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The study of relationship between taste and wheat bran protein isolate fortification during high-protein yogurt manufacturing

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ABSTRACT

High-protein (HP) food is becoming a popular and nutritionally valuable commodity. The most common protein fortifiers are milk whey or soy protein isolates. However, one of the potentially promising sources of quality protein isolate is wheat bran, the secondary product of wheat milling. Wheat bran protein isolate (WBPI) can be obtained using a sustainable and environmentally friendly pH shift method. Considering its nutritional properties, incorporating WBPI into people's diet is convenient. This work focuses on protein fortification of plain yogurt using WBPI while maintaining sensory acceptability. Direct fortification of yogurts with WBPI to achieve 5 % and 10 % protein content revealed two main sensory defects – bitterness and sandiness. Several approaches, such as grinding WBPI, protein blending, and WBPI encapsulation, were applied to improve the overall impression score of HP yogurts. It was found that eliminating the sandiness of WBPI-fortified yogurt is not possible by reducing WBPI particle size. Blending WBPI with dried milk decreased bitterness, but the products lacked amino acid diversity. The most effective procedure to mask the sensory defects was WBPI encapsulation using alginate, revealing an appropriate way to incorporate natural plant proteins into HP food.

1. Introduction

In recent years, the global food industry has witnessed a remarkable surge in demand for functional foods that offer both enhanced nutritional value and sensory satisfaction. A significant part of the functional foods field focuses on high protein products, aiming to enhance the protein content of standard foodstuffs. One such product at the forefront of this trend is high-protein yogurt, offering consumers a convenient source of essential nutrients. The strong position of high protein yogurts in the market is supported by various market research findings. The global high protein yogurt market valuation in 2022 was 35.19 billion US dollars, with a projected global market growth rate of 7 % from 2023 to 2033 [1].

The production requirements for high-protein yogurt are still not legislated in most of the countries. The closest benchmark for this type of product is concentrated fermented milk with a minimum of 5.6 % protein content [2]. The enhancement of yogurt with proteins can be achieved through different methods of milk base concentration (after and prior to fermentation) or by direct fortification of the milk base with protein isolates. Direct fortification includes the possibility of enriching the product with various types of protein isolates, both of animal and plant origin, or adding of protein blends. A common and almost exclusive representative from the group of animal proteins is whey protein isolate. However, in the field of plant protein isolates, the choices are significantly more varied. In addition to commonly used soy and wheat proteins, proteins from crops such as pea, fava bean, mung bean, rice, oat, chickpea, potato, and wheat bran can be utilized [3–5].

Another promising candidate with substantial potential for incorporation into dairy products is wheat bran protein isolate (WBPI). This isolate is derived from the external layer of the wheat grain, involving several procedural stages, including defatting, solubilization, and precipitation [6]. A key advantage of WBPI lies in its sourcing from readily available wheat bran, a cost-effective and environmentally sustainable material abundantly generated during the milling process. WBPI is characterized by a well-balanced amino acid composition similar to plant-derived proteins like those from soy and pea. Its Protein

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Digestibility Corrected Amino Acid Score (PDCAAS) closely resembles that of these plant proteins. The isolate is rich in asparagine, glutamic acid, leucine, arginine, and proline. Beyond its protein content, WBPI contains bioactive components such as polyphenols, phytosterols, and lignans. These components have been correlated with advantageous health effects, including the enhancement of cardiovascular well-being, antioxidative efficacy, and potential anticancer properties [7–10].

According to its chemical composition, WBPI has the potential to be integrated into high-protein yogurt formulations. An interesting amino acid profile and the potential physiological benefits make WBPI an attractive option for fortification. However, the integration of WBPI into high-protein yogurt formulations prompts an intriguing consideration: the influence of manipulating WBPI fortification levels on sensory attributes, especially taste and texture. It is common for plant protein isolates to have an astringent and bitter taste and can produce sandiness in specific types of products [11]. The aim of this article was to evaluate the impact of different fortification approaches on the sensory and functional attributes of high protein yogurt. The optimization of the production technology was focused on producing a high-protein product with properties similar to standard yogurt. There is currently no published scientific article on the application of WBPI in yogurts, and it is thus a new contribution to the field of research and development of functional dairy products.

2. Materials and methods

2.1. Isolation and characterization of WBPI

Wheat bran was obtained from flour manufacturer (Mlýny J. Voženílek, Czech Republic). The process of WBPI isolation from wheat bran was described in detail in the work published by Pořízka et al. [6]. The pH-shift method was used for isolation of WBPI. The extraction of proteins from wheat bran was carried out by 0.05 M NaOH (pH 11, 1 h, 20 °C). The de-proteined wheat bran was removed from the protein extract by centrifugation (8000 rcf, 15 min, 20 °C). The precipitation of extracted proteins was provided by pH adjustment to 4 ± 0.05 by H_2SO_4. The sedimented WBPI was collected after centrifugation and freeze-dried.

Protein content of WBPI and of its encapsulated forms was determined by determination of total nitrogen using elemental analyzer (Eurovector EA 3100, Pavia, Italy) in triplicate. For the conversion of total nitrogen amount to protein content, the factor 6.31 corresponding to wheat bran was used.

The amino acid composition was measured by automatic amino acid analyzer (AAA-400, Ingos, Prague, Czech Republic) with post-column derivatization by ninhydrin and photometric detection. The hydrolysis of peptide bonds was carried out by 6 M HCl for 23 h at temperature 110 °C. The concentration of cysteine and methionine was determined after their oxidation by solution of performic acid, phenol, and hydrogen peroxide. Tryptophane was determined after hydrolysis by saturated solution of barium hydroxide at 110 °C for 20 h.

