

Article

Valorization of Whey as a Natural Functional Ingredient in Gluten-Free Rice Biscuits: Formulation, Optimization, and Chemical Profiling

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Abstract

The present study investigates the effect of whey powder incorporation on the nutritional composition, structural characteristics, and functional properties of rice flour-based gluten-free systems. Composite flours and biscuits were formulated by substituting rice flour with 5%, 10%, and 15% whey powder. Proximate composition, mineral profile, and structural modifications were evaluated using standard analytical methods, complemented by Fourier Transform Infrared Spectroscopy (FTIR) and Small-Angle X-ray Scattering (SAXS). The results showed that whey addition significantly improved the protein content of both flours and biscuits, increasing from 8.45% in the control to 15.06% at the highest enrichment level. Whey powder showed elevated phosphorus (912 mg/kg), sodium (434.65 mg/kg), and calcium (526.49 mg/kg) contents compared to rice flour. Consequently, mineral levels increased progressively in the composite flours, with phosphorus rising from 528 mg/kg to 647 mg/kg, sodium from 105.66 mg/kg to 132.81 mg/kg, and calcium from 102.15 mg/kg to 137.33 mg/kg as the whey incorporation level increased. Iron content showed minor variations among the gluten-free biscuit formulations (76.01–95.16 mg/kg). Whey incorporation led to a progressive increase in copper content, from 8.91 mg/kg in the control biscuits to 15.50 mg/kg, while zinc levels decreased from 27.47 mg/kg to 18.47 mg/kg with increasing whey addition. FTIR analysis revealed clear structural changes associated with whey addition, including the progressive intensification of amide I and II bands and a reduction in starch-specific signals, confirming the incorporation of whey proteins into the starch matrix and the formation of protein–starch interactions. These findings were supported by SAXS analysis, which indicated modifications in the internal structural organization of the systems. Sensory evaluation indicated good overall acceptability of the fortified biscuits at moderate whey incorporation levels, while higher whey addition slightly reduced taste scores due to the characteristic salty flavor associated with acid whey. Overall, the study demonstrates that whey powder is an effective functional ingredient



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for enhancing the nutritional and structural properties of gluten-free products. However, achieving an optimal balance between improved nutritional quality, technological performance, and mineral composition remains essential for the development of high-quality gluten-free formulations.

Keywords: gluten free; proximate composition; mineral profile; Fourier Transform Infrared Spectroscopy (FTIR); Small-Angle X-ray Scattering (SAXS)

1. Introduction

Bakery products are among the most widely consumed staple foods worldwide due to their accessibility, convenience, and sensory acceptability. The bakery sector continues to expand globally, driven by growing consumer demand for functional, convenient, and gluten-free products. As bread and bakery products contribute substantially to daily energy and carbohydrate intake in many populations, considerable scientific interest has focused on improving their nutritional quality through reformulation strategies and the incorporation of functