



Physicochemical and sensory properties of biscuits fortified with whey protein isolate and wheat bran

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ABSTRACT

Overweight and obesity are major public health concerns that necessitate innovative nutritional strategies. This study aimed to investigate the effects of fortifying biscuits with wheat bran (WB) and whey protein isolate (WPI) on their physicochemical and sensory characteristics. Four formulations were developed: a control, biscuits fortified with WB, biscuits fortified with WPI, and biscuits fortified with both. In all formulations, 40% of sucrose was replaced with date syrup. Physicochemical (moisture, ash), nutritional (sugar, fat, protein, fiber), color, texture, and sensory attributes were evaluated using standard analytical methods. Statistical analysis was performed by Tukey's post-hoc test at a significance level of $P < 0.05$. Fortification with WB and WPI significantly increased moisture, ash, protein, and fiber contents, while fat and total sugar levels remained unchanged. Biscuits containing WPI received the highest color scores, whereas the control and WB samples exhibited more desirable texture. No significant differences were observed in taste or overall acceptability among formulations. Fortifying biscuits with WB and WPI improves nutritional quality without compromising sensory acceptability, offering a feasible strategy for developing healthier snack options.

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1. Introduction

Overweight and obesity are among the leading global public health issues, imposing substantial economic and healthcare burdens worldwide (1).

Contemporary dietary strategies for weight management have shifted from mere caloric restriction toward improving diet quality and selecting foods that facilitate appetite regulation and balanced energy intake (2).

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Increasing the consumption of protein- and fiber-rich foods has been shown to promote satiety, decrease hunger, and support long-term weight control (3,4). Among dietary fibers, wheat bran, a key by-product of wheat milling, is recognized for its functional and nutritional attributes. It consists mainly of non-starch polysaccharides such as arabinoxylans, cellulose, and β -glucan (8), conferring high water-holding capacity and desirable textural properties (9). Additionally, wheat bran provides essential minerals (e.g., iron, zinc, magnesium) and vitamins (B-complex and E), along with bioactive compounds such as alkylresorcinols and ferulic acid that contribute to its antioxidant and health-promoting effects (10,11). Recent evidence highlights its beneficial role in improving metabolic parameters, moderating glycemia and lipidemia, and promoting gastrointestinal well-being (12,13). Similarly, whey protein, valued for its high biological quality and complete amino acid profile, has demonstrated significant metabolic and physiological benefits. It enhances satiety by modulating gastrointestinal hormones including GLP-1, ghrelin, and leptin (14-17), and studies after 2020 have confirmed its potential to improve metabolic profiles and cardiovascular health (20,21). Consequently, incorporation of whey protein into cereal-based products has attracted growing attention due to its favorable nutritional and sensory effects (18,19). Nevertheless, previous research indicates that excessive supplementation of wheat bran or protein ingredients may unfavorably affect sensory or technological properties of baked products (22,23). Optimal formulation adjustments, however, can balance nutritional enhancement with consumer

acceptability (24-26). Considering the rising prevalence of obesity, the growing consumer demand for nutritionally enhanced snack products, and the technological feasibility of incorporating fiber and protein fortification, this study aimed to formulate and evaluate biscuits enriched with wheat bran and whey protein. The physicochemical, sensory, and nutritional properties of the fortified biscuits were comparatively assessed to develop a standardized, high-quality formulation that offers consumers a healthier and functionally improved snack option.

2. Materials and Methods

2.1. Ingredients and Chemicals

Wheat flour with an extraction rate of 85% (Khosheh Co., Iran), whey protein isolate with 94% purity (Karen Pharmaceutical and Nutritional Supplements Co., Iran), wheat bran, refined white sugar, date syrup, trans-fat-free margarine, potable water, fresh eggs, sodium chloride, sodium bicarbonate, sodium pyrophosphate, and lecithin were used as the primary ingredients for biscuit preparation. Additional food-grade cereal ingredients were obtained from reputable retail suppliers in Shiraz, Iran. All analytical-grade chemicals required for physicochemical analyses were procured from Merck (Germany).

2.2. Biscuit Sample Preparation

In this study, four distinct biscuit formulations were prepared:

1. Biscuits fortified with whey protein isolate and wheat bran.
2. Biscuits fortified with whey protein isolate.
3. Biscuits fortified with wheat bran.
4. Control biscuits without fortification.

