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Original Article

Effects of Tetrasodium Pyrophosphate and Whey Protein Concentrate on Quantitative and Qualitative Characteristics of Gluten-free Breads

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ABSTRACT

Background and Objectives: Gluten-free breads are essential for individuals with celiac disease and gluten-linked disorders; however, the absence of gluten commonly results in poor texture, low loaf volume and decreased sensory quality. This study aimed to assess the synergistic effects of tetrasodium pyrophosphate and whey protein concentrate on the physicochemical, textural and sensory characteristics of gluten-free breads.

Materials and Methods: Gluten-free bread samples were produced by partially substituting base flours with whey protein concentrate at 0, 10, 15 and 20% (w/w) and incorporating tetrasodium pyrophosphate at concentrations of 0, 0.2 and 0.4% (w/w). Bread quality was assessed through measurements of specific volume, moisture content, baking weight loss, protein content, color parameters, texture profile analysis and sensory evaluation. Data were analyzed using ANOVA and Duncan's multiple range test at $p < 0.05$.

Results: Increasing whey protein concentrate levels significantly improved protein and moisture content, yellowness (b*), firmness and specific volume, while decreasing baking weight loss and lightness (l*). Sensory analysis showed that the formulation containing 0.4% tetrasodium pyrophosphate and 15% whey protein concentrate received the highest overall acceptability from the panelists.

Conclusions: WPC and TSPP synergistically improved the specific volume, protein content, and firmness of gluten-free bread while reducing baking weight loss, with the 15% WPC–0.4% TSPP formulation achieving the highest overall sensory acceptability, indicating its potential for industrial application.

Keywords: Gluten-free, Whey protein concentrate, Tetrasodium pyrophosphate, Bread fortification, Textural quality, Functional characteristics

Highlights

- Whey protein concentrate significantly increased protein content, moisture retention, firmness and specific volume of gluten-free breads.
- Tetrasodium pyrophosphate improved gas retention and decreased baking weight loss, particularly at 0.4% concentration.
- The combination of 15% whey protein concentrate and 0.4% tetrasodium pyrophosphate received the highest sensory acceptability from the panelists.
- Structural, physicochemical and sensory quality of gluten-free bread were significantly enhanced.

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Introduction

Bread is a globally consumed staple food and one of the most widely produced bakery products, considered a strategic commodity in the human diets (1). Wheat flour, commonly used in bread production, contains gluten—a protein complex responsible for the viscoelastic characteristics of dough, which plays a critical role in bread quality (2). However, a significant portion of the global population suffers from gluten-linked disorders, such as celiac disease (CD), non-celiac gluten sensitivity and irritable bowel syndrome (IBS), which are often triggered by the consumption of wheat or gluten-containing grains (3). Celiac disease is a chronic autoimmune disorder in genetically predisposed individuals, induced by gluten intake, leading to atrophy and flattening of the intestinal villi. This impairs nutrient absorption and, if untreated, may result in complications such as anemia, osteoporosis, vomiting, small bowel cancer and endocrine disorders (4). The primary treatment is strict lifelong adherence to gluten-free diets (5). With approximately 1% of the global population affected by gluten intolerance, the demand for gluten-free products has increased significantly (6).

However, gluten-free breads usually show inferior texture, decreased volume and lesser sensory appeal due to the lack of gluten, which is essential for dough structure and elasticity (7). Substituting wheat flour with gluten-free alternatives such as chickpea or corn flour often leads to further sensory issues and undesirable taste. Moreover, gluten-free doughs lack cohesion and elasticity, often resembling liquid cake batters and are difficult to handle (8). Numerous studies have tried to overcome these challenges by incorporating various functional ingredients such as pseudocereals, dairy proteins, emulsifiers, fibers and hydrocolloids to mimic the gluten network, enhance nutritional value and improve processing (7). Rice flour is within the most popular bases in gluten-free formulations due to its high digestibility, hypoallergenic characteristics and pleasant flavor (9;8). Nonetheless, gluten-free breads generally offer lesser nutritional value with decreased protein, fiber, vitamins and minerals and higher fat content, compared to traditional wheat breads (10). This nutritional gap has urged efforts to fortify gluten-free bread using nutrient-dense ingredients to enhance its quality and health benefits (11). Consumers increasingly expect breads to deliver functional benefits such as low glycemic index and enriched content of proteins, fibers, vitamins and minerals (12). Hence, current research focused on integrating such components into gluten-free formulations to bridge the nutritional gap and improve sensory and textural attributes.

