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Enrichment of Bread with Whey Treated with High Hydrostatic Pressure

Ekmeğin Yüksek Hidrostatik Basınçla İşlenmiş Peynir Altı Suyuyla Zenginleştirilmesi

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ABSTRACT

There are some deficiencies in the nutritional profile of bread due to the use of refined flour in modern production. Therefore, various additives are employed to enhance the nutritional profile of bread. Whey is a valuable source of functional proteins, lipids, vitamins, and minerals. However, bread volume and water absorption decrease when whey is added directly to the bread dough. Therefore, studies are being carried out to investigate pretreatments that can improve the technological properties of whey. This study aimed to develop a bread product fortified with whey treated with high hydrostatic pressure (HHP) and evaluate its quality. Properties of bread enriched with whey treated with HHP (300 MPa, 3 min, 20°C) (HHPWB) were examined using crumb images, experimental (moisture, ash, and unsalted ash content, acidity, weight, dough yield, volume, specific volume, and crust/crumb ratio), texture, color, and sensory analyses. The HHPWB formulation resulted in a decrease in gas cells, hardness, and chewiness, as well as an increase in ash content, acidity, weight, volume, and *a** color values. HHPWB received better sensory acceptance for crust, symmetry, and color. The HHP treatment may have the potential to boost the benefits of fortifying bread with whey.

Keywords: High hydrostatic pressure, Bread, Whey, Enrichment

ÖZ

Modern üretimde rafine un kullanılması nedeniyle ekmeğin besin profilinde bazı eksiklikler bulunmaktadır. Bu nedenle, ekmeğin besin profilini geliştirmek için çeşitli katkı maddeleri kullanılmaktadır. Peynir altı suyu fonksiyonel proteinler, lipitler, vitaminler ve mineraller açısından değerli bir kaynaktır. Ancak peynir altı suyu doğrudan ekmek hamuruna eklendiğinde ekmek hacmi ve su emilimi azalmaktadır. Bu nedenle, peynir altı suyunun teknolojik özelliklerini geliştirebilecek ön işlemler ile ilgili araştırmak için çalışmalar yürütülmektedir. Bu çalışma, yüksek hidrostatik basınç (HHP) ile işlenmiş peynir altı suyuyla zenginleştirilmiş bir ekmek ürünü geliştirmeyi ve kalitesini değerlendirmeyi amaçlamıştır. HHP (300 MPa, 3 dakika, 20°C) ile işlenmiş peynir altı suyuyla zenginleştirilmiş ekmeğin (HHPWB) özellikleri, kırıntı görüntüleri, deneysel (nem, kül ve tuzsuz kül içeriği, asitlik, ağırlık, hamur verimi, hacim, özgül hacim ve kabuk/iç oranı), doku, renk ve duyusal analizler kullanılarak incelenmiştir. HHPWB formülasyonu gaz hücrelerinde, sertlikte ve çiğnenebilirlikte azalmanın yanı sıra kül içeriği, asitlik, ağırlık, hacim ve *a** renk değerlerinde artışa neden olmuştur. HHPWB kabuk, simetri ve renk açısından daha iyi duyusal kabul görmüştür. HHP uygulaması ekmeğin peynir altı suyuyla zenginleştirilmesinin faydalarını artırma potansiyeline sahip olabilir.

Anahtar Kelimeler: Yüksek hidrostatik basınç, Ekmek, Peyniraltı suyu, Zenginleştirme

Introduction

Bread, which can be produced in various forms, is one of the most basic traditional foods consumed by people all over the world and can meet a large part of their daily nutritional needs (Lockyer & Spiro, 2020). Although there are varieties of bread made with different grain flours (rye, corn, and barley), wheat flour is the most preferred basic raw material due to its structure, which allows it to form a dough when in contact with water in traditional bread making (Kourkouta et al., 2017). Bread, which is produced by fermenting and baking dough prepared with wheat flour, water, salt, and yeast. However, rafinated wheat flour used in bread production may cause malnutrition in people whose diet mainly consists of bread, due to the low lysine content in wheat protein. In low-income societies where malnutrition is common due to being bread as main food source, research on enriching bread with vitamins, minerals, flavonoids, anthocyanins, fatty acids, carotenoids, folic acid, protein, and/or amino acids is very important (Betoret & Rosell, 2020). In addition, issues such as the production of low-calorie and improved-quality bread that can aid in the treatment of diseases such as obesity in high-income societies have started to receive more attention. For this reason, researchers are focusing on studying the addition of functionality to bread, especially by using additives with proven health benefits (Martins et al., 2017; Meral & Erim Köse, 2019; Lachowicz et al., 2021).