The SDS-PAGE analysis of WBPI was performed according to Czubinski et al. [12]. The proteins from homogenized WBPI were extracted by shaking of 20 mg of sample with 0.15 mL of buffer consist of 8 M urea, 2 M thiourea, 50 mM trizma base, 0,1 M SDS and 75 mM DTT for 30 min. After centrifugation, the supernatant with extracted proteins was collected and directly analyzed.

The digestibility of the WBPI proteins was verified by simulating the conditions of the digestive tract. Determination of digestibility was always carried out in 2 repetitions. 1 g of WBPI was first mixed with 15 mL of 0.03 M HCl and shaken for 5 min at 37 °C. Then the pH of this suspension was adjusted to 1.9 ± 0.05 (1 M NaOH/1 M HCl) and 1 mL of pepsin solution (4 mg/mL; 2000 U/g) was added and the suspension was shaken and incubated for another 30 min in an IKA KS 3000 shaking incubator. Subsequently, the pH of the suspension was adjusted to 7.5 using 1 M NaOH, 4.5 mL of phosphate buffer and 1 mL of pancreatin solution (5 mg/mL, 4 USP) were added. The suspension was further incubated and shaken at 37 °C for 24 h. After the given time, the suspension was centrifuged (8000 rcf, 10 min, laboratory temperature). Undigested dried residue was analyzed by elemental analyzer (Eurovector EA 3100, Pavia, Italy). For the conversion of total nitrogen amount to protein content, the factor 6.31.

2.2. Manufacturing and fortification of yogurt

Whole fat, standardized, UHT milk purchased from a retail was used for manufacturing the yogurt samples. The dry matter of the milk was determined by drying a 3 mL milk sample to a constant weight in a laboratory oven (Memmert UFE550, Memmert, Büchenbach, Germany) maintained at 105 °C. The milk characteristics are presented in Table 1.

As a starter culture, freeze-dried YF-L812 (Chr. Hansen, Denmark) containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus* was used to create a 1 % w/w inoculum. The milk was initially heated to 70 °C for 10 min, and after cooling to laboratory temperature, the inoculum was added to the milk in a 1:100 ratio. The samples were then homogenized and placed in the yogurt maker (43 °C) until the pH reached 5 (approximately 8 h). The samples were stored in a refrigerator at 4 °C until further analysis.

For the production of high-protein yogurts, WBPI, dried milk, or a combination of both were used to enhance the protein content. The dried milk (Bohemilk, Czech Republic) contained 31 % protein.

The appropriate amount of WBPI, dried milk, or encapsulated WBPI was added directly to the milk and homogenized before the fermentation process. Yogurt samples with a total protein content of 5 % and 10 % were prepared, in addition to a reference yogurt sample. The precise amounts of individual protein materials were calculated based on their protein content, and they are detailed in Table 2.

Table 1
Characterization of milk used for yogurt manufacturing.

Energy value	266 kJ
Components [g/100 mL]	
Fat	3.5
Carbohydrates	4.7
Proteins	3.3
Dry matter	12.5

Table 2
Composition of individual yogurt samples per 100 mL of milk.

Sample	WBPI [g]	Dried milk [g]	Encapsulated WBPI [g]	Total protein content [%]
Reference	–	–	–	3.30
5 % WBPI	2.01	–	–	5.00
10 % WBPI	7.91	–	–	10.0
5 % MIX1	1.00	2.74	–	5.00
5 % MIX2	1.33	1.84	–	5.00
10 % MIX1	3.96	10.8	–	10.0
10 % MIX2	5.27	7.19	–	10.0
5 % E-WBPI	–	–	3.15	5.00
10 % E-WBPI	–	–	12.4	10.0

2.3. Grinding and particle distribution of WBPI

The raw WBPI was ground using a ball mill (HK 40, HK Laboratory Equipment, Turnov, Czech Republic) to obtain smaller WBPI particles and prevent sensory defects such as sandiness. The size of the resulting WBPI particles was measured using a Dispersing unit (Rodos, Sympatec, Clausthal, Germany) and Laser diffraction (Helos, Sympatec, Clausthal, Germany).

2.4. Encapsulation of WBPI

The raw whey protein isolate (WBPI) was encapsulated using sodium alginate. A solution of alginate and WBPI in distilled water was prepared. Initially, alginate was dissolved in water at a temperature of 60 °C and mixed for 1 h. Subsequently, WBPI was added, mixed, and homogenized for an additional hour at a temperature of 35 °C. The ratio of alginate to WBPI in the resulting solution was 1:5.

The solution was then pumped through a tube with a diameter of 0.2 mm using a peristaltic pump and dropped into a 10 % solution of $CaCl_2$ to form encapsulated balls. These formed encapsulates were filtered, washed three times with distilled water, and dried at laboratory temperature for 12 h. They were subsequently stored in a dark, dry place until further use. The encapsulation efficiency was verified by determining the protein content in the dry WBPI encapsulates. The size of the WBPI encapsulates was determined using a vibratory sieve shaker and test sieves (Retsch, Haan, Germany).

2.5. Sensory analysis of yogurt samples

The sensory analysis of all prepared samples was conducted one day after storage at the sensory laboratory of the Faculty of Chemistry, Brno University of Technology, Czech Republic. This laboratory is equipped in accordance with the requirements of the ISO 8589 standard. The sensory panel consisted of 20 participants with relevant experience and knowledge of dairy products, trained in general sensory analysis of various food products. Before the analysis, the problematics, and conditions of sensory analysis of dairy products were explained to all participants. Sensory descriptors were evaluated for all yogurt samples and compared to the reference. Maximum 4 samples were analyzed at the same time. The water and bread were used as an odor neutralizer for aroma and taste descriptors. The yogurt samples had the temperature of about 5 °C, which corresponds to usual temperature for eating yogurt.