Whey protein concentrate (WPC), a byproduct of cheese production, is rich in essential amino acids (EAA) and valued for its emulsifying, foaming, gelling and water-holding capacities. These characteristics make WPC a promising additive for bakery products, particularly gluten-free breads, where it can enhance crust color, texture and flavor (13; 14; 15; 16; 17). Despite its potential, WPC is still underuse in gluten-free bread research (18). Tetrasodium pyrophosphate (TSPP), a water-soluble polyphosphate, is widely used in the food industry to stabilize proteins, improve moisture retention and preserve textural quality during freezing and baking (19; 20; 21). The TSPP capacity to bind proteins and regulate pH makes it a valuable additive in processed foods. However, excessive phosphate intake may pose health risks, particularly for individuals with kidney or cardiovascular diseases and individuals may experience mild gastrointestinal irritation (22). When used at approved levels, TSPP concentrations used in this study (0.2–0.4%) were fit within the regulatory limits established for phosphate food additives and considered safe for consumption, while providing technological advantages in food processing (23). Regarding the persistent quality challenges in gluten-free bread and the nutritional shortcomings of current formulations, this study aimed to investigate the combined effects of WPC and TSPP on the physicochemical and sensory characteristics of gluten-free breads.

Materials and Methods

Materials and methods

The WPC was purchased from Pegah Azerbaijan Tabriz, Iran. The WPC was used at substitution levels of 0, 10, 15 and 20% and its physicochemical characteristics are present in Table 1. The TSPP was purchased from OEM, China, and used at concentrations of 0, 0.2 and 0.4% as outlined in Table 2. Other dry ingredients, including rice flour, chickpea flour and corn starch, were provided by Golha, Iran, and stored in sealed containers under dry conditions until further use.

Table 1. Physicochemical characteristics of whey protein concentrate used in the gluten-free bread formulation

Nu	Characteristics	Value
1	Acidity	0.075
2	Moisture (%)	1.5-4.5
3	Fat (%)	1.5
4	Scorched particle (%)	< 0.5
5	Protein (%)	13
6	Lactose (%)	67
7	Ash (%)	9.5
8	pH	5.6-6.3

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Table 2. Specifications of tetrasodium pyrophosphate used in gluten-free bread formulation

Nu	Characteristics	Value
1	$Na_2H_2P_2O_7$	97.22%
2	Water Insoluble	0.20%
3	Arsenic (As)	2ppm
4	Loss On Drying	Conforms
5	Cadmium (ppm)	0.2
6	Lead (ppm)	0.2
7	Mercury (ppm)	0.3
8	Heavy Metal (Pb)	2ppm
9	Fluoride (ppm)	Conforms
10	pH Value	4.2
11	Purity Appearance	96.5%
12		White Powder Conform

Sample preparation

Gluten-free bread samples were prepared by substituting chickpea flour, rice flour and cornstarch with WPC at levels of 0, 10, 15 and 20% and incorporating TSPP at concentrations of 0, 0.2 and 0.4%, as described by (24). All powdered ingredients were first sifted and then mixed with sugar and *Saccharomyces cerevisiae* yeasts. The mixture was transferred into a dough mixer and mixed for two min. Liquid oil was added and mixing continued for an additional 5 min until a smooth homogeneous dough was formed. Each dough sample (100 g) was weighed and transferred into eight heat-resistant polyethylene baking molds per treatment. The molds were placed in a proofing chamber at 85% relative humidity and left to rest for 15 min to improve fermentation. The samples were baked in a convection oven at 260 °C for 20 min.