In the food industry, cheese processing plants produce whey in large quantities as waste during cheese making or the coagulation of the milk casein step. Whey causes serious environmental pollution due to its high chemical oxygen demand (COD: 50-80 g/L), biochemical oxygen demand (BOD: 40-60 g/L), and organic content (lactose, proteins, lipids, vitamins) (Das et al., 2016; Zandona et al., 2021). In addition, difficulties in separating whey components pose a significant challenge for treatment plants. It is vital to manage, process, and reuse whey properly. For this purpose, advanced technologies are used to manage high organic and inorganic nutrients in whey, and alternative methods are being explored to maximize benefits and further processing (Yadav et al., 2015). Whey is a byproduct rich in functional proteins and peptides, lipids, vitamins, minerals and lactose. Whey proteins are crucial because they contain essential amino acids such as leucine, isoleucine, and valine. The positive effects of whey proteins on boosting immunity, lowering the risk of heart disease, and reducing cancer incidence have been reported (Pillai et al., 2024; Thampy et al., 2024). Due to these properties, whey was included in bread in various studies (Madenci & Bilgiçli, 2014; Zhou et al., 2018; Tsanasidou et al., 2021; Ingrassia et al., 2022). Lactose in whey increases the Maillard reaction (Dıblan et al., 2024; Zhang et al., 2024). However, it can reduce the volume of the bread by creating high osmotic pressure, slowing down yeast activity. When whey is added directly to the bread dough, it increases protein, mineral, thiamine, and riboflavin contents, but reduces water absorption. In addition, whey protein caused to ruptures of gluten network in dough (Van Riemsdijk et al., 2011). The study aimed to reduce or eliminate adverse conditions in whey by exploring the effects of various technological applications.

There has been increasing attention in high hydrostatic pressure (HHP) due to its ability to increase the extraction efficiency of functional elements and enhance the health efficacy of foods (Huang et al., 2020). HHP is a non-thermal technology that meets consumer requirements by extending the shelf life and enhancing the safety of foods. It is endorsed as reliable by the U.S. Food and Drug Administration (FDA) (Wang et al., 2016). HHP can be applied to inactivate of pathogenic microorganisms that, deactivate enzymes, increase the shelf life of foods, and induce desired conformational and chemical modifications in the food matrix (Aganovic et al., 2021). Previous studies have demonstrated that HHP treatment can increase the surface hydrophobicity, enhance solubility, and improve foaming features of whey proteins (Lim et al., 2008a; Ambrosi et al., 2016). However, no study has been found on the contribution of HHP treated whey to bread quality. Therefore, this study investigated the effects of HHP treated whey on certain quality parameters of bread.

Methods

Materials

Wheat flour and whey were provided from Ankara Public Bread and Flour Factory Inc. (Ankara, Türkiye) and Atatürk Forest Farm Milk Factory (Ankara, Türkiye), respectively. Wheat flours (Type 550, Type 650, and Type 85) were mixed in equal proportions, and the mixed flour was utilized in bread making. Good quality refined salt and baker's yeast (Saccharomyces cerevisiae) were provided from Ankara Public Bread and Flour Factory Inc. (Ankara, Türkiye).

Material Analysis

Moisture content, wet gluten, dry gluten, Zeleny sedimentation, falling number, ash content, acidity, sieve analysis, farinograph, and extensograph properties of flour were analyzed following the methods outlined by Elgün et al. (1998). Dry matter, ash, oil, acidity, and pH values of whey were analyzed using the methods specified by Kurt et al. (1996).

High Hydrostatic Pressure Treatment of Whey

High hydrostatic pressure treatment was conducted at Middle East Technical University. In previous studies, the features of the device used were detailed (Erkan et al., 2011; Önür et al., 2018). Deionized water was used as the medium for isostatic pressure transduction. HHP treatment conditions for whey were justified (R = 0.999). The whey samples were exposed to HHP treatment for 3 minutes at 300 MPa and 20±1°C. Pressurization times did not contain the times for pressure rise and release. The sterile falcon tubes containing whey, wrapped with stretch film, were placed inside the cylindrical vessel of the HHP equipment. The chamber was then closed, and the samples were left for 2 minutes for temperature equilibration. After the HHP treatment, the samples were immediately removed from the chamber and stored at 4°C (Çiçek, 2011).