Descriptors of homogeneity, firmness, and density described the textural perception of samples and were determined visually or mechanically by stirring with a teaspoon. Aroma of the samples was evaluated by smelling. Creaminess, sweetness, sourness, and bitterness described the taste properties and were evaluated after the perception of the samples in mouth. It was recommended to roll the samples on the tongue for several seconds and then evaluated the descriptor. Overall impression was determined by participants according to their personal preferences and requirements to yogurts.

Table 3
Sensory analysis descriptors.

Descriptor	1	3	5
Homogeneity	Inhomogeneous, big particles	Inhomogeneous, small particles	Homogenous, no particles
Firmness	Easy to stir, teaspoon is not standing	Firm, teaspoon is not standing	Firm, teaspoon is standing
Density	Thin, fluid	Thin, compact	Solid, compact
Creaminess	Sandy, lumpy	Creamy, small particles	Creamy, no particles
Aroma	Unpleasant, unnatural smell	Imperceptible	Pleasant, yogurt like
Sweetness	Imperceptible	Pleasant	Very intensive
Sourness	Imperceptible	Pleasant	Very intensive
Bitterness	Imperceptible	Middle intensive	Very intensive
Overall impression	I dislike	Acceptable	I like it very much

Descriptor ratings were measured by 5-point scales characteristic for each descriptor. The points 1, 3 and 5 were defined according to Table 3. The median of the rating was used for evaluation using Statistica software (Tibco, Palo Alto, USA, version 14.1). The results of the sensory analysis were presented using radar graphs created in Microsoft Excel software (Microsoft, Redmond, USA, version 2019).

2.6. Syneresis of yogurt samples

Syneresis measurement was conducted to characterize the release of whey in yogurt over time. This measurement was performed after 7 days of storing the yogurt samples. A predetermined amount of each yogurt sample was placed on pre-wetted filter paper in a funnel. The whey released was collected in a graduated cylinder. Syneresis was determined as the volume of released whey after 3 h per 100 g of the yogurt sample in duplicate.

3. Results and discussion

3.1. Characterization of WBPI

The WBPI was extracted for wheat bran according to the procedure described in 2.1. Protein material was subjected to analysis. The protein content in WBPI was 84.7 ± 0.3 %, comparable to commercially available protein isolates from soy or whey [3]. According to SDS-PAGE analysis (Fig. 1), WBPI contains protein fractions with molecular weights ranging from 6.5 to 145 kDa. The used isolation method did not cause the cleavage of wheat bran proteins into new shorter peptides (under 40 kDa), which could be. This is evident when comparing the WBPI bands with the bands of the raw wheat bran sample. Short peptides could be responsible for the bitter taste of plant-protein foods, making it undesirable to use them in higher amounts for food fortification [13].

Another nutritionally important factor is the amino acid profile (AAP) of WBPI. The high-quality protein isolates contain all essential amino acids (EAA) in high quantities. However, the intake of non-essential amino acids in the diet (NEAA) can also provide several health benefits. The content of semi-essential amino acids (SEAA) is particularly important for children and youth. The amino acid composition of WBPI is presented in Table 4.

The WBPI contains all essential amino acids (EAA), making it a nutritionally significant plant-protein supplement or fortifier, similar to soy protein isolate [14]. The most abundant amino acids in WBPI are glutamic acid, proline, and the semi-essential arginine. According to the amino acid profile (AAP), WBPI could be utilized in sports nutrition due to its relatively high content of amino acids responsible for muscle growth or regeneration. It could also be nutritionally beneficial for elderly individuals, helping to prevent or slow down the loss of muscle mass [15]. In addition to the influence of WBPI amino acids on the development of muscle mass, WBPI can be a good supplement to reduce blood pressure and improve outcomes in diabetes. It is also known, that

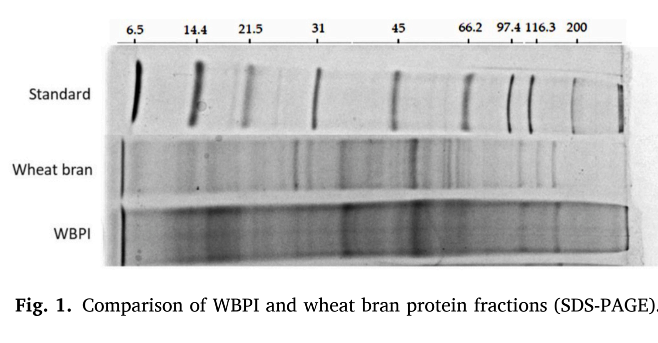

Fig. 1. Comparison of WBPI and wheat bran protein fractions (SDS-PAGE).

Table 4
Amino acid composition of WBPI [mg/100g].

EAA		SEAA		NEAA	
Val	4.6	Arg	7.7	Gly	5.2
Leu	6.8	His	3.3	Ala	4.6
Ile	2.8	–	–	Ser	4.7
Thr	3.3	–	–	Cys	1.6
Met	1.8	–	–	Asp	9.2
Lys	4.0	–	–	Glu	20.1
Phe	4.1	–	–	Tyr	3.7
Trp	0.9	–	–	Pro	9.5

EAA – essential amino acids; SEAA – semi-essential amino acids; NEAA – non-essential amino acids.

increased intake of certain amino acids positively affects the endocrine system include that of glucocorticoids, thyroid function, glucagon-like peptide 1, ghrelin, insulin-like growth factor-1 and leptin [16]. Lysine is the limiting amino acid in WBPI. However, even a single dose of WBPI (typically 30 g) covers, according to WHO, about 57 % of the recommended daily intake of lysine for 70 kg individual.

