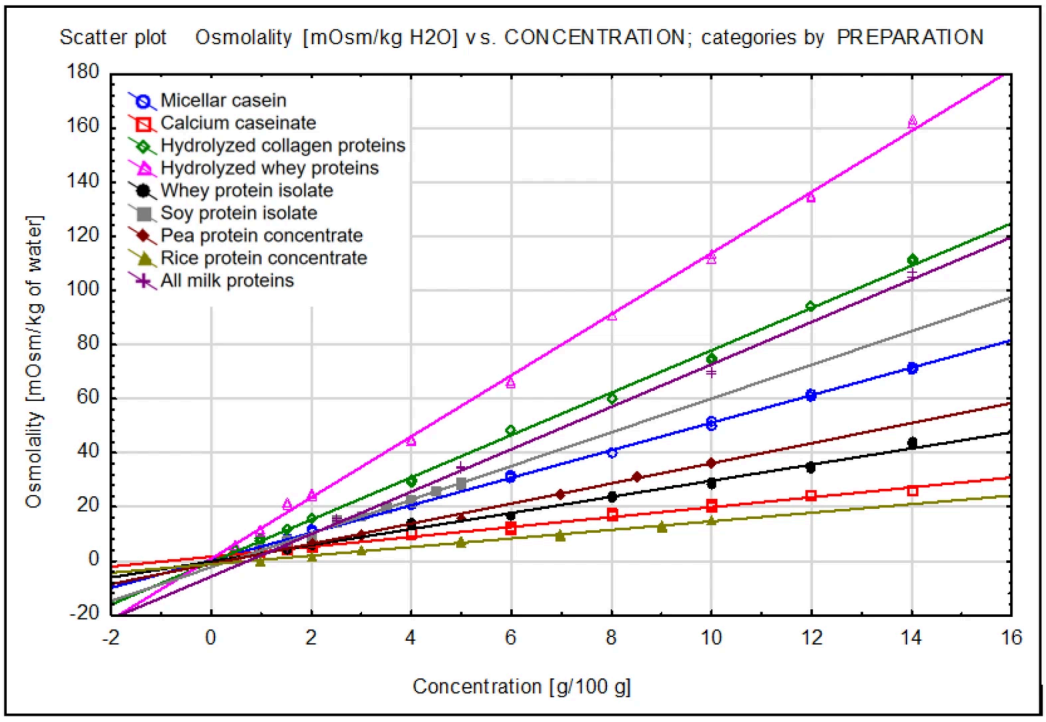


Figure 4. Relationship between the osmolality [mOsm/kg H_2O] of aqueous solutions and the mass concentration [%] of the nine analyzed protein preparations, along with fitted linear regression lines ($p < 0.001$; $R^2 \geq 0.99$).

Table 6. Parameters of linear regression equations y = ax + b, describing the relationship between osmolality [mOsm/kg H_2O] and mass concentration [%] for the studied protein preparations.

Protein Preparation	Slope (a ± SE)	95% CI * for a	Intercept (b ± SE)	95% CI * for b	Significance (p)	Coefficient of Determination (R^2)
All milk proteins	7.83 ± 0.15	[7.52; 8.23]	−5.71 ± 0.14	[−9.23; −3,12]	0.000000	0.997
Micellar casein	5.09 ± 0.03	[5.01; 5.15]	0.30 ± 0.25	[−0.24; 0.83]	0.000000	0.998
Calcium caseinate	1.83 ± 0.04	[1.78; 1.94]	1.65 ± 0.28	[1.01; 2.13]	0.000000	0.992
Whey protein isolate	2.98 ± 0.06	[2.86; 3.10]	−0.05 ± 0.41	[−1.04; 0.82]	0.000000	0.993
Hydrolyzed whey proteins	11.29 ± 0.10	[11.07; 11.51]	0.89 ± 0.78	[−0.76; 2.52]	0.000000	0.998
Hydrolyzed collagen proteins	7.82 ± 0.09	[7.64; 8.00]	−0.37 ± 0.64	[−1.72; 0.98]	0.000000	0.997
Soy protein isolate	6.23 ± 0.10	[6.02; 6.44]	−2.27 ± 0.32	[−2.94; −1.61]	0.000000	0.995
Pea protein concentrate	3.72 ± 0.09	[3.52;3.91]	−1.06 ± 0.56	[−2.22; 0.09]	0.000000	0.993
Rice protein concentrate	1.58 ± 0.03	[1.52; 1.65]	−1.14 ± 0.02	[−1.55; −0.73]	0.000000	0.995

* 95% CI —95% confidence interval.