Bread Baking Procedure

The dough was prepared by combining 45 g of refined salt, 203 g of bread yeast, 3 kg of flour, and 1800 mL of water in a stainless-steel mixing bowl. Water absorption capacity of the flour (59.5%) was determined using a farinograph, as per standard methods. Whey (treated with high hydrostatic pressure or untreated) was added at a ratio of 25% of the water absorption capacity of the flour, with the exact amount of whey added being 450 mL (Dinçoğlu & Ardıç, 2012; Iuga et al., 2020). The ingredients were mixed using a spiral kneader, initially at slow speed for 4 min and then at fast speed for 18 minutes to form the dough. The dough was then weighed and divided into three equal portions, each of which was allowed to rest for 10 min at room temperature. After this, the dough portions were shaped and allowed to rest for an additional 10 min. The dough was transferred to a fermentation room and left to ferment for 75 min at 25±1°C. The oven was preheated to an inlet temperature of 190°C and an outlet temperature of 242°C, and the dough was baked for 28 min, following the AACC 10-10B guidelines with slight modifications. After baking, the bread was removed from the oven, weighed, and allowed to cool to room temperature. Finally, texture analysis was performed on the cooled bread 24 hours after baking.

Bread Quality

Stereo microscope images of bread samples

Bread samples were sliced (3 mm thick) after 24, 48, and 120 hours and photographed under a stereo microscope (Nikon SMZ 1500, Japan, 40X magnification).

Experimental analysis

The moisture, ash, and unsalted ash content, acidity, weight, dough yield, volume, specific volume, and crust/crumb ratio of bread samples were analyzed using the methods specified by Nwosu et al. (2014).

Texture analysis

Texture profile analyse was carried by a texture analyzer (Lloyd TA Plus, UK). The diameter of the cylinder probe was 3.5 cm. The bread was cut into 1.25 cm thick slices using a saw blade after 24, 48, and 72 hours. Two slices were taken from the loaf, stacked on top of each other, and measured to be a total of 2.5 cm thick (Basman et al., 2002; Çetiner et al., 2017). Hardness values were obtained by compressing the bread at a rate of 25% with a stroke of 1 mm/s. The overall average value of three measurements was reported.

Color analysis

Values of brightness (L^*), redness (a^*), and yellowness (b^*) of bread samples were determined using the CIE $L^*a^*b^*$ system with a Minolta Spectrophotometer CM-3600d. The color of bread samples was determined by averaging three L^*, a^*, and b^* readings. In this context, L^* indicates brightness (ranging from 0=black to 100=white), $+a^*$ indicates redness, $-a^*$ indicates greenness, $+b^*$ indicates yellowness, and $-b^*$ indicates blueness. A white calibration plate was used to standardize the equipment before conducting color measurements (Adamczyk et al., 2021). Color measurements were taken at four points on the surface of the bread.

Sensory analysis

Sensory features of bread samples were scored by 10 panelists, consisting of six females and four males aged 23-35. The bread samples were labeled with letters, and the order of sample presentation was entirely randomized before serving them to the panelists. All bread samples were served simultaneously on the same day. The sensory quality characteristics of bread samples (structure of the bread inside, crust, symmetry of shape, color, taste, and smell) were scored on a 1–5 scale, where 1 indicated "dislike extremely" and 5 indicated "like extremely" (Bilgiçli & İbanoğlu, 2015).

Statistical Analysis

All measurements were examined using SPSS 15.0 software (SPSS Inc., Chicago, IL, USA). The least significant difference was calculated at the $p < .05$ level. Significant differences amongs means were determined using Duncan's multiple range test. All analyses were conducted with at least 3 replications. The values are given as ± standard error.

Results

The physical and chemical parameters of the flour and whey used in the study are provided in Table 1.

The images of the internal structure of the bread samples at 24, 48, and 120 hours are presented in Figure 1.