The composition of encapsulated WBPI (E-WBPI) differs from raw WBPI due to the alginate content covering the protein isolate. According to analysis, the protein content in E-WBPI was 53.9 %. Due to this decrease, the dosage of E-WBPI needed to be higher to maintain the required protein content of fortified yogurt compared to the dosage of raw WBPI (Table 2). Encapsulation substantially increased the particle size. The average size of E-WBPI determined by sieve analysis was 4.4 mm.

Important nutritional aspect of protein isolates is digestibility. Native plant proteins in general have lower digestibility compared to animal proteins due to the presence of antinutritional factors which can be exogenous (tannins, phytates and lectins) or endogenous (cross-linking or hydrophobicity). Determination of WBPI digestibility was proceeded according to the procedure described in 2.1. Total digestibility of WBPI was 93,4 ± 0,2 %. It is obvious, that isolation process significantly increased digestibility when compared to standard native plant proteins, which is normally between 75 and 80 % [17].

3.2. Raw WBPI fortification of yogurt

Individual samples of high-protein yogurts were prepared using direct fortification by raw WBPI. Yogurts with a protein content of 5 and 10 % were prepared according to the procedure described in chapter 2.2.

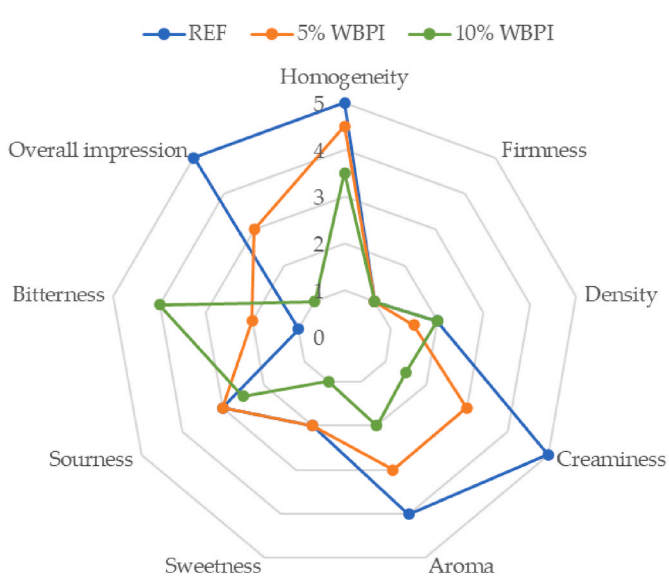

Fig. 2. Sensory descriptor scores of raw WBPI fortified yogurt samples.

According to the proposed technology, it was possible to prepare yogurts with a homogeneous structure and texture close to the reference yogurt. Produced samples were subjected to sensory analysis which revealed several sensory defects of fortified yogurts. The descriptor scores for WBPI-fortified yogurt samples were compared to the reference, which was plain reference yogurt (Fig. 2).

The physical characteristics of yogurt samples, such as firmness and density, were not affected by the WBPI fortification according to the sensory analysis and its statistical evaluation. All samples, including the reference, were evaluated as thin and easy to consume. However, the scores for other sensory descriptors changed with the addition of WBPI. The homogeneity score slightly decreased with increasing WBPI content, indicating uneven distribution or larger WBPI particles, the changes were also confirmed by ANOVA, which showed significant differences between samples ($P = 0.0122$). Most sensory panel participants evaluated the 10 % WBPI yogurt as inhomogeneous, with evidence of small lumps (score 3.5). The homogeneity perception of 5 % WBPI fortified yogurt was slightly better, but still inferior to that of the reference.

The creaminess feeling of the yogurt samples is closely related to homogeneity. The reference yogurt structure was described as mild and creamy in the mouth, with no lumps. On the other hand, WBPI-fortified yogurts were perceived as less creamy, and the 10 % WBPI sample structure was evaluated as sandy and lumpy. According to statistical evaluation the high protein samples and the reference were significantly different with the P value = 0.00001.

The aroma of yogurt samples was also affected by the addition of WBPI. The aroma of the reference sample was mostly evaluated as pleasant. It was revealed that WBPI additions led to the development of an aroma that was rather unpleasant for the sensory panel, which was confirmed by multiple comparison test. At a lower addition of WBPI, this phenomenon was not statistically significant, but the 10 % high-protein yogurt already showed a statistically significant deterioration of the aroma compared to the reference. WBPI has a characteristic grain aroma due to its origin, and this aroma persists in yogurts after WBPI fortification. The fat content in yogurts can even amplify this aroma. However, according to the sensory panel, this type of aroma is not natural for yogurts, and its presence reduced the aroma descriptor score.

Preferences for yogurt taste vary among individuals, but an appropriate perception of sweetness and sourness is generally acceptable. Yogurt should not exhibit any undesired or unpleasant taste, such as bitterness. These yogurt standards were maintained in the reference sample production, with sweetness slightly lower than the sensory participants' preferences and a moderate sourness. With WBPI addition, the taste perception of the fortified samples changed. The most significant difference was observed in the bitterness score ($P = 0.0004$). The 5 % WBPI yogurt was mostly described as moderately bitter (score 3), and the 10 % WBPI sample was perceived as even more bitter (score 4). The sourness of the fortified samples was almost identical to the reference, but the sweetness was likely masked by the bitterness and in the 10 % WBPI sample, it was described as unrecognizable (score 1). Statistically significant differences between sourness and sweetness perception of the samples and the reference were not observed.