Table 3. Formulation of gluten-free breads used in the study

Nu	Characteristics	Value
1	Rice Flour	25
2	Corn Starch	58.3
3	Chickpea Flour	16.7
4	Oil (Liquid)	2
5	Sugar (Sucrose)	3
6	Carboxymethyl Cellulose	1
7	Saccharomyces cerevisiae (a type of yeast)	3
8	Acidity	0.02
9	Moisture	40
10	Fat	0.7
11	Crude Fiber	0.3
12	Total Solids	60
13	pH	5.6 - 6

Volume

The volume of gluten-free bread was measured using cubic samples from the crumb, excluding the crust with a defined weight. The volume was assessed by the seed displacement method using Talaye variety canola seeds. These seeds specifications from Behshehr Oilseeds, Iran, are detailed in Table 4. Specific volume ($cm^3\ g^{-1}$) was calculated as the ratio of the measured volume to the sample weight (6). The use of Talaye canola seeds enhanced the accuracy and reproducibility of the volume measurements.

Table 4. Physical characteristics of canola seeds (Talaye variety) used in the volume displacement assay.

Particle density (Kg/m^3)	**1145.77**
Bulk Density (Kg/m^3)	698.02
Weight (g)	0.0031
Volume (mm^3)	2.667
MC (wb%)	5.4
Cultivar	Brassica napus

Protein

Protein content was assessed to investigate the effect of WPC on gluten-free breads, as TSPP did not affect protein levels. The Dumas combustion method (AOAC 992.23) was used, providing a rapid, accurate reliable assessments of nitrogen and protein contents. In this method, samples were combusted in an oxygen-rich environment using high-temperature combustion chamber at 1030 °C, resulting in the release of CO_2, H_2O and N_2 gases. The CO_2 and H_2O were removed by passage through selective absorption columns, while nitrogen gas was passed through a reduction column at 750 °C and detected using thermal conductivity detector. Nitrogen content was then converted to protein using conversion factor of 6.25.

Color

Color analysis of the bread crust and crumb was carried out one day after baking by measuring three color parameters of l*, a* and b*. The l* value indicated lightness, ranging from 0 (black) to 100 (white). The a* value represented the red-green axis, with negative values indicating green as well as positive values indicating red. The b* value corresponded to the yellow-blue axis, with negative values for blue as well as positive values for yellow. Bread samples were fully transferred into a high-resolution imaging device (300 dpi) to capture images. The images were analyzed using ImageJ software to assess the color parameters (25).

Moisture and Weight Loss

Moisture content measurement of the bread samples was assessed based on the approved AACC method no. 40-44 (2000). Weight loss was calculated by measuring

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the sample weight before baking (A) and after baking (B) using the following equation (26):

$$\text{Weight Loss}\ (\%) = \frac{A - B}{A} \times 100$$

Where, A was weight of the sample before baking and B was weight of the sample after baking.

Texture Measurement

Texture profile analysis (TPA) was carried out on 2 × 2 × 2 cm cubic samples cut from the central part of the bread using texture analyzer (TA-XT Plus, Stable Micro Systems, Surrey, UK). The parameters included hardness, maximum force required to compress the sample during the first cycle (n); cohesiveness, ratio of the area under the force–time curve during the second compression to that of the first compression; springiness, ratio of the time duration of the second compression to the first compression; resilience, ratio of the area during the release phase of the first compression to the area of the compression phase; and chewiness, calculated as the product of hardness, cohesiveness and springiness (N). The assessment was carried out using 25-mm diameter flat aluminum probe (P/25) with a double compression cycle. The interval between compressions was 30 s and the test speed was set at 2 mm s^{-1}. All assessments were carried out on the first day after baking, with two replicates for each sample (25).