An analysis of the linear regression models (Table 6) revealed a statistically significant ($p < 0.001$) nearly linear relationship between the concentration of a protein preparation and its osmolality within the ranges analyzed. This is confirmed by high coefficients of determination ($R^2 > 0.99$), indicating that the variation in mass concentration accounts for more than 99% of the variance in solution osmolality.

In most of the analyzed preparations, the calculated values of the free term (b) did not differ significantly from zero (the 95% confidence intervals (CI) included the value 0), which, in physicochemical terms, corresponds to the osmolality of the pure solvent (water). The exception was four preparations for which a statistically significant shift in the y-intercept relative to zero was observed. A shift toward negative values was observed for the preparation containing the full set of milk proteins (b = −5.71 ± 0.14), soy isolate (b = −2.27 ± 0.32), and rice concentrate (b = −1.14 ± 0.03). However, calcium caseinate had a significant shift in the free-form toward positive values (b = 1.65 ± 0.28), indicating a higher baseline level of the tested parameter than in the pure solvent.

The high reproducibility of the regression models suggests that these concentration-dependent equations may provide a useful tool for theoretically predicting the osmolality of aqueous solutions prepared using various commercially available protein preparations. From a technological point of view, the slope of these relationships is an important parameter, represented by the coefficient (a), as it defines the dynamics of osmolality changes that occur as the concentration of the preparation increases. This phenomenon is visually illustrated by the distinct, fan-like divergence of the regression lines on the combined plot as the concentration rises (Figure 4). For preparations characterized by a steeper regression line, with the most pronounced increase observed for whey protein hydrolyzate (a = 11.29 ± 0.10), the maximum concentration used (e.g., to maintain an isoosmotic solution) must be lower than for preparations with a flatter regression line, as is the case for rice protein concentrate (a = 1.58 ± 0.03).

A formal comparison of the slopes of the linear regression lines, performed using analysis of covariance (ANCOVA), revealed a highly statistically significant interaction between the type of protein preparation and its concentration ($F(8, 137) = 2019.61$; $p < 0.001$). This confirms that the dynamics of osmolality increase (the values of the slopes a) differ significantly among the individual studied protein sources.

Based on the analysis of the residuals, it can be concluded that the estimated linear regression models exhibit statistical validity and a good fit for all analyzed preparations. An assessment of the normality plots of the residuals showed that the data points lie close to a straight line, confirming that the error distribution is normal. Furthermore, plots of residuals versus predicted values revealed a random, uniform dispersion of data points around zero across the entire plot, without any systematic curvature or funnel effect, confirming that the assumptions of linearity and constant variance had been met within the concentration ranges studied (plots not included).

The observed differences in osmolality may stem from the number of dissolved, osmotically active molecules present in the various preparations studied. The process of hydrolysis can increase the number of lower-molecular-weight molecules at the expense of intact (non-degraded) proteins; however, the present study did not determine the degree of hydrolysis, the molecular weight distribution, or the proportion of low-molecular-weight non-protein components. Consequently, these factors should be viewed as plausible explanations rather than experimentally verified mechanisms.

Figure 5 presents the results concerning the antioxidant properties of the studied preparations. It should be noted that the aqueous extraction procedure employed in this study allowed for the assessment of antioxidant potential of only the hydrophilic fraction—that is, water-soluble proteins and peptides. Nevertheless, the results provide

valuable information on the readily available soluble fraction of the preparations, which directly scavenges ABTS+• radical cations. This analysis shows the capability of the components present in the sample to react with ABTS radical cations under specific experimental conditions; it does not, however, allow for a determination of antioxidant effects "in vivo". Since the amino acid composition, peptide profiles, and concentrations of individual compounds with antioxidant properties were not determined, the observed activity cannot be attributed to specific components of the samples. Further compositional analyses would be necessary to find the mechanisms underlying the differences between commercially available preparations. This paper discusses the differences among the preparations studied and—drawing on the literature—attempts to explain the most likely reasons for the significant discrepancies in the values obtained. The protein preparations varied in terms of their antioxidant activity. Milk-based protein preparations had higher antioxidant capacity than plant preparations. Calcium caseinate had the highest capacity for deactivating synthetic ABTS radical cations (40.533 ± 0.147 µmol TE/1 g), followed by hydrolyzed whey proteins (37.478 ± 0.154 µmol TE/1 g) and all milk proteins (33.507 ± 0.148 µmol TE/1 g). Plant-based protein preparations showed significantly weaker antioxidant properties with the lowest values recorded for the rice protein concentrate (15.524 ± 0.073 µmol TE/1 g).