It is well known that plant-based protein isolates can cause a bitter and astringent perception. The compounds responsible for bitterness can vary among proteins from different plant species [11]. Bitterness is commonly caused by the presence of small molecular weight peptides [18] or phenolic compounds [19]. Most plant protein isolates have an intense odor of their raw material. Although wheat protein aroma is less intense compared to various plant protein isolates [8], the grain aroma in WBPI yogurt was detected and described as less pleasant. Sandiness is also a natural characteristic of plant proteins and is associated with their low solubility. The solubility of protein isolates can be influenced by the extraction method, protein fraction content [20], or storage conditions [21]. The potential sandy perception of food limits the application of plant protein isolates. For yogurt fortification, it is necessary to treat the plant protein isolates to eliminate or reduce their sandy perception.

Table 5
Correlation coefficients between sensory descriptors and overall impression of WBPI fortified yogurt samples.

	Homogeneity	Firmness	Density	Creaminess
Overall impression	0.4905	0.0734	0.1054	0.7489

	Aroma	Sweetness	Sourness	Bitterness	Fortification
Overall impression	0.5789	0.4719	0.2366	−0.7933	−0.8294

According to the correlation coefficients provided by Spearman correlation test (Table 5), the overall impression score of individual samples was mainly affected by bitterness (−0.7933), aroma (0.5789), and creaminess (0.7489) of the yogurts. These descriptors are directly related to WBPI fortification. Consumers rated creamy, non-bitter, and pleasantly smelling yogurt samples with higher overall impression scores. It was also observed significant negative correlation between overall impression and degree of WBPI fortification (0.8294).

To improve the overall impression score of WBPI-fortified yogurt samples, it is necessary to mitigate or reduce the negative effects of adding WBPI on sensory descriptors. Adjusting the particle size of WBPI can enhance the creaminess score. Additionally, efforts should be made to mask the aroma and bitterness of WBPI with components more typical of yogurt. However, these enhancement procedures should not diminish the scores of other sensory descriptors of the yogurt samples or alter the natural characteristics of yogurt.

3.3. *Enhancing of sensory properties of high-protein yogurts*

The identification of the negative sensory properties of WBPI-fortified yogurt prompted modifications to both the raw WBPI and the yogurt recipe and manufacturing process. The objective was to significantly reduce or eliminate the negative sensory perceptions and enhance the overall impression score.

3.3.1. *Grinding of WBPI*

The negative evaluation of the creaminess of fortified yogurt was addressed by grinding and reducing the particle size of raw WBPI. Incorporating finely ground WBPI into the yogurt was intended to prevent the perception of sandiness in the product. The raw WBPI had a median particle size (×50) of 94.6 μm, which was reduced to a median particle size (×50) of 38.3 μm through ball mill grinding (referred to as G-WBPI).

The fortification of yogurt with ground WBPI was carried out following a process similar to the original manufacturing, and sensory analysis of these samples was conducted after 1 day of storage. The creaminess perception of yogurts fortified with raw and ground WBPI was compared, and the results are presented in Fig. 3.

Reducing the particle size of WBPI to less than 40 μm did not effectively eliminate the perception of sandiness in the yogurt. The textural sensory characteristics remained almost the same as those of yogurts fortified with raw WBPI. Additionally, the aroma perception of G-WBPI yogurt samples was evaluated to be even worse than that of yogurts fortified with raw WBPI. Furthermore, the sweetness score of the G-WBPI sample decreased. This reduction in sweetness could be attributed to the significant increase in bitterness of G-WBPI, which masked the sweet perception of the sample. The final low overall impression (score 1 – unacceptable) of yogurt samples fortified with ground WBPI revealed the potential adverse effects of fortifying high-protein (HP) foods with ground plant proteins on sensory properties.

Sandiness is considered a common sensory defect in all protein powders. Depending on the target food, achieving a specific minimum protein particle size is necessary to prevent sandiness. While reducing sandiness perception through grinding has been demonstrated in various studies [22,23], grinding WBPI did not effectively reduce sandiness perception. On the contrary, it led to a significant deterioration in other sensory properties. Creaminess is a crucial sensory property of yogurt, and any defects in this aspect are readily perceived by consumers. Addressing the sandiness issue in WBPI-fortified yogurt through particle size reduction alone proves challenging. Using alternative protein forms, such as balls instead of powder, could potentially help mitigate product sandiness while maintaining the creaminess of the original yogurt.

3.3.2. *Protein blending*

To enhance the taste of WBPI-fortified yogurts, protein blending was employed. A portion of the WBPI was substituted with dried milk while maintaining the 5 % and 10 % protein content in the yogurts. The blends were created to maintain a protein content ratio of 1:2 (MIX1) and 1:1 (MIX2) between WBPI and dried milk. Besides the protein fraction, the MIX samples were enriched with components from dried milk, such as fat and carbohydrates, which could also influence the sensory perception of the yogurt samples.

Sensory analysis of yogurts with 5 % and 10 % protein content was conducted individually to determine the acceptability limits of the yogurts. The descriptor scores for yogurt samples fortified with protein

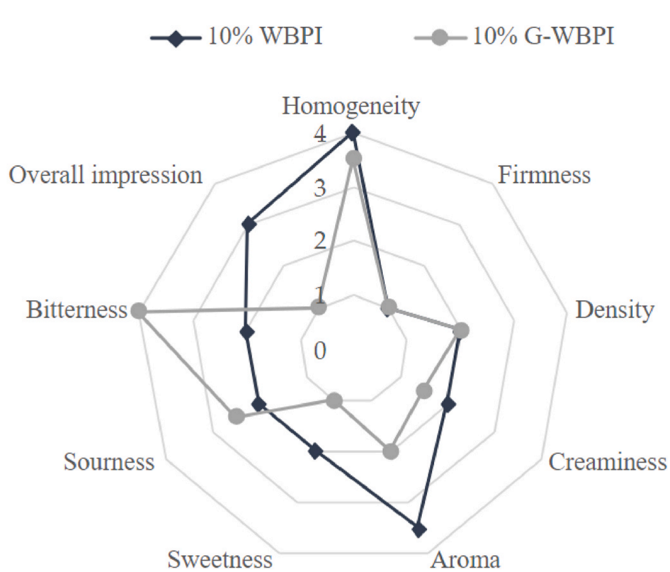

Fig. 3. Sensory descriptor scores of ground WBPI fortified yogurt sample.