Sensory Evaluation

The sensory characteristics of gluten-free bread samples, including taste, appearance, texture and overall acceptability, were assessed by a panel of 12 trained assessors one day after baking. A linear scoring method was used through 15-cm unstructured scales with endpoints labeled as "weak" and "strong" for each attribute. Scores were recorded by measuring the distance from the origin to the assessor mark on the scale and normalizing it by the total scale length (0–1) (27). Panelists were trained prior to evaluation to familiarize them with the attributes and the scoring procedure. Sensory assessments were carried out in a controlled environment under uniform lighting conditions and samples were coded and present in a randomized order. Water was provided for palate cleansing between the samples.

Statistical Analysis

The present study used a factorial experimental design to investigate the individual and interactive effects of two independent variables on the physicochemical, sensory and textural characteristics of gluten-free breads. The first factor was WPC, assessed at four levels (0, 10, 15 and 20%) used by substitution of rice flour, chickpea flour and corn starch in the formulation. The second factor was TSPP, assessed at three levels (0, 0.2 and 0.4% w/w). All experimental treatments were arranged in a completely randomized design with replicates to ensure statistical reliability. Data from various analyses were subjected to analysis of variance (ANOVA) to assess the significance of major effects and their interactions. Post-hoc mean comparisons were carried out using Duncan's multiple range test at a significance level of $p < 0.05$ to identify statistically various groups within the treatments. Statistical analyses were carried out using SAS software v.9.1 (SAS Institute, Cary, NC, USA). Results were present as mean ±SD (standard deviation) and differences were considered significant when *p*-values were less than 0.05.

Results and Discussion

Specific Volume

The specific volume of gluten-free breads was significantly affected by the addition of WPC. As the substitution levels of chickpea flour, rice flour and corn starch with WPC increased, the specific volume of the bread samples significantly increased (Figure 1). This enhancement in specific volume suggested improved gas retention and crumb structure attributed to the incorporation of WPC.

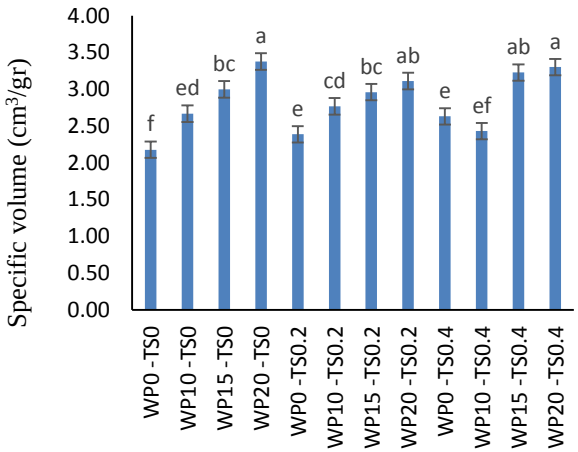


Figure 1. Effects of whey protein concentrate and tetrasodium pyrophosphate on the specific volume of gluten-free bread samples. Bars represent mean ±SD (n = 2). Different lowercase letters indicate statistically significant differences within the treatments ($p < 0.05$) based on Duncan's multiple range test.

The interaction effects of 0.2% TSPP and 10% WPC resulted in a significant improvement in the specific volume of the samples, compared to the control. The individual effect of TSPP on gluten-free bread was significant and positive, likely due to its capacity to retain and trap gases within the bread matrix. This effect is particularly important in gluten-free breads, where the absence of a proper gluten network compromises volume

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and texture development (28). However, increasing WPC levels greater than 10%, in combination with TSPP, did not result in further significant increases in specific volume. This might be attributed to interactions between TSPP and WPC, as well as associated changes in pH. These findings were similar to those investigating the effects of WPC and various flour blends on gluten-free bread quality. Specifically, (29) reported that increasing WPC levels enhanced the specific volume of gluten-free bread samples. Moreover, Sahagún and Gómez (2018) reported that various protein sources could variably affect bread volume (34).