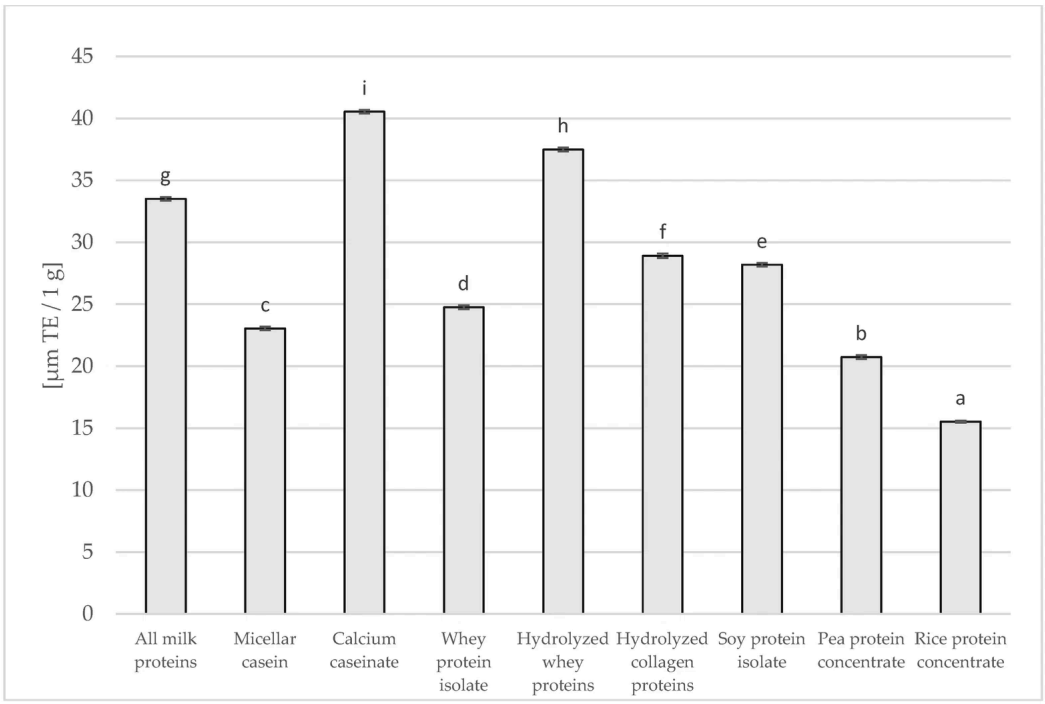


Figure 5. Antioxidant activity in the tested protein preparations. The letters a–i in the bars indicate statistically significant differences between the results obtained ($p < 0.05$).

The ability of the studied compounds to neutralize the ABTS+• radical cation depended primarily on the spatial structure of the proteins, the steric accessibility of the functional groups, and their solubility in the aqueous phase. The higher activity of calcium caseinate—contrasted with the low result obtained for micellar casein (despite their similar amino acid profiles)—may have stemmed from the disruption of the compact micellar structure in the caseinate. The stretching of casein polypeptide chains allows the ABTS+ radical cation free access to numerous tyrosine and tryptophan residues, which are trapped within colloidal aggregates in micellar casein [36]. A similar conformational effect has been observed in whey proteins, where a hydrolyzate exhibited sig-

nificantly stronger antioxidant properties than a native whey isolate; in the latter, aromatic residues and cysteine thiol (-SH) groups remain hidden within the core of the globular proteins [37,38]. Meanwhile, the high antioxidant capacity of collagen hydrolyzate may be attributed to its low molecular weight and complete solubility within the reaction medium. Lower activity was observed in pea and rice protein concentrates —a finding confirmed by comparative studies of plant-based preparations in the ABTS assay [39]. This may result from their limited solubility in aqueous buffers and the presence of insoluble storage protein fractions [40]. This reduces the effective surface area available for interaction with the radical compared to more soluble soy isolates or milk-based preparations.

3.3. Model Beverage Formulations and Their Osmolality

This study focused on developing base formulations for beverages with the addition of protein preparations that had been examined in the first part of the research. It was assumed that the beverages would differ in terms of osmolality. The following ingredients were used to develop the formulations: freeze-dried raspberry powder, cocoa, sugar, salt, steviol glycosides, hydrolyzed collagen protein, and whey protein isolate. The quantities of each ingredient required for the formulations were determined based on values calculated from regression equations, which are presented in Table 7. Some of these regression equations, those for sugar and salt, were derived from previous work published by our research team [12,13]. The regression equations for freeze-dried raspberry powder and cocoa were developed based on osmolality measurements taken at concentrations ranging from 2.5 to 10% and 0.5 to 14% for freeze-dried raspberry powder and cocoa, respectively. Steviol glycosides were excluded from the linear regression model because this sweetener does not significantly affect the osmolality of the final beverage, which was confirmed by Mroczek et al. (2023) [41]. Establishing these regression equations for the beverage components allowed for the calculation of their expected osmolality, which was subsequently compared with the values determined through analytical measurement.