Table 1.
Physical and chemical parameters of the flour and whey

Parameters for flour	Values (Unit)
Moisture	12.90 (%)
Dry matter	87.10 (%)
Above sieve (the sieve pore size: 224 µm)	0.05 (%)
Under sieve (the sieve pore size: 224 µm)	99.96 (%)
Ash	0.56 (%)
Acidity	0.03 (%)
Wet gluten	31.97 (%)
Dry gluten	11.19 (%)
Sedimentation	26 (cm^3)
Falling number	869 (s)
Parameters for whey	**Values (Unit)**
Dry matter	5.73 (%)
Ash	0.38 (%)
Acidity	0.17 (%)
pH (at 28.5℃)	4.93
Fat	0.30 (%)

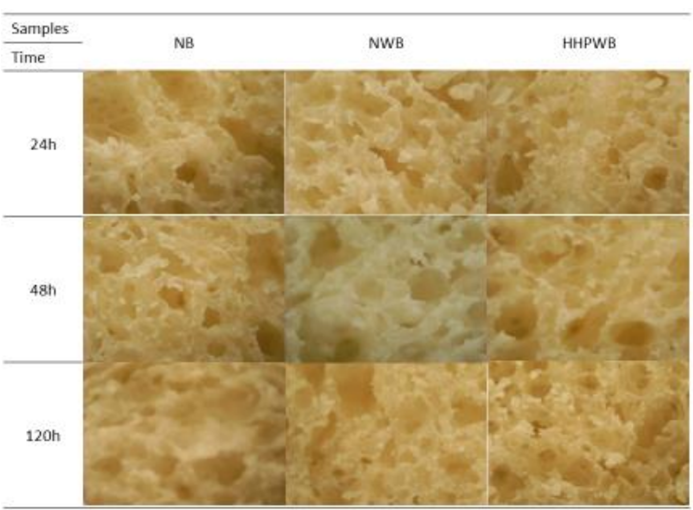

Figure 1.
The images of the internal structure of the bread samples (NB: Normal bread, NWB: Normal whey added bread, HHPWB: HHP treated whey added bread)

The results of the experimental analysis of the bread samples are presented in Table 2.

Table 2.
Experimental Values of the Bread Samples

Experimental analysis	Bread Samples		
	NB	NWB	HHPWB
Moisture (%)	37.63±0.03^{a}	37.62±0.02^{a}	38.79±0.01^{b}
Ash (%)	2.28±0.01^{a}	2.28±0.01^{a}	2.21±0.01^{b}
Unsalted Ash (%)	0.55±0.00^{a}	0.53±0.00^{a}	0.53±0.01^{a}
Acidity	2.85±0.07^{a}	3.71±0.05^{b}	3.82±0.03^{b}
Weight (g)	143.50±1.53^{a}	148.50±1.53^{b}	148.20±0.18^{b}
Dough Yield (%)	166.60±0.32^{a}	169.40±1.83^{a}	171.30±1.46^{a}
Volume (cm^3)	355±0.03^{a}	377.50±3.53^{b}	365±0.00^{c}
Specific volume (cm^3/g)	2.47±0.03^{a}	2.54±0.05^{a}	2.46±0.00^{a}
Crust/crumb ratio	0.76±0.00^{a}	0.71±0.00^{a}	0.63±0.00^{a}

NB: Normal bread, NWB: Normal whey added bread, HHPWB: HHP treated whey added bread; $^{a, b}$: Means with different lowercase letters in the same column are statistically different at $p<.05$

The results of the texture analysis of the bread samples are presented in Table 3.

Table 3.
Texture Values of the Bread Samples

Texture Parameters	Bread Samples		
	NB	NWB	HHPWB
Hardness (N)	13.34^{a}	10.16^{b}	9.88^{b}
Gumminess	0.54^{a}	0.54^{a}	0.54^{a}
Elasticity (mm)	5.40^{a}	5.44^{a}	5.70^{b}
Chewability (Nmm)	38.31^{a}	29.64^{b}	30.67^{b}

NB: Normal bread, NWB: Normal whey added bread, HHPWB: HHP treated whey added bread; $^{a, b}$: Means with different lowercase letters in the same column are statistically different at $p<.05$

The results of the color analysis of the bread samples are presented in Table 4.

Table 4.
Color Values of the Bread Samples

Color Values	Bread Samples		
	NB	NWB	HHPWB
L^*	67.46±5.66^{a}	65.09±6.39^{b}	65.10±4.96^{b}
a^*	7.69±6.37^{a}	8.77±6.89^{b}	8.80±6.93^{b}
b^*	28.35±1.20^{a}	27.68±0.78^{a}	28.11±0.63^{a}

NB: Normal bread, NWB: Normal whey added bread, HHPWB: HHP treated whey added bread; $^{a, b}$: Means with different lowercase letters in the same column are statistically different at $p<.05$

The results of the sensory analysis of the bread samples are presented in Table 5.