In all formulations, 40% of the sucrose was replaced with date syrup. This substitution level was selected based on preliminary sensory tests conducted with 15 trained panelists, which suggested that the 40% replacement provided the most acceptable overall sensory quality. The choice of this ratio was further supported by prior findings on date syrup use in bakery products (28). In addition, trans-fat-free margarine was substituted for traditional bakery fats in the preparation of all biscuit samples.

2.3. Dough Preparation and Baking Process

All ingredients, except flavoring and water, were weighed according to the predetermined formulation and transferred to a dough mixer. After initial mixing, food grade coconut flavoring and potable water were gradually added to the mixture until a homogeneous and cohesive dough was formed. The flavoring concentration was kept constant across all formulations. The prepared dough was rested at ambient temperature for 20 min, then molded into uniform shapes using a biscuit forming machine. Baking was carried out in an electric oven at 180 °C for 20 min. Following baking, the biscuits were cooled to 25 °C, packaged, and stored under dry, cool conditions (< 25 °C) until analysis.

2.4. Physicochemical Properties of Biscuits

2.4.1. Physical analyses

Moisture content of the biscuits was determined according to the AACC Official Method (2000). Water activity (*a_w*) was measured at 25 °C using a Lab Master-*a_w* water activity meter (Switzerland) (29).

2.4.2. Chemical analyses

Total sugar, fat, protein, fiber, and ash contents of the biscuit samples were determined following the AACC standard protocols (2000) (29).

2.4.3. Texture analysis

Textural properties of the biscuits were evaluated using a TA-XT2 texture analyzer (Germany) employing the three-point bending test (30). Biscuit samples with uniform dimensions were subjected to fracture under a loading force of 5 g-force, with the probe speed set at 5 mm s⁻¹. Young's modulus (*E*) was calculated using Equation 1:

$$E = \frac{a^3}{4bh^3} \times \frac{F}{d}$$

Where:

E: Young's modulus (MPa), *F*: applied force (N), *a*: distance between the two supports (m), *d*: deflection prior to fracture (m), *b*: biscuit thickness (m), *h*: biscuit width (m)

2.4.4. Sensory evaluation

To comprehensively evaluate the sensory attributes of the biscuit samples, a standardized questionnaire was developed. Sensory characteristics, including color, texture, flavor, and overall acceptability, were assessed. All biscuit samples were coded with unique identifiers and presented to a panel of 15 trained evaluators. A ranking test was employed to compare color, texture, and flavor among the four biscuit formulations. Overall acceptability was evaluated using a five point hedonic scale ranging from "very poor" to "very good" (25).



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2.4.5. Color evaluation

For precise measurement of surface color characteristics, each biscuit sample was placed in a chamber with controlled lighting conditions and photographed. The images were analyzed using Adobe Photoshop software, and the color parameters L, a*, and b* were calculated based on the Lab mode technique. The L value represents lightness/darkness, a* indicates the green-to-red range, and b* corresponds to the blue to yellow range. These parameters were measured within a fixed, predefined area of the biscuit surface (31).

2.5. Statistical analysis

Statistical analysis of the collected data was performed using SPSS software (version 22) within the framework of a completely randomized design (CRD). Mean values were compared using Tukey's post-hoc test at a 95% confidence level ($P < 0.05$).

3. Results

3.1. Biscuit production and formulation

In this study, four biscuit formulations were developed to investigate the influence of specific food additives on the nutritional value and quality attributes of the final product. The formulations were as follows:

1. Biscuit fortified with WPI (1.24%) and WB (10.94%),
2. Biscuit fortified with WPI (1.24%),
3. Biscuit fortified with WB (10.94%),
4. Plain biscuit (control).

All samples were prepared under identical processing conditions using the same raw materials, with partial substitution of wheat flour by WPI and/or WB in the fortified formulations. This experimental design allowed for direct comparison of the individual and

combined effects of these ingredients on the physicochemical, textural, and sensory properties of the biscuits. The subsequent sections present the results of these evaluations in a comparative framework.

3.2. Moisture Content and Water Activity

As shown in Table 1, biscuits fortified with both WPI and WB exhibited significantly higher moisture content compared with the other formulations ($P < 0.05$). Biscuits containing WPI also showed significantly higher water activity (aw) values than the WB-only and control samples ($P < 0.05$).

3.3. Ash, Fat, Protein, Sugar, and Fiber Content

According to Table 1, the ash content of biscuits fortified with both WPI and WB was significantly higher than that of all other groups ($P < 0.05$). No significant differences were observed among the formulations in terms of total sugar or fat content ($P > 0.05$). Protein content was significantly higher in WPI + WB and WPI-only biscuits compared with WB-only and control samples ($P < 0.05$). In contrast, fiber content was significantly higher in WPI + WB and WB-only biscuits than in the control and WPI-only formulations ($P < 0.05$).