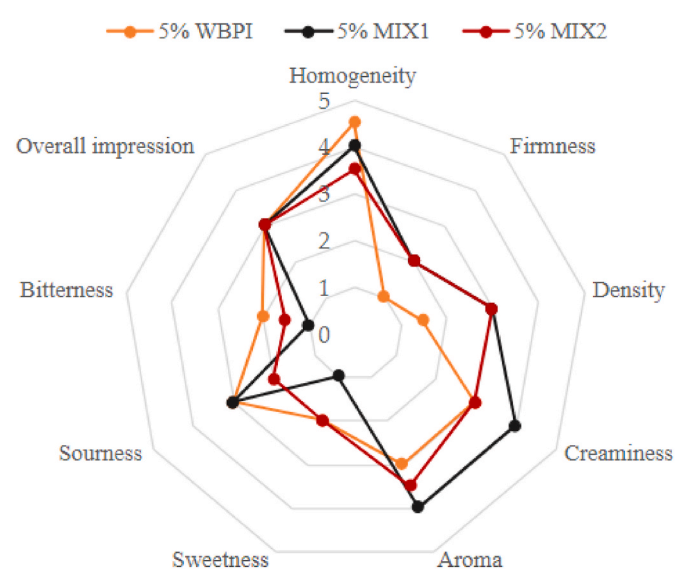

Fig. 4. Sensory descriptor scores of 5 % MIX fortified yogurt samples.

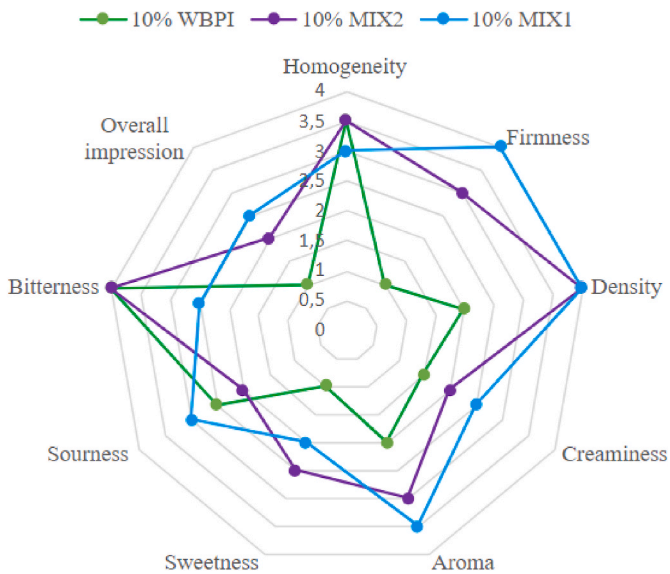

Fig. 5. Sensory descriptor scores of 10 % MIX fortified yogurt samples.

blends (MIX1 and MIX2, prepared according to the recipes listed in Table 2) were compared to those of raw WBPI-fortified yogurt. The results are presented in Figs. 4 and 5.

The scores for individual sensory descriptors differed between yogurts with 5 % and 10 % protein content, but only few descriptors of the samples were evaluated as statistically different according to multiple comparison test. Fortifying with blended protein materials to maintain 5 % protein content did not result in significant differences in taste sensory descriptors among the yogurt samples. However, the samples with dried milk were perceived as denser ($P = 0.0025$) and creamier ($P = 0.0486$). Combination of milk and WBPI led to a stiffer and more compact structure. Better synergy of mixed systems of milk and plant proteins for stabilizing the emulsions was also described in the research of Lima Nascimento et al. [24]. Additionally, milk proteins, specifically caseins, are more compatible with casein micelles due to their unique structural properties, such as open and flexible conformations and the presence of calcium phosphate. Plant proteins, on the other hand, have different properties that make them less compatible with casein micelles, including low solubility and lack of structural compatibility. Blending of plant proteins with milk proteins can improve this problem and increase solubility in aqueous solutions [25].

The aroma of yogurts with dried milk content was also rated as more pleasant. Conversely, the sample with 5 % WBPI was described as more homogeneous compared to the 5 % MIX yogurt samples with P value = 0.0339.

The differences in individual descriptors for 5 % protein yogurts did not result in a variation in the overall impression score. For yogurts with lower protein content, protein blending did not significantly increase scores for any sensory descriptor, which are generally important for yogurt consumers. It was also not proved the influence of WBPI fortification degree on overall impression.

The fortification using a blend of dried milk and WBPI to maintain a 10 % protein content in yogurts (Table 2) resulted in more notable changes in the perception of taste, structure, and aroma of MIX yogurt samples compared to the 10 % WBPI sample.

Homogeneity among the evaluated samples was similar, but the textural characteristics, such as firmness and density, of MIX samples were described as much more solid compared to the 10 % WBPI yogurt and were evaluated as significantly different ($P = 0.0001$ and 0.00001 respectively). The higher density of MIX samples was also observed in yogurts with a total protein content of 5 %. As mentioned earlier, a higher amount of dry matter in yogurts leads to a stiffer yogurt. Additionally, the presence of milk proteins contributes to stabilizing the yogurt structure. This aligns with the results of the sensory analysis, where the highest density was observed in the sample 10 % MIX 1, which contains 10.8 g of dried milk.

Creaminess was also influenced by the addition of dried milk and statistical differences were evaluated ($P = 0,0294$). There were no significant observations of statistical differences between aroma and taste descriptors of yogurt samples, except of the bitterness perception ($P = 0.0444$).