Table 5.
Sensory values of the bread samples

Sensory Parameters	Bread Samples		
	NB	NWB	HHPWB
Bread Crumb	3.70±0.95[a]	4.40±0.70[a]	3.50±0.97[a]
Bread Crust	3.20±0.92[a]	4.10±0.88[b]	4.40±0.70[b]
Symmetry	3.10±0.57[a]	4.10±0.57[b]	4.20±0.42[b]
Taste	3.40±1.07[a]	3.80±0.79[a]	4.00±1.15[a]
Odour	3.60±1.17[a]	3.70±1.06[a]	4.10±0.99[a]
Color	2.90±0.99[a]	4.00±1.05[b]	3.90±0.99[b]

NB: Normal bread, NWB: Normal whey added bread, HHPWB: HHP treated whey added bread; [a, b]: Means with different lowercase letters in the same column are statistically different at $p<.05$

Discussion

The parameters, such as moisture content, dry matter, ash content, acidity, wet gluten, and dry gluten of the mixed flour, were found to be suitable for bread making according to the Turkish Food Codex Wheat Flour Communique (Communique Number: 2013/9) (Table 1). The low volume of the normal bread observed in this study may be attributed to the very low amylase activity in the flour, as indicated by the falling number of 869 s. This reduced amylase activity limits the availability of fermentable sugars for the yeast, which can result in insufficient fermentation and lower bread volume.

The whey was characterized by the following parameters: dry matter, ash, acidity, pH, and fat values were 5.73%, 0.38%, 0.17%, 4.93, and 0.30%, respectively (Table 1). It has been reported that the dry matter content of whey from six dairy plants ranged from 5% to 7% (Alsaed et al., 2013). In another study, it has been demonstrated that large variations were observed in the ash content of acid whey (0.57-1.88) and sweet whey (0.37-0.58) (Gernigon et al., 2010). The characterization parameters of whey mostly depend on the processing techniques used in cheese factories.

The addition of HHP-treated and untreated whey to the bread dough did not led to a significant change in bread properties in terms of unsalted ash, dough yield, specific volume, and crust/crumb ratio according to the control group (normal bread: NB). It has been reported that whey affects the specific volume of bread depending on the presence of hydrocolloids in the dough formulation. Whey, which increases the specific volume of bread in the presence of hydrocolloids such as hydroxypropylmethylcellulose (HPMC), yeast β-glucan, and whey protein isolate (WPI), decreases the specific volume of bread in the absence of hydrocolloids (Kittisuban et al., 2014). The decrease in the specific volume can be explained by the low water absorption of proteins. In this case, a more intense breadcrumb feature can be encountered (Kittisuban et al., 2014). A significant decrease in bread acidity, as well as a notable increase in bread weight and volume, were observed with the addition of whey. The addition of HHP-treated whey to bread dough resulted in significant differences in moisture, ash content, and bread volume compared to bread with untreated HHP whey and normal bread. Gluten proteins have a greater capacity to form hydrogen bonds with water molecules compared to whey proteins. Lower water absorption and water binding capacity of whey proteins may be responsible for bread with low moisture content. It has been reported that bread prepared with 20% whey protein concentrate (WPC) had lower moisture content compared to normal bread (Ferreyra et al., 2021). Because high hydrostatic pressure can be applied at low temperatures, it provides an advantage in modifying food products while preserving their quality (Carullo et al., 2021). The incorporation of untreated WPC (5%) into wheat flour decreased water absorption by 66% compared to the control group (100% classical wheat flour with 70% water absorption). Conversely, the inclusion of HHP-treated WPC (5%) at 85 Kpsi for 30 minutes resulted in an increase in water absorption to 70% (Kadharmestan et al., 1998). Pressure treatment up to 300 MPa can cause reversible protein denaturation at low protein concentrations, while pressures above 300 MPa can cause irreversible denaturation of proteins. High pressure causes conformational and structural changes in proteins by deprotonating charged groups, disrupting salt bridges, and hydrophobic interactions. Here, HHP treatment improved the hydrogen bonding of proteins in whey with water molecules (Table 2).