Moisture

Moisture Data analysis revealed a significant positive correlation between WPC and the moisture content of gluten-free bread samples in the presence and absence of 0.2% TSPP. Specifically, the highest moisture content was observed in samples with 20% substitution of the major formulation flours by ultrafiltration WPC within various levels of TSPP (Figure 2). This increase was attributed to the ability of WPC components—particularly hydrophilic proteins—to bind water molecules; thereby, enhancing moisture retention after baking. In contrast, wheat flour-based breads, which typically contain nearly 27% moisture, depends further on gluten capacity to form and retain water through hydrogen bonding, a characteristic superior to that of WPC (30).

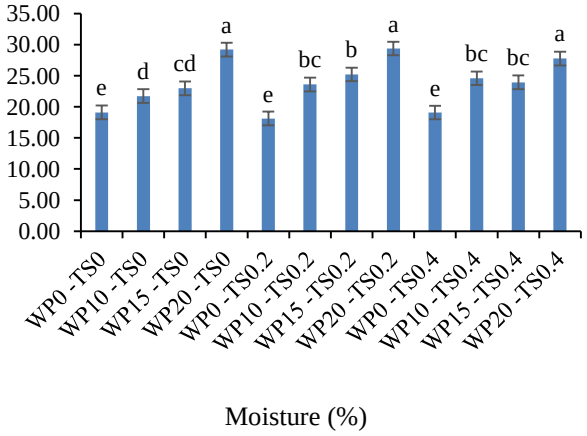


Figure 2. Effects of whey protein concentrate and tetrasodium pyrophosphate on the moisture content of gluten-free bread samples. Bars represent mean ±SD (n = 2). Different lowercase letters indicate statistically significant differences between the treatments (p < 0.05) based on Duncan's multiple range test.

Weight Loss

The assessment of individual factor effects revealed that the highest weight loss occurred in samples containing 0.2% of TSPP. In samples without TSPP, increasing the level of WPC substitution significantly decreased weight loss. However, when TSPP was included as a factor, weight loss increased at the 0.2% level but decreased at 0.4% (Figure 3). Generally, products with higher specific volumes demonstrated greater water loss during baking due to increased surface area exposed to air (31). Nevertheless, the findings of this study suggested an atypical effect of WPC on weight loss, indicating its potential to enhance moisture retention in the gluten-free bread formulation. This might be attributed to hydrophilic interactions between WPC and water molecules.

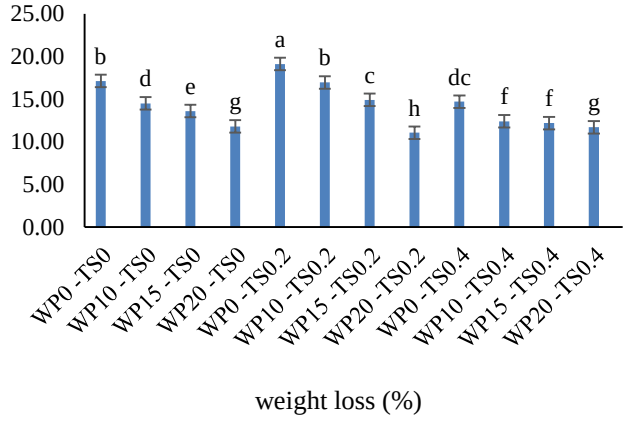


Figure 3. Effects of whey protein concentrate and tetrasodium pyrophosphate on the baking weight loss of gluten-free bread samples. Bars represent mean ±SD (n = 2). Different lowercase letters indicate statistically significant differences within the treatments (p < 0.05) based on Duncan's multiple range test.