3.4. Texture Analysis

The results of texture profile analysis are presented in Table 2. No statistically significant differences were observed among the four biscuit formulations in terms of Young's modulus values ($P = 0.26$).

Table 1. Physicochemical characteristics and nutritional composition of different biscuit formulations.

Parameter	Biscuit fortified with WPI + WB	Biscuit fortified with WPI	Biscuit fortified with WB	Control biscuit	P-value*
Ash (%)	1.79 ± 0.02 ^a	1.35 ± 0.03 ^b	1.17 ± 0.14 ^b	0.86 ± 0.01 ^c	<0.05
Moisture (%)	7.60 ± 0.22 ^a	5.87 ± 0.007 ^b	3.75 ± 0.74 ^c	4.02 ± 0.10 ^c	<0.05
Water activity (aw)	0.54 ± 0.009 ^a	0.42 ± 0.001 ^b	0.22 ± 0.0007 ^c	0.19 ± 0.002 ^d	<0.05
Total sugar (g)	9.87 ± 0.42	10.13 ± 1.41	10.80 ± 0.96	11.07 ± 0.39	>0.05
Reducing sugar (g)	2.78 ± 0.17 ^a	2.37 ± 0.18 ^a	3.75 ± 0.13 ^b	2.89 ± 0.06 ^a	<0.05
Fat (%)	15.00 ± 0.70	13.50 ± 1.21	15.50 ± 1.76	15.00 ± 1.07	>0.05
Protein (%)	29.92 ± 0.80 ^a	29.60 ± 0.63 ^a	8.56 ± 0.57 ^b	8.24 ± 0.42 ^b	<0.05
Fiber (%)	1.28 ± 0.14 ^a	0.15 ± 0.05 ^b	1.97 ± 0.07 ^c	0.17 ± 0.02 ^b	<0.05

Values are expressed as mean ± standard deviation; *P-values are derived from one-way ANOVA; different lowercase letters within the same row indicate significant differences between groups according to Tukey's post-hoc test.

Table 2. Comparison of texture analysis in different biscuit samples

Sample	Young's modulus (kg/m ²)	P-value*
Biscuit fortified with WPI and WB	7006.43 ± 2480.67	>0.05
Biscuit fortified with WPI	6123.10 ± 369.93	
Biscuit fortified with WB	6245.47 ± 1610.45	
Control biscuit	2359.11 ± 160.15	

*P-value obtained from one-way ANOVA.

Table 3. Comparison of sensory characteristics of different biscuit samples

Sensory attribute	WPI+WB fortified biscuit	WPI fortified biscuit	WB fortified biscuit	Control biscuit
Color‡	76	91	74	81
Texture‡	67 ^a	73 ^{ab}	92 ^b	89 ^{ab}
Taste and flavor‡	79	76	92	86
Overall product acceptability†	4.20±0.76 ^{ab}	4.08±0.81 ^a	4.60±0.50 ^b	4.48±0.58 ^{ab}

† Values are reported as mean ± standard deviation.

‡ Values are reported as rankings.

* A rank difference equal to or greater than 24 indicates a statistically significant difference between the tested groups.

** P-value (P = 0.02) obtained from one-way ANOVA. Different lowercase superscript letters in each row indicate statistically significant differences between groups (Tukey's post-hoc test).

Table 4. Color comparison of different biscuit samples

Parameter	Biscuit fortified with WPI and WB	Biscuit fortified with WPI	Biscuit fortified with WB	Control biscuit	P-value*
L* (lightness/darkness)	41.1±0.52 ^a	41.1±0.54 ^a	63.5±0.70 ^b	71.5±0.70 ^c	<0.05
a* (greenness to redness)	19.0±1.41 ^a	24.5±2.12 ^a	7.5±0.70 ^b	5.5±0.70 ^b	<0.05
b* (blueness to yellowness)	47.5±0.70 ^a	53.0±0.00 ^b	40.5±0.70 ^c	46.0±1.41 ^a	<0.05

*P-value from one-way ANOVA. Different lowercase superscripts in the same row indicate statistically significant differences between groups according to Tukey's post hoc test.

3.5. Sensory Evaluation

The sensory evaluation results for the four biscuit formulations are presented in Table 3. Biscuits fortified

with whey protein (WP) received the highest color score. In terms of texture, biscuits fortified with wheat bran (WB) and the control were rated more favorably than the two WP-containing formulations. No



significant differences in taste and flavor were detected among the four samples. Overall acceptability scores for the three fortified formulations (WP + WB, WP only, and WB only) did not differ significantly from those of the control biscuits.