Table 7. Parameters of the linear regression equations y = ax + b, describing the relationship between osmolality [mOsm/kg H_2O] and mass concentration [%] for the components of the developed beverages with varying osmolality.

Protein Preparation	Slope (a ± SE)	Intercept (b ± SE)	Significance (p)	Coefficient of Determination (R^2)
Cocoa	12.33 ± 0.27	3.39 ± 1.42	0.000000	0.997
Freeze-dried raspberry powder	24.00 ± 4.64	−3.33 ± 31.74	0.029496	0.942
Salt	330.75 ± 5.23	7.87 ± 4.99	0.000000	0.997
Sucrose	31.72 ± 0.46	4.89 ± 4.20	0.000000	0.997

Analysis of the linear regression models (Table 7) revealed a statistically significant (in most cases $p < 0.001$), nearly perfectly linear relationship between the concentration of beverage components, namely cocoa, freeze-dried raspberry powder, sugar, and salt, and their osmolality, within a concentration range of 0.5% to 10%. This linear relationship is confirmed by high coefficients of determination ($R^2 > 0.94$), indicating that variations in mass concentration account for over 94% of the variance in the osmolality values of the solutions. Notably, the trend lines for all systems studied originate from nearly the same point on the Y-axis (the ordinate) at a concentration of 0% (Figure 6). These equations, which take into account the concentrations of individual basic beverage ingredi-

ents, can be a useful tool for theoretically predicting the osmolality of aqueous solutions containing these ingredients. Also noteworthy is the slope of these relationships (the "a" coefficients); for the components in question, it is steeper than in the case of the protein solutions studied. This indicates that the protein preparations had the least impact on the osmolality of the developed beverages. The greatest influence was exerted by the salt solution, followed by sugar (sucrose) and raspberry powder, with cocoa having the smallest effect.

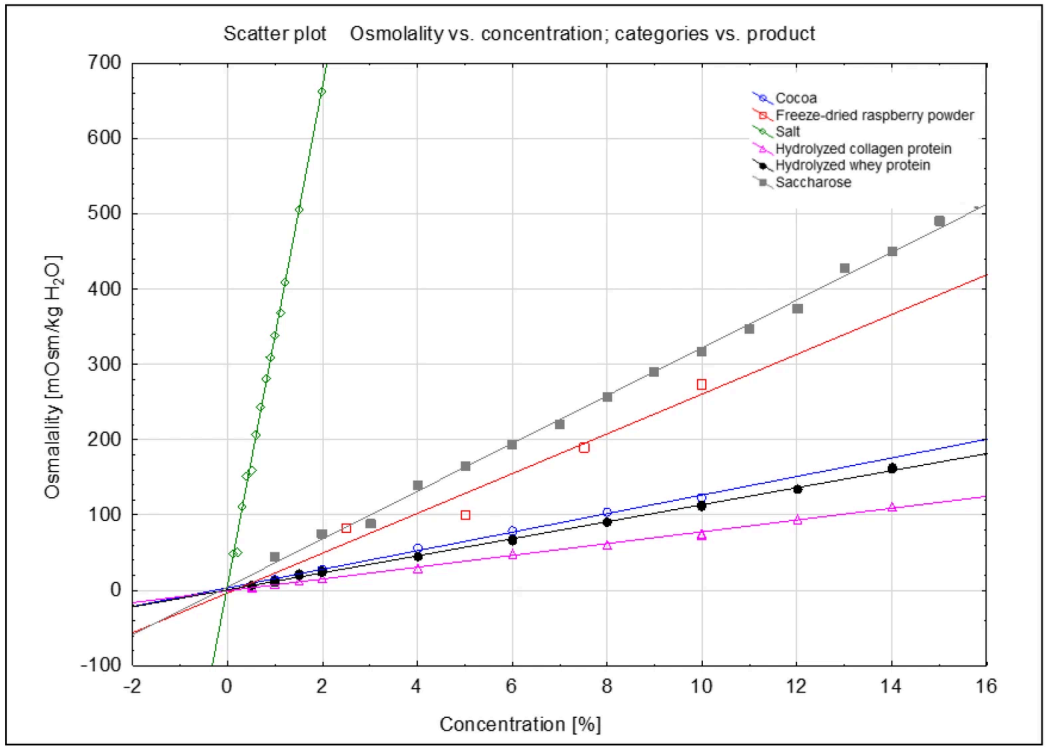


Figure 6. Relationship between the osmolality [mOsm/kg H_2O] of aqueous solutions of the formulation components used in the developed beverages and their mass concentration [%], with fitted linear regression lines ($p < 0.03$; $R^2 \geq 0.94$).