Acidity increased significantly in breads with whey added, both in the HHP-treated and untreated samples. This increase in acidity can be attributed to the presence of whey proteins, which contribute additional amino acids to the bread formulation. In the case of HHP-treated whey, structural changes in the proteins may enhance the availability of amino acids, potentially influencing the acidity further. The alteration of the whey proteins, especially through protonation of their amine groups in the acidic environment, could increase the release of free protons (H^+), contributing to the overall rise in acidity in the bread (Table 2) (Falade et al., 2021; Yang et al., 2019).

The specific volume of bread increased when 25% whey was added compared to normal bread, but this increase was not significant (Table 2). Previous studies have shown that adding 5%, 10%, and 15% whey protein to bread resulted in a decrease in specific volume, while adding 20%, 25%, and

30% whey protein led to an increase in specific volume compared to the control group. However, these changes in specific volume were not statistically significant (Zhou et al., 2018). The presence of whey proteins in bread dough in a higher concentration than the gluten content of wheat flour can result in a better specific volume by increasing the gelation of whey proteins with heat and strengthening the expanding cells of the dough. The utilization of HHP-treated whey resulted in the lowest specific volume of bread, although this difference was not statistically significant. HHP treatment results in a reduction in the specific volume of proteins. When water molecules permeate the polypeptide core, they cause the tertiary and quaternary structures to unfold as the spaces become occupied. In this way, conformational and structural changes of proteins occur (Carullo et al., 2021).

A significant decrease was seen in the hardness and chewability properties of HHP-treated and untreated whey-added breads compared to normal breads (Table 3). In previous studies, it was reported that the hardness and chewiness of bread increased when whey was added compared to normal bread. However, some studies have reported that bread made with enhanced whey was softer than normal bread after 30 minutes of baking but tended to become harder over time. Dissolved proteins in whey can play a role by creating a homogeneous structure (Guiné et al., 2020; Hossein, 2009; Ozturk & Mert, 2018). Organic acids and calcium in whey can contribute to a softer texture by influencing the activity of protease and amylase in bread dough. In addition, the high amount of whey added to the bread dough can also contribute to the softness of the bread and increase the fermentation ratio. The elasticity of the bread with whey added and treated with HHP was significantly increased according to the other bread samples. Previous studies have indicated that the HHP treatment affects the foaming, surface hydrophobicity, solubility, gelling, and emulsifying properties of whey, particularly whey proteins (Lee et al., 2006; Lim et al., 2008b). It has been reported that HHP application provides accessibility for antibodies and increases the antigenicity of epitopes embedded in whey proteins. Conformational changes induced by HHP can impact enzyme activity, enhance enzymatic digestion efficiency, alter hydrolysis reaction kinetics, influence flavor binding, and affect overrun and foam stability (Ambrosi et al., 2016; Liu et al., 2005; Lim et al., 2008b).

Color measurements showed that high hydrostatic pressure (HHP)-treated and untreated whey-added breads had a darker but less yellowish color, although there was a decrease in the brightness of normal breads. It can be seen that the *a** value increased in both HHP-treated and untreated whey-added breads, whereas there was no significant change in the *b** values. *L** values were lower in high hydrostatic pressure (HHP)-treated and untreated whey-added breads compared to normal breads (Table 4). It has been demonstrated that bread supplemented with whey protein concentrate (WPC) had a lower *L** value of crumb compared to normal bread. In addition, it has been observed that the *L** value decreased further when the addition of WPC was increased from 5% to 10% (Zhou et al., 2018). In another study, a decrease in *L** values and an increase in *a** values were observed in the crust and crumb color parameters of bread prepared with 20% WPC-added sourdough compared to the control group (Ferreyra et al., 2021). The slightly yellowish hue of bread may be due to vitamin B2 (riboflavin) found in whey (Chudy et al., 2020). The HHP application may have caused the release of riboflavin by affecting the disintegration of micelles in whey. The reduction in micelle size can increase the yellowness of whey color. With the application of pressure in the range of 300 MPa to 676 MPa, it is possible to recombine the dispersed micelles. HHP application can affect non-covalent bonds, such as hydrogen, ionic, and hydrophobic bonds, as well as high molecular weight compounds due to their sensitivity to pressure (Chawla et al., 2011). The darker color can be explained by the active role of the amino acid lysine and lactose in whey in the Maillard reaction. The Maillard reaction is a series of sequential reactions that occur between the α and ε ends of amino acids, including lysine, and the carbonyl group in the reduced carbohydrate monomers at high temperatures (Mzoughi et al., 2024; Xiang et al., 2021). It is important for the desired brown color formation in foods such as bakery products (Wang et al., 2013). The dark color tone and lighter yellow bread tone obtained in this study are consistent with findings from other studies on bread enrichment with whey (Divya & Rao, 2010; Tsanasidou et al., 2021).