3.6. Color Evaluation of Biscuits

Color parameters of biscuit samples are presented in Table 4. Significant differences were observed among the formulations for L^* , a^* , and b^* values ($P < 0.001$). The lowest L^* values were recorded for biscuits fortified with WPI and WB, whereas the control biscuits showed the highest lightness. Biscuits containing WPI, particularly in combination with WB, exhibited significantly higher a^* values compared with the WB-only and control samples ($P < 0.05$). The highest b^* values were observed in WPI-fortified biscuits, while WB-fortified biscuits showed the lowest b^* values ($P < 0.05$).

4. Discussion

4.1. Effect of WPI and WB on Moisture Content and Water Activity

The increased moisture content and water activity observed in WPI and WB fortified biscuits can be attributed to the strong water binding capacity of whey proteins and the hydrophilic nature of wheat bran fibers (32). Whey proteins interact with the dough matrix through hydrogen bonding and protein water interactions, while the hydroxyl groups present in wheat bran polysaccharides promote water adsorption. Although higher moisture levels are generally associated with reduced shelf life, previous studies have reported no significant microbial instability following whey protein incorporation in biscuit formulations (12,14).

4.2. Nutritional Enhancement through WPI and WB Fortification

The elevated ash, protein, and fiber contents in fortified biscuits reflect the nutritional contribution of WPI and WB. Whey protein isolate is a rich source of high-quality protein (11), whereas wheat bran contributes dietary fiber and essential minerals (2, 11, 13, 18). The absence of significant changes in fat and total sugar contents indicates that fortification enhanced nutritional quality without increasing caloric density. Similar improvements in the nutritional profile of bakery products fortified with whey protein and wheat bran have been reported in previous studies (2, 11, 13, 18). The lack of significant changes in fat content observed in the present study contrasts with some earlier reports that documented increased fat levels following the incorporation of whey protein concentrate (12, 24). This discrepancy can be explained by the use of whey protein isolate in the current formulation, which is characterized by higher protein purity and negligible lipid content compared with whey protein concentrate.

4.3. Texture Properties of Fortified Biscuits

Biscuit texture is known to be influenced by multiple formulation and processing factors, including ingredient composition, dough preparation, and baking conditions such as time and temperature (3). With respect to wheat bran incorporation, earlier studies have demonstrated that low to moderate substitution levels do not negatively affect textural properties, whereas excessive substitution rates (above 30%) may result in increased hardness (2, 7, 13, 18). However, it has also been reported that acceptable textural characteristics can be maintained even at

higher wheat bran inclusion levels when formulation parameters are properly optimized (13). Overall, the present findings confirm that the combined use of WPI and WB allows for nutritional enhancement of biscuits without inducing undesirable changes in texture.

4.4. Sensory Acceptability of WPI- and WB-Fortified Biscuits

The sensory results indicate that fortification with whey protein isolate (WPI) and wheat bran (WB) did not compromise the overall acceptability of the biscuits, despite some variations in individual attributes.

Color. The highest color score was observed for WPI-fortified biscuits, which aligns with previous reports that whey protein can enhance the visual appearance of baked products through intensified Maillard browning and increased surface redness (a^*) (15). This improvement in color perception may contribute positively to consumer preference, as visual appeal is a key driver of initial acceptance.

Texture. Texture scores were more favorable for WB-fortified and control biscuits compared to the WPI-containing formulations. This finding is consistent with earlier studies noting that whey protein incorporation can reduce crispness and increase firmness, potentially due to its high water-binding capacity and the formation of a more cohesive protein network that limits air-cell expansion during baking (15). However, the absence of significant differences in overall acceptability suggests that the slight textural alteration induced by WPI was not severe enough to deter consumers. Previous work has similarly reported that, although specific texture attributes may decline with whey protein enrichment, overall product acceptability remains unaffected (9, 20).

Taste and flavor. No significant differences in taste and flavor were detected among the four formulations. This indicates that the addition of WPI and WB at the studied levels did not introduce off-flavors or alter the basic taste profile of the biscuits. The masking effect of date syrup (used as a partial sucrose replacer) and the mild flavor profile of both ingredients likely contributed to this result.