Tables 8–10 present the formulations of the developed beverages. These feature varying osmolarities characteristic of hypertonic, isotonic, and hypotonic drinks. The osmolality values for these beverages were determined both by calculation—using regression equations for all components included in a given formulation—and by direct measurement. The discrepancies between the calculated values and the measured results arise from interactions between the individual components present in the beverages—interactions that are not accounted for by the regression equations. These differences are in the range of approximately 11–15%. Similar patterns were observed in previous studies conducted by our research team [12.13]. Three formulations of powdered beverages were prepared and evaluated after being dissolved in water. The measured osmolality of these beverages was approximately 245, 302, and 554 mOsm/kg H_2O, respectively. Based on the osmolality values of the developed beverages, they can be classified as hypo-, iso-, or hypertonic drinks, respectively [42].

Table 8. Composition of the model beverage with the highest measured osmolality.

Ingredient	g/100 g of the Beverage
Water	79.34
Freeze-dried raspberry powder	6.50

Ingredient	g/100 g of the Beverage
Collagen hydrolysate	5.00
Sugar (Sucrose)	9.16
Calculated osmolality	493.35 mOsm/kg H_2O
Measured osmolality	554.68 ± 9.87 mOsm/kg H_2O

Table 9. Composition of the model beverage with osmolality in the isotonic range.

Ingredient	g/100 g of the Beverage
Water	88.39
Collagen hydrolysate	5.00
Freeze-dried raspberry powder	4.00
Salt	0.10
Sugar (Sucrose)	2.50
Stevia (Steviol glycosides)	0.01
Calculated osmolality	256.55 mOsm/kg H_2O
Measured osmolality	301.67 ± 8.96 mOsm/kg H_2O

Table 10. Composition of the model beverage with the lowest measured osmolality.

Ingredient	g/100 g of the Beverage
Water	85.98
Whey protein isolate	5.00
Cocoa powder	5.00
Sugar (Sucrose)	4.00
Stevia (Steviol Glycosides)	0.02
Calculated osmolality	211.77 mOsm/kg H_2O
Measured osmolality	245.43 ± 3.35 mOsm/kg H_2O

The formulations differed in their protein source and other ingredients, which included, among others, powdered raspberries, cocoa, sucrose, sodium chloride, and steviol glycosides. According to the literature, combining protein supplements with carbohydrates can improve protein balance, both at rest and during physical exertion, as well as during the post-workout recovery period [43]. This justifies the deliberate inclusion of a protein source alongside carbohydrate components (and in this study, powdered raspberries and sucrose) in the first and second beverage formulations developed. Furthermore, the use of collagen protein hydrolyzate in the developed formulations deserves special emphasis. From a technological perspective, collagen protein hydrolyzate is completely soluble and virtually tasteless; it does not precipitate from solution at pasteurization temperatures or at low pH. Two raspberry-flavored variants contained collagen hydrolyzate. The cocoa-flavored variant contained whey protein isolate. The presented recipes are only examples of possible ingredient combinations and are not products whose effectiveness in hydration or regeneration has been verified in experimental studies.

The raspberry-flavored variant containing collagen hydrolyzate and sucrose (Table 8) exhibited the highest measured osmolality (554.67 ± 9.87 mOsm/kg H_2O). This high value may be due to the combined contribution of sucrose, the collagen preparation, and the soluble components of the raspberry powder. Compared to the second raspberry-flavored variant, this formulation contained larger amounts of sucrose and

raspberry powder; the influence of individual ingredients was not investigated in isolation. The drink had a deep red color, a sweet-tart taste, distinct notes of raspberry, and visible pieces of fruit.

The second beverage contained collagen hydrolyzate, powdered raspberries, sucrose, sodium chloride, and steviol glycosides (Table 9). Its measured osmolality was 301.67 ± 8.96 mOsm/kg H_2O, which falls within the range for beverages with isotonic properties. Compared to the variant with a higher osmolality, this formulation contained a smaller amount of sucrose and powdered raspberries. Sodium chloride was included as a source of electrolytes, while steviol glycosides provided a sweet taste despite the limited amount of added sucrose. Osmolality alone is not sufficient to determine whether a given product is suitable for rehydration during physical exertion. The prepared beverage had a pinkish-red color and a sweet, fruity taste; it also contained sediment derived from raspberry particles.