Protein

The individual effect of WPC on the protein content of gluten-free breads demonstrated a significant increase in nutritional value with higher substitution levels. The highest protein content was observed in the sample containing 20% WPC replacement of the major flour ingredients. Within the base flours, rice flour included the lowest protein content, whereas chickpea flour contained more protein than WPC. These findings were similar to those reporting that incorporation of WPC significantly increased protein content in breads with increases exceeding 22%, compared to control samples (30). Electrophoresis gel analyses revealed that β-lactoglobulin, α-lactalbumin, bovine serum albumin (BSA) and immunoglobulin were the predominant proteins in WPC, comprising approximately 50, 20, 10 and 10% of total protein content (TPC), respectively (32). The type and structure of proteins used in gluten-free bread formulation play a critical role not only in enhancing nutritional value but also in improving bread structure, especially due to the absence of gluten networks. Functional characteristics of WPC—including high nutritional value, gel formation, thermal stability and foaming and emulsifying capacities—are essential contributors to these improvements (15).

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Color

Analysis of the crust color of gluten-free breads revealed that samples with 20% WPC substitution included the greatest decrease in lightness (l*), compared to other treatments. In contrast, samples without WPC showed the highest brightness. This decrease in lightness might be attributed to the high lactose content in WPC, similar to previous studies investigating the supplementation of gluten-free breads with non-gluten proteins and their effects on dough rheology and bread characteristics (33). Regarding the redness parameter (a*), a significant positive correlation was observed between increasing levels of WPC and the a* value (Table 6). The WPC, rich in lactose and proteins, participates in Maillard reactions during baking, contributing to darker crust coloration. Similar findings were reported by (13), who reported that samples containing dairy powders showed darker crusts than others. For browning, an important attribute of baked product color, the sample containing 20% WPC and 0.4% TSPP showed a higher browning index, compared to the control.

Texture Assessment

The results of the internal texture analysis of gluten-free breads indicated that TSPP, as a strengthening agent, significantly increased the firmness of samples without WPC. When the two factors were overall combined, increasing substitution levels resulted in a progressive increase in sample firmness, compared to the control. The highest firmness was observed in the treatment containing 0.4% TSPP and 15% WPC. These findings were similar to those reporting that increased protein content led to greater firmness and negative correlation between hardness and bread volume (30). Regarding chewiness and springiness, the two factors contributed to increased values, compared to the control samples. Additionally, cohesiveness—an important attribute affecting staling—was enhanced by WPC alone in samples without TSPP. While the effect of 0.2% TSPP on cohesiveness was not significant, increasing its level to 0.4% resulted in a significant improvement, compared to the control. The highest cohesiveness was recorded in the treatment combining 0.4% TSPP and 20% WPC, indicating improved textural characteristics (Table 7). This enhancement was particularly critical in gluten-free breads due to the absence of a gluten network. Similar studies on WPC incorporation up to 20% reported increased firmness and decreased elasticity with higher protein levels (30).

Table 5. Effects of whey protein concentrate substitution on the protein content of gluten-free bread samples.

Sample	Whey protein concentrate (% 0) - control	Whey protein concentrate (%10) - control	Whey protein concentrate (%15) - control	Whey protein concentrate (% 20) - control
Result	4.87 ± 0.28^{d}	5.59 ± 0.18^{c}	5.88 ± 0.35^{b}	6.55 ± 0.58^{a}

Different lowercase superscript letters indicate statistically significant differences between the treatments ($p < 0.05$) based on Duncan's multiple range test. Protein content was assessed using Dumas combustion method. Values are means of two replicates.

Table 6. Effects of whey protein concentrate and tetrasodium pyrophosphate on crust color parameters of gluten-free bread samples.