With the addition of whey to bread, the sensory scores for bread crust, symmetry, and color were significantly higher compared to normal bread (Table 5). These high scores can be attributed to the role of whey in the Maillard browning reaction. Considering the protein content of whey, it is known to be particularly rich in lysine. The amino acid lysine is highly reactive in the Maillard reaction. In addition, the high lactose content of whey positively affects the Maillard reaction (Komeroski & Oliveira, 2023; Pořízka et al., 2023). In studies on the antioxidant effects of Maillard reaction products, it has been stated that they gain antioxidant properties by scavenging peroxyl radicals in the Maillard reaction, which occurs with the cooperation of lactose and lysine (Feng et al., 2022). The application of high hydrostatic pressure to the whey enhanced the overall sensory perception of bread, with the exception of the crumb structure and color. However, there was no statistical difference in sensory analysis between normal whey-added bread and HHP-treated whey-added bread. Bread made with HHP-treated whey had the lowest score in crumb properties, but there was no significant difference compared to other bread samples.

The taste and odour of the bread improved with the addition of HHP and untreated whey, but no significant difference was observed. HHP treatment does not affect low molecular weight compounds responsible for nutritional and sensory properties, such as vitamins and aroma compounds (Tirpanci et al., 2022). In the study examining the flavor binding properties of high hydrostatic pressure (HHP) treatment (600 MPa at 50°C) on whey protein concentrate (WPC), it was reported that there were significant increases in the binding sites of WPC for heptanone and octanone (Liu et al., 2005). Hydrophobic, electrostatic, and steric properties of protein molecules affect their functionality. For example, the hydrophobic surfaces of proteins affect their emulsion capacity and stability. With the increase in the hydrophobicity of the proteins, the oil-binding capacity can increase. In this case, the flavor properties of the foods can be positively affected (Liu et al., 2005). Β-lactoglobulin (β-Lg), which constitutes over 50% of whey proteins, plays a crucial role in determining the properties of whey, including solubility, gelling, foaming, emulsification, and flavor. It has been reported that the application of high hydrostatic pressure causes openings in the protein structure. This leads to the exposure of embedded hydrophobic groups, thereby increasing the surface hydrophobicity and binding affinities of β-Lg (López-Fandiño, 2006). It has been reported that whey proteins enhance the crust quality of gluten-free breads made with rice flour and corn starch. The bread crust has a high pyrazine content, contributing to its dark color. Additionally, whey proteins increase the volatile compound content through lipid oxidation, thereby influencing the taste of the bread (Pico et al., 2019). Therefore, the concentration and ratio of whey used should be carefully selected to control the development of a rancid taste due to the increase in volatile compounds originating from lipid oxidation. In this study, only one concentration of whey was investigated, as the focus was on evaluating the specific effects of HHP-treated whey on the quality of the bread, particularly physicochemical, texture, sensory, and color. Future studies could explore a range of concentrations to assess how different levels of whey affect lipid oxidation and the associated flavor changes.

Conclusion and Recommendations

This study investigated the impact of HHP-treated whey on the physicochemical, texture, color, and sensory properties of bread. The application of HHP treatment resulted in reduced bread hardness, while increasing the ash content, acidity, weight, and volume of the bread. In addition, there was a decrease in L^* values (lightness) and an increase in a^* values (redness). Sensory evaluation showed improvements in the crust, symmetry, and overall color of the bread compared to the control. The HHP treatment also influenced the ash content and elasticity of the whey in the bread formulation. This study provides valuable insights into the conformational changes in whey proteins induced by HHP, which enhance its potential for enriching bread. HHP treatment has gained significant attention for its ability to induce desired properties by altering the structure of large molecules held together by noncovalent bonds, especially in dairy products. Given the nutritional and technological benefits of dairy products, including whey, their incorporation into bakery products is common. Therefore, further research into the application of HHP-treated whey in other bakery and food products is recommended.

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