The third formulation contained whey protein isolate, nonfat cocoa powder, sucrose, and steviol glycosides (Table 10). Its measured osmolality was 245.43 ± 3.35 mOsm/kg H_2O, the lowest among the three formulations and within a hypotonic range relative to the other samples. This formulation did not contain collagen hydrolyzate or raspberry powder. The measured osmolality reflects the combined contribution of the whey protein preparation, sucrose, cocoa constituents, and other soluble components. The reconstituted beverage was brown and opaque, with a cocoa flavor, a mild dairy note, and some sediment.

Previous research conducted by our team focused on the osmolality of individual ingredients and their application in the development of recovery beverages [12,13]. The present comparison extends this work to a specific set of commercially available protein preparations. The relationships between concentration and osmolality were determined for each preparation in aqueous systems. The osmolality of multi-ingredient beverages also depends on the content of sugars, salts, and fruit-based components, as well as the interactions between them. Since the beverage variants developed differed in more than just their protein source, the measured osmolality values do not allow for the isolation of the independent effect of the protein type—an objective that was not within the scope of this chapter. In this chapter, an attempt was made to develop a base composition for beverages with varying osmolality, incorporating the previously studied protein preparations. Although osmolality is a useful parameter for formulation, it is not sufficient on its own to determine hydration efficacy, gastrointestinal tolerance, or recovery benefits. Further research in these areas is required.

4. Conclusions

The results of this study revealed significant differences in the physicochemical, technological, sensory, and antioxidant properties (determined via “in vitro” methods) of nine protein preparations of animal and plant origin. These differences are relevant when selecting protein ingredients for the development of functional beverages with specific physicochemical parameters. In summary, this study indicates that the selection of protein preparations for functional beverages should be based not only on protein content and nutritional value but also on their impact on osmolality (which is concentration-dependent) and their technological and sensory properties. The findings provide a practical basis for the preliminary formulation of protein-enriched beverages with a specific, predetermined osmolality. Before broader technological or physiological conclusions can be drawn, further controlled studies on such formulations are required, including, among other things, the verification of predicted osmolality values based on data obtained under experimental conditions.

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

Funding: This research was prepared as part of the statutory activity of the Department of Functional Foods, Organic Foods, and Nutritional Sensory Science, Faculty of Health Sciences, Warsaw University of Life Sciences.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

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

References

1. Gupta, A.; Sanwal, N.; Bareen, M.A.; Barua, S.; Sharma, N.; Olatunji, O.J.; Nirmal, N.P.; Sahu, J.K. Trends in functional beverages: Functional ingredients, processing technologies, stability, health benefits, and consumer perspective. *Food Res. Int.* **2023**, *170*, 113046. [Google Scholar] [CrossRef] [PubMed]
2. Sugajski, M.; Buszewska-Forajta, M.; Buszewski, B. Functional Beverages in the 21st Century. *Beverages* **2023**, *9*, 27. [Google Scholar] [CrossRef]
3. Panou, A.; Karabagias, I.K. Composition, properties, and beneficial effects of functional beverages on human health. *Beverages* **2025**, *11*, 40. [Google Scholar] [CrossRef]
4. Nasrabadi, M.N.; Doost, A.S.; Mezzenga, R. Modification approaches of plant-based proteins to improve their techno-functionality and use in food products. *Food Hydrocoll.* **2021**, *118*, 106789. [Google Scholar] [CrossRef]
5. Anema, S.G. On heating milk, the dissociation of κ-casein from the casein micelles can precede interactions with the denatured whey proteins. *J. Dairy Res.* **2008**, *75*, 415–421. [Google Scholar] [CrossRef] [PubMed]
6. Zhu, D.; Damodaran, S.; Lucey, J.A. Physicochemical and Emulsifying Properties of Whey Protein Isolate (WPI)−Dextran Conjugates Produced in Aqueous Solution. *J. Agric. Food Chem.* **2010**, *58*, 2988–2994. [Google Scholar] [CrossRef] [PubMed]
7. Truong, V.-D.; Clare, D.A.; Catignani, G.L.; Swaisgood, H.E. Cross-Linking and Rheological Changes of Whey Proteins Treated with Microbial Transglutaminase. *J. Agric. Food Chem.* **2004**, *52*, 1170–1176. [Google Scholar] [CrossRef] [PubMed]
8. Du, Q.; Ji, X.; Lyu, F.; Liu, J.; Ding, Y. Heat Stability and Rheology of High-Calorie Whey Protein Emulsion: Effects of Calcium Ions. *Food Hydrocoll.* **2021**, *114*, 106583. [Google Scholar] [CrossRef]
9. McKenna, A.B.; Singh, H. Age Gelation in UHT-Processed Reconstituted Concentrated Skim Milk. *Int. J. Food Sci. Technol.* **1991**, *26*, 27–38. [Google Scholar] [CrossRef]
10. Datta, N.; Deeth, H.C. Age Gelation of UHT Milk—A Review. *Food Bioprod. Process.* **2001**, *79*, 197–210. [Google Scholar] [CrossRef]
11. Xie, A.; Dong, Y.; Liu, Z.; Li, Z.; Shao, J.; Li, M.; Yue, X. A Review of Plant-Based Drinks Addressing Nutrients, Flavor, and Processing Technologies. *Foods* **2023**, *12*, 3952. [Google Scholar] [CrossRef] [PubMed]
12. Sadowska, A.; Świderski, F.; Laskowski, W. Osmolality of Components and Their Application in the Design of Functional Recovery Drinks. *Appl. Sci.* **2020**, *10*, 7663. [Google Scholar] [CrossRef]
13. Sadowska, A.; Świderski, F.; Kulik, K.; Waszkiewicz-Robak, B. Designing Functional Fruit-Based Recovery Drinks in Powder Form That Contain Electrolytes, Peptides, Carbohydrates and Prebiotic Fiber Taking into Account Each Component's Osmolality. *Molecules* **2021**, *26*, 5607. [Google Scholar] [CrossRef] [PubMed]
14. *EN ISO 8586:2012*; Sensory Analysis—General Guidelines for the Selection, Training and Monitoring of Selected Assessors and Expert Sensory Assessors. European Committee for Standardization (CEN): Brussels, Belgium, 2012.
15. *PN-ISO 6658:2017*; Sensory Analysis—Methodology—General Guidance. Polish Committee for Standardization (PKN): Warsaw, Poland, 2017.
16. *EN ISO 5492:2009*; Sensory Analysis—Vocabulary. European Committee for Standardization (CEN): Brussels, Belgium, 2009.