WP (%)	TS (%)	Specifications			
		l*	a*	b*	BI
0%	Negative	62.31^{a}	14.78^{b}	38.21^{l}	103.94^{g}
10%		53.81^{bc}	18.21^{a}	40.54^{i}	$142.68g^{f}$
15%		48.94^{d}	16.65^{b}	41.03^{h}	170.93^{ef}
20%		46.83^{de}	19.66^{b}	52.71^{c}	440.38^{b}
0%	0.2 %	60.44^{a}	13.24^{b}	39.2^{j}	104.04^{g}
10%		54.56^{b}	16.31^{b}	44.5^{f}	161.28^{f}
15%		47.11^{de}	18.24^{b}	46.15^{e}	223.38^{d}
20%		43.01^{e}	19.25^{b}	53.16^{b}	391.5^{c}
0%	0.4 %	60.72^{a}	14.12^{b}	38.65^{k}	109.82^{g}
10%		54.98^{b}	16.41^{b}	43.43^{g}	152.55^{f}
15%		49.28^{cd}	17.92^{b}	46.8^{d}	210.76^{de}
20%		45.25^{de}	23.85^{ab}	53.6^{a}	502.23^{a}

l*, a* and b* values represent color parameters measured from crust surface using image analysis. Different lowercase superscript letters within columns indicate statistically significant differences ($p < 0.05$) based on Duncan's test. BI, Browning index calculated as per standard methodology.

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Table 7. Effects of whey protein concentrate and tetrasodium pyrophosphate on textural characteristics of gluten-free bread samples.

WP (%)	TS %)(	Specifications				
		Hardness (N)	Chewiness (N)	Springiness (mm)	Cohesiveness	Resilience
0%	Negative	5.79^{d}	2.57^{h}	0.95b	0.53^{f}	0.74^{dfe}
10%		6.65^{c}	3.07^{f}	0.932e	0.65^{c}	0.76^{cde}
15%		7.21^{b}	3.26^{e}	0.937d	0.65^{c}	0.8^{a}
20%		7.19^{b}	3.92^{a}	0.83i	0.72^{a}	0.79^{acb}
0%	0.2 %	5.85^{cd}	2.72^{gh}	0.99a	0.53^{f}	0.72^{f}
10%		6.58^{c}	3.33^{d}	0.94c	0.57^{e}	0.74^{dfe}
15%		6.64^{c}	3.30^{e}	0.95b	0.59^{d}	0.73^{ef}
20%		6.72^{c}	3.63^{b}	0.93d	0.66^{c}	0.8^{ab}
0%	0.4 %	6.79^{c}	2.78^{g}	0.92f	0.59^{de}	0.73^{de}
10%		7.64^{a}	3.06^{f}	0.92f	0.65^{c}	0.77^{c}
15%		7.85^{a}	3.47^{cd}	0.92f	0.69^{b}	0.78^{b}
20%		7.72^{a}	3.68^{b}	0.90h	0.73^{a}	0.8^{a}

Values are expressed as mean ±SD (n = 2). Different lowercase superscript letters within columns indicate statistically significant differences (p < 0.05) based on Duncan's multiple range test. Textural attributes were assessed using texture profile analysis with a double compression test.