The perception of bitterness in MIX samples varied. Bitterness reduction was observed only in the 10 % MIX1 sample, which had a higher content of dried milk. In the 10 % MIX2 and 10 % WBPI samples, bitterness was very intense, significantly affecting the overall impression (correlation coefficient −0.6966). The 10 % WBPI sample received the lowest overall impression score. The scores of MIX samples were higher, with the panel mostly describing them as acceptable. In these samples, the fortification by WBPI had great influence on the overall impression (cor. coefficient = −0,5019), but in comparison to samples fortified only by raw WBPI, the correlation between the fortification degree and the overall impression was not so significant.

It was demonstrated that substituting WBPI with dried milk positively influenced the sensory perception of fortified yogurts, although the diversity of amino acids was reduced. The lower amount of added WBPI and the presence of dried milk components increased the overall impression score of the 10 % MIX samples compared to 10 % WBPI-fortified yogurt. Whey and milk proteins are, based on their physical characteristics, the most suitable for incorporation into the yogurt structure. Furthermore, other milk components can effectively mask the aroma and bitter taste of WBPI [26] and improve the textural properties of yogurts. According to Karam, adding 1–2% whey protein concentrate to yogurt leads to a creamier perception, but the firmness and density of yogurt are low. Conversely, adding skimmed milk powder increases viscosity and reduces lumpiness in yogurts [27].

3.3.3. WBPI encapsulation

The encapsulation of WBPI was performed to mitigate the perception of sandiness, bitter taste, and enhance the aroma in WBPI-fortified yogurt. The WBPI was immobilized in alginate according to the procedure described in 2.4. Zhang et al. (2016) proved, that alginate is good delivery system for encapsulation and release of proteins in human nutrition. Release of proteins occurs in the small intestine environment at slightly acidic pH around 6.3 This pH is suitable for almost absolute release of proteins from alginate beads [28,29].

Encapsulation had significant effect on sensory properties of prepared yogurts. The scores for sensory descriptors of E-WBPI fortified yogurt samples are presented in Fig. 6.

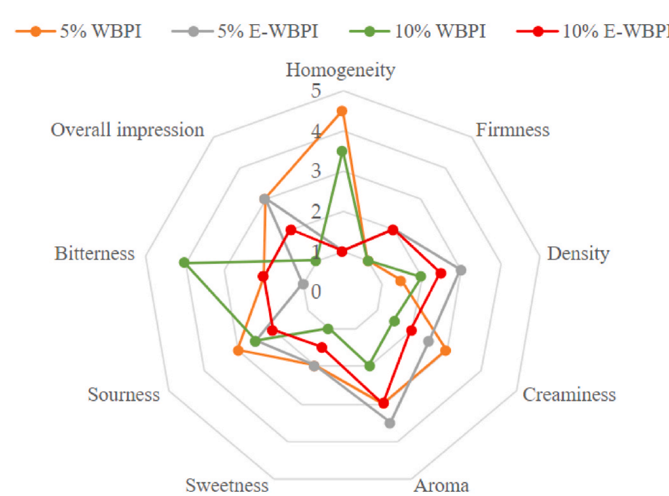

Fig. 6. Sensory descriptor scores of E-WBPI fortified yogurt samples.

The sensory analysis of 5 % and 10 % E-WBPI yogurts was compared to samples of raw WBPI. The encapsulation of WBPI altered the perception of E-WBPI yogurts compared to WBPI yogurt samples. The resulting WBPI encapsulate balls ranged in size from 0.25 to 0.63 cm. The addition of WBPI encapsulate strongly influenced the homogeneity score ($P = 0.0001$), with the yogurt samples described as non-homogeneous with visible particles. The other texture descriptors, as firmness and density, were not evaluated as statistically different. The creaminess of the samples was also not affected by the addition of E-WBPI, differences in this descriptor were not significant.

One advantage of WBPI encapsulation is the prevention of yogurt color alteration and masking of WBPI grain odor. The addition of raw WBPI turns the color of yogurt to brown, while the yogurt with E-WBPI maintains its original white color. The aroma of E-WBPI and 5 % WBPI yogurts was described as acceptable. However, a 10 % addition of raw WBPI to the yogurt caused the development of a less pleasant aroma, which also influenced the overall impression score.

Perceptions of sourness was not significantly different in any of the tested yogurt samples. However, differences in the perception of sweetness ($P = 0.0379$) and bitterness ($P = 0.0122$) between individual yogurt samples were observed. The encapsulation of WBPI reduced the bitterness of E-WBPI yogurt samples compared to 10 % WBPI yogurt and therefore sweet taste was perceived more intensively. In the 5 % E-WBPI sample, the bitterness was almost eliminated. The bitterness perception of 10 % E-WBPI was similar to the bitterness of the 5 % WBPI yogurt sample.

The overall score of the yogurt samples was mainly influenced by bitterness sweetness and creaminess perception (cor. coefficients = −0.6471, 0.6113, 0.6253 respectively). The 10 % WBPI yogurt sample was evaluated as unacceptable compared to E-WBPI samples. The overall impression score of the yogurt sample with 10 % E-WBPI was higher, but the yogurt was still not considered good enough to be acceptable for consumers. The presence of larger particles in yogurt is also undesirable [30]. The 5 % WBPI and 5 % E-WBPI received similar and the highest overall impression score among the compared yogurt samples.

3.4. Syneresis of high-protein yogurts

Syneresis of whey is one of the primary sensory defects observed in yogurts. This is mainly caused by the low dry matter content in yogurts. However, factors such as high incubation temperature, storage conditions, or an inappropriate ratio of casein and whey proteins can also contribute to whey separation. Commercially, it is recommended to use milk with a 15 % dry matter content to produce yogurt. Nevertheless, adding non-milk material to the yogurt increases the dry matter content, which can disrupt the yogurt micelle structure and increase product syneresis. The dry matter content and the degree of syneresis for each yogurt sample are presented in Fig. 7.