17. *EN ISO 8589:2010/A1:2014*; Sensory Analysis—General Guidance for the Design of Test Rooms. European Committee for Standardization (CEN): Brussels, Belgium, 2014.
18. *ISO/CIE 11664-4:2019*; Colorimetry—Part 4: CIE 1976 L*a*b* Colour Space. International Organization for Standardization (ISO): Geneva, Switzerland, 2019.
19. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [Google Scholar] [CrossRef] [PubMed]
20. *PN-ISO 1842:1999*; Fruit and Vegetable Products—Determination of pH. Polish Committee for Standardization (PKN): Warsaw, Poland, 1999.
21. *ISO 18787:2017*; Foodstuffs—Determination of Water Activity. International Organization for Standardization (ISO): Geneva, Switzerland, 2017.
22. Sosulski, F.W.; Humbert, E.S.; Bui, K.; Jones, J.I. Functional Properties of Rapeseed Flours, Concentrates and Isolate. *J. Food Sci.* **1976**, *41*, 1349–1352. [Google Scholar] [CrossRef]
23. Phosanam, A.; Chandrapala, J.; Huppertz, T.; Adhikari, B.; Zisu, B. Changes in physicochemical and surface characteristics in milk protein powders during storage. *Dry. Technol.* **2022**, *40*, 638–652. [Google Scholar] [CrossRef]
24. Kneifel, W.; Paquin, P.; Abert, T.; Richard, J.P. Water-holding capacity of proteins with special regard to milk proteins and methodological aspects—A review. *J. Dairy Sci.* **1991**, *74*, 2027–2041. [Google Scholar] [CrossRef]
25. Loveday, S.M.; Hindmarsh, J.P.; Creamer, L.K.; Singh, H. Physicochemical changes in intermediate-moisture protein bars made with whey protein or calcium caseinate. *Food Res. Int.* **2010**, *43*, 1321–1328. [Google Scholar] [CrossRef]
26. Blais, H.N.; Schroën, K.; Tobin, J.T. A review of multistage membrane filtration approaches for enhanced efficiency during concentration and fractionation of milk and whey. *Int. J. Dairy Technol.* **2022**, *75*, 749–760. [Google Scholar] [CrossRef]
27. Karuppuchamy, V.; Heldman, D.R.; Snyder, A.B. A review of food safety in low-moisture foods with current and potential dry-cleaning methods. *J. Food Sci.* **2024**, *89*, 793–810. [Google Scholar] [CrossRef] [PubMed]
28. Quinn, J.R.; Paton, D. A practical measurement of water hydration capacity of protein materials. *Cereal Chem.* **1979**, *56*, 38–40. [Google Scholar]
29. Möller, A.C.; van der Padt, A.; van der Goot, A.J. Influence of the fractionation method on the protein composition and functional properties. *Innov. Food Sci. Emerg. Technol.* **2022**, *81*, 103144. [Google Scholar] [CrossRef]
30. Malik, M.A.; Saini, C.S. Improvement of functional properties of sunflower protein isolates near isoelectric point: Application of heat treatment. *LWT* **2018**, *98*, 411–417. [Google Scholar] [CrossRef]
31. Boukid, 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. [Google Scholar] [CrossRef]
32. Ellis, Z.M.; Tan, H.S.G.; Embleton, N.D.; Sangild, P.T.; van Elburg, R.M. Milk feed osmolality and adverse events in newborn infants and animals: A systematic review. *Arch. Dis. Child. Fetal Neonatal Ed.* **2019**, *104*, F333–F340. [Google Scholar] [CrossRef] [PubMed]