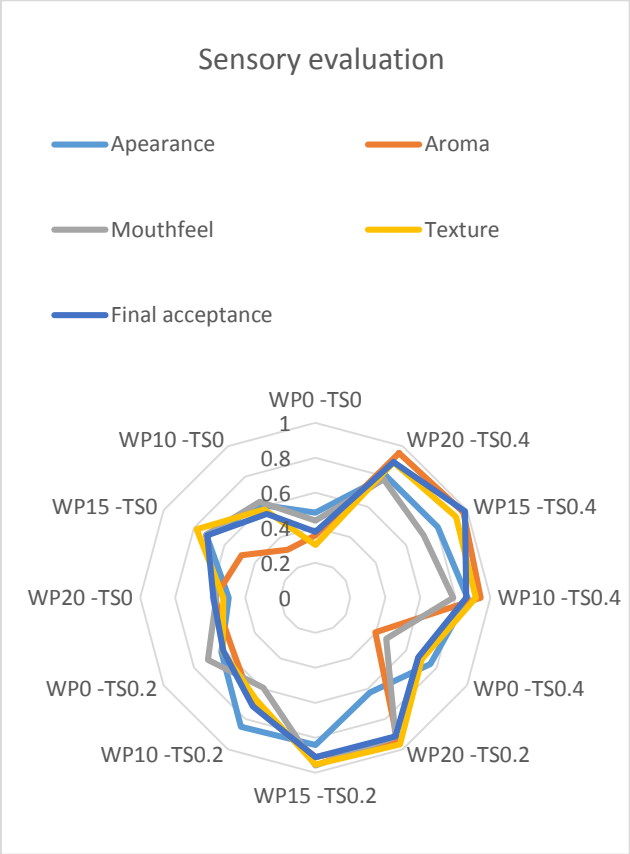


Figure 4. Effects of whey protein concentrate and tetrasodium pyrophosphate on the sensory attributes of gluten-free bread samples. Sensory attributes, including appearance, aroma, texture, mouthfeel and overall acceptability, were assessed using normalized scale ranging from 0 (lowest acceptability) to 1 (highest acceptability). Values represent mean sensory scores (n = 12). Different lowercase letters within the same sensory attribute indicate statistically significant differences (p < 0.05) based on Duncan's multiple range test.

Sensory Evaluation

Appearance is a critical factor affecting the perceived quality, taste, shelf life and nutritional characteristics of food products. Sensory evaluation of the gluten-free bread samples was particularly important due to the absence of wheat flour in the formulation. After informing the panelists that the samples were gluten-free breads, results revealed a positive effect of WPC on the aroma and mouthfeel. Panelists reported that as the substitution level of WPC increased, the samples further closely resembled conventional breads. Regarding texture, samples containing TSPP were preferred over the control, with the highest overall acceptability score assigned to the sample containing 0.4% TSPP and 15% WPC (Figure 4). These sensory improvements were similar to those investigating the effects of whey protein and mixed flour blends on gluten-free bread quality parameters (29).

Limitation

A limitation of this study was that several instrumental measurements were carried out in duplicate (n = 2), which might decrease statistical power. Nevertheless, consistent trends were observed within the treatments and statistically significant differences were detected using ANOVA followed by Duncan's multiple range test. Further studies are recommended to increase replication (e.g., $n \geq 3$) to validate the findings.

Conclusion

This study demonstrated that WPC significantly enhanced the gas retention capacity of gluten-free bread formulations primarily due to its major proteins of β-lactoglobulin and α-lactalbumin, leading to increased protein content and specific volume. The incorporation of TSPP further contributed to volume improvement, particularly at a 0.4% level, where it effectively decreased

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weight loss, compared to the control. Moreover, WPC positively affected the color attributes of the bread by decreasing the lightness (l*) and increasing the yellowness (b*), while textural analysis revealed increased firmness and cohesiveness and decreased elasticity. Sensory evaluation verified that the combination of 0.4% TSPP and 15% WPC could be recommended as the optimal formulation, receiving the highest overall acceptability from the panelists.

Although WPC contains residual lactose, the estimated lactose content in the recommended formulation is expected to be low due to the moderate level of WPC. Therefore, this formulation may be appropriate for individuals with mild lactose sensitivity but not be appropriate for those with severe lactose intolerance. Overall, these findings suggest that the synergistic use of WPC and TSPP can effectively improve the quality and consumer acceptance of gluten-free breads, addressing key challenges associated with gluten substitution. From an industrial perspective, the suggested formulation may be used to improve the quality of commercial gluten-free breads. Further studies can investigate shelf-life stability and *in-vivo* digestibility of the optimized formulations.

Ethics declaration

This study involved sensory evaluation with human panelists only; no animal testing was carried out. All participants received informed consents and their safety and confidentiality were ensured. The research focused on physicochemical, nutritional and sensory analyses of gluten-free breads made from a composite of rice, chickpea and corn starch flours with WPC.

Financial disclosure

The authors declared no financial interest.

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