The syneresis of the reference yogurt was 31.8 ± 1.6 mL/100 g, a volume of whey separated from yogurt that was similar to the one determined by Erkaya et al. [31]. The addition of 5 % WBPI, in any form, caused a slight increase in yogurt syneresis. According to Akin et al. [32], the addition of plant protein materials to fermented milk products increases their syneresis due to their low water-holding capacity. This was observed and confirmed by the syneresis of 5 % WBPI yogurt samples.

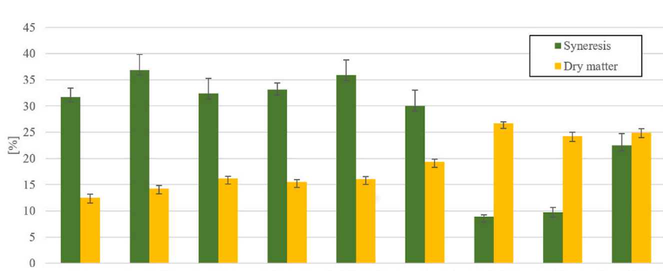

Fig. 7. The syneresis and dry matter content of yogurt samples; syneresis [mL/100 mL], dry matter [g/100 mL].

However, the syneresis of yogurt samples with 10 % WBPI content decreased compared to the reference yogurt. The lowest syneresis was observed in sample 10 % MIX1 with the addition of a blend of dry milk and WBPI. Due to its high content of dry matter and relatively low content of plant-origin WBPI, the syneresis of the 10 % MIX samples reduced by more than 20 mL/100 g. Delikanti et al. [33] also described the reduction of syneresis after the addition of whey protein. This reduction was confirmed in the case of the combination of dry milk and WBPI, but only up to a total 10 % protein content of the yogurt samples. The addition of E-WBPI to the 10 % protein yogurt also caused a reduction in whey syneresis. In addition to proteins, E-WBPI yogurt samples are fortified by carbohydrates, especially alginate. The presence of alginate could significantly influence the syneresis of the yogurt sample due to its water-holding capacity. The same phenomenon was described by Free-Manjarrez et al. [34] after the addition of encapsulated bean protein hydrolysate by arabic gum and whey protein concentrate to the yogurt.

The correlation coefficient between dry matter content and syneresis volume was −0.891, revealing that an increase in the yogurt's dry matter decreases whey release. The indirect addition of carbohydrates, together with the protein fraction, to the yogurts can improve the strength of the yogurt structure. Therefore, fortifying yogurts with plant proteins of high purity can be inconvenient and may lead to excessive whey release.

4. Conclusions

Analysis of WBPI proved, that WBPI is good source of dietary proteins. WBPI contains complete spectrum of essential amino acids. Another significant positive feature of this isolate is its good digestibility (93.4 ± 0.2 %). However, fortifying yogurts with plant proteins is limited by the occurrence of sensory defects, notably sandiness, aroma, and bitterness, as confirmed by the production of WBPI-fortified yogurts.

It was revealed that the form in which WBPI is added to the yogurt samples has a significant impact on individual sensory descriptors and the final overall impression of the HP yogurts. Raw powder form of WBPI had negative impact on overall impression which was directly connected with textural and flavour descriptors bitterness, aroma and creaminess.

To reduce sandiness, and thus increase the degree of creaminess, WBPI was ground to a finer, analytically defined powder. This modification proved to be inefficient. It did not solved problems with sandiness and the yogurt samples were also perceived as significantly more bitter, which was further reflected in the deterioration of the overall evaluation of the product.

Substituting WBPI with dried milk was demonstrated to positively influence the sensory perception of fortified yogurts, despite a reduction in amino acid diversity. The inclusion of dried milk components, alongside a lower quantity of WBPI, elevated the overall impression score of yogurt samples fortified with a 10 % mixture compared to those fortified solely with 10 % WBPI. Wheat bran protein and milk proteins, due to their physical characteristics, are deemed highly suitable for integration into the yogurt matrix. Moreover, other constituents of milk effectively mask the aroma and bitter taste of WBPI while also improving the textural properties of yogurts.

Encapsulating WBPI in alginate improved yogurt by reducing sandiness and bitterness while masking unpleasant aroma. Sensory analysis showed differences between E-WBPI and raw WBPI yogurts, with E-WBPI maintaining yogurt color and aroma better. Texture-wise, E-WBPI affected homogeneity but not creaminess, firmness, or density.

Bitterness was reduced in E-WBPI, enhancing sweetness perception. Larger particles in proved have negative impact on overall impression.

Syneresis was observed to increase with 5 % added WBPI but decreased with 10 % addition, especially when combined with dry milk. This reduction is attributed to improved yogurt structure. Encapsulated plant proteins also showed a reduction in whey release due to added carbohydrates. A strong negative correlation (−0.891) between dry matter content and syneresis volume suggests that increasing dry matter decreases whey release.

WBPI proved to be a suitable raw material to produce high-protein yogurt. The raw isolate treatment procedures proposed herein are effective enough to minimize the additive's effects on sensory properties.

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CRediT authorship contribution statement

Zuzana Slavíková: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Pavel Diviš:** Writing – review & editing, Supervision, Funding acquisition, Formal analysis. **Wojciech Bialas:** Methodology. **Magdalena Montowska:** Methodology. **Michaela Adamczyková:** Investigation. **Jaromír Pořízka:** Writing – review & editing, Validation, Supervision, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jaromir Porizka reports financial support was provided by Brno University of Technology. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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