33. Bijl, E.; van Valenberg, H.J.F.; Huppertz, T.; van Hooijdonk, A.C.M. Protein, casein, and micellar salts in milk: Current content and historical perspectives. *J. Dairy Sci.* **2013**, *96*, 5455–5464. [Google Scholar] [CrossRef] [PubMed]
34. Shanthakumar, P.; Klepacka, J.; Bains, A.; Chawla, P.; Dhull, S.B.; Najda, A. The Current Situation of Pea Protein and Its Application in the Food Industry. *Molecules* **2022**, *27*, 5354. [Google Scholar] [CrossRef] [PubMed]
35. Huppertz, T. Milk Protein Concentrate. In *Advanced Dairy Chemistry: Volume 1B: Proteins: Applied Aspects*, 4th ed.; McSweeney, P.L.H., O'Mahony, J.A., Eds.; Springer: New York, NY, USA, 2016; pp. 235–261. [Google Scholar]
36. Zhao, X.; Cui, Y.-J.; Bai, S.-S.; Yang, Z.-J.; Cai, M.; Megrous, S.; Aziz, T.; Sarwar, A.; Li, D.; Yang, Z.-N. Antioxidant Activity of Novel Casein-Derived Peptides with Microbial Proteases as Characterized via Keap1-Nrf2 Pathway in HepG2 Cells. *J. Microbiol. Biotechnol.* **2021**, *31*, 1163–1174. [Google Scholar] [CrossRef] [PubMed]
37. Mann, B.; Kumari, A.; Kumar, R.; Sharma, R.; Prajapati, K.; Mahboob, S.; Athira, S. Antioxidant activity of whey protein hydrolysates in milk beverage system. *J. Food Sci. Technol.* **2015**, *52*, 3235–3241. [Google Scholar] [CrossRef] [PubMed]
38. Mehdipour Biregani, Z.; Ahari, H. Effect of alcalase-mediated hydrolysis on the free radical scavenging activity and reducing power of whey protein isolate. *J. Food Bioprocess Eng.* **2021**, *4*, 58–62. [Google Scholar]
39. Sawicki, T.; Jabłońska, M.; Danielewicz, A.; Przybyłowicz, K.E. Phenolic Compounds Profile and Antioxidant Capacity of Plant-Based Protein Supplements. *Molecules* **2024**, *29*, 2101. [Google Scholar] [CrossRef] [PubMed]
40. Rashwan, A.K.; Osman, A.I.; Abdelshafy, A.M.; Mo, J.; Chen, W. Plant-based proteins: Advanced extraction technologies, interactions, physicochemical and functional properties, food and related applications, and health benefits. *Crit. Rev. Food Sci. Nutr.*

2025, *65*, 667–694. [Google Scholar] [CrossRef] [PubMed]

41. Mroczek, K.; Saletnik, B.; Bajcar, M.; Saletnik, A.; Puchalski, C.; Zaguła, G. Effect on Ionic Composition and Tonic Parameters of Sweeteners Used in the Production of Functional Beverages. *Beverages* **2023**, *9*, 98. [Google Scholar] [CrossRef]
42. Rowlands, D.S.; Kopetschny, B.H.; Badenhorst, C.E. The Hydrating Effects of Hypertonic, Isotonic and Hypotonic Sports Drinks and Waters on Central Hydration During Continuous Exercise: A Systematic Meta-Analysis and Perspective. *Sports Med.* **2022**, *52*, 349–375. [Google Scholar] [CrossRef] [PubMed]
43. Koopman, R.; Pannemans, D.L.E.; Jeukendrup, A.E.; Gijsen, A.P.; Senden, J.M.G.; Halliday, D.; Saris, W.H.M.; van Loon, L.J.C.; Wagenmakers, A.J.M. Combined ingestion of protein and carbohydrate improves protein balance during ultra-endurance exercise. *Am. J. Physiol. Endocrinol. Metab.* **2004**, *287*, E712–E720. [Google Scholar]

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.