Overall acceptability. The overall acceptability scores of all fortified samples were statistically comparable to those of the control. This demonstrates that the nutritional improvements achieved through WPI and WB fortification (increased protein and fiber contents) can be realized without sacrificing sensory appeal. Comparable findings have been documented for WB inclusion levels of 10% and 20%, which showed no adverse impact on sensory quality (24). Moreover, one investigation reported satisfactory acceptability even at a 30% WB substitution level (29), reinforcing the notion that moderate WB addition is well-tolerated in biscuit matrices.

Practical implications. The sensory outcomes support the feasibility of producing nutritionally enriched biscuits that meet consumer expectations. The slight textural trade-off associated with WPI can be mitigated by optimizing formulation parameters (e.g., hydration balance, leavening agents) and processing conditions. Meanwhile, the neutral flavor and favorable texture contribution of WB make it an ideal ingredient for boosting dietary fiber without compromising sensory attributes. In summary, the sensory evaluation confirms that biscuits fortified with WPI and WB at the studied inclusion levels maintain acceptable color, texture, taste, and overall acceptability. The results are



in agreement with prior literature and validate the practical suitability of these fortificants for developing nutritious, sensorially appealing baked snacks.

4.5. Color Changes Induced by WPI and WB Incorporation

The differences observed in the color parameters of the biscuit formulations can be primarily attributed to compositional changes and the occurrence of non-enzymatic browning reactions during baking. The reduction in lightness (L^*) in biscuits fortified with whey protein isolate (WPI) and wheat bran (WB) indicates the formation of darker surfaces, which is commonly associated with intensified Maillard reactions between reducing sugars and amino-containing compounds under baking conditions. Similar reductions in lightness following the incorporation of protein and bran into bakery products have been reported previously (12, 16, 18, 25). The lower L^* values observed in WB-fortified biscuits compared with the control can also be explained by the intrinsic color of bran particles and their influence on heat-induced browning reactions. Wheat bran contains phenolic compounds and dietary fiber that can promote darker coloration in baked products by interacting with proteins and sugars during thermal processing (2, 11, 25). Regarding the a^* coordinate, the increased redness observed particularly in WPI-containing biscuits, and more prominently in the combined WPI + WB formulation, may be attributed to enhanced non-enzymatic browning intensity and the higher availability of free amino acids and reducing sugars derived from whey proteins (11, 18). In addition, phenolic compounds naturally present in wheat bran may contribute to the development of red-brown

pigments, further intensifying the redness of the final product (2, 3, 11). In contrast, bran alone exerted a less pronounced effect on redness compared with protein-containing formulations. Significant variations were also observed in the b^* coordinate (yellowness). Biscuits fortified with WPI exhibited higher b^* values, which may be explained by the thermal degradation of carotenoid pigments and the formation of yellow-brown Maillard-derived chromophores in protein-rich systems during baking (12, 18). Conversely, the lower b^* values observed in WB-fortified biscuits may be related to the higher fiber content and phenolic compounds in bran, which can attenuate yellowness intensity and mask bright color tones in baked goods (25). Overall, enrichment of biscuits with WPI and WB resulted in darker products with enhanced red and yellow hues. Although such color modifications may influence visual perception, the sensory evaluation results demonstrated that these changes did not negatively affect overall consumer acceptability. Therefore, careful selection of protein and bran type, along with controlled inclusion levels, can successfully balance nutritional enhancement with the preservation of desirable visual quality in fortified biscuit formulations.

5. Conclusion

Enrichment of biscuits with WPI and WB effectively enhanced their nutritional value without adversely affecting texture or overall sensory acceptability. The combined inclusion of WPI and WB resulted in a nutrient-dense snack alternative to conventional biscuits, with the added advantage of a potentially

lower glycemic index compared to the control formulation. These findings demonstrate the feasibility of developing healthier bakery products through the strategic incorporation of protein- and fiber-rich ingredients while maintaining consumer-acceptable quality attributes. One limitation of the study is that only one level of WPI and WB enrichment was investigated, so it may not fully reflect the range of possible effects on product quality. Future research could investigate different levels of enrichment.

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Authorship contribution

Zahra Hassanzadeh Rostami: Conceptualization, Methodology, Investigation, Data curation, Writing original draft, Visualization.

Shiva Faghih: Conceptualization, Methodology, Writing review & editing, Supervision.

Maryam Safarirad: Investigation, Formal analysis, Data curation.

Azam Abbasi: Conceptualization, Methodology, Supervision, Writing review & editing, Project administration, Funding acquisition.

Conflict of interest

The authors declare no competing financial interests or personal relationships that could have influenced the work reported in this article.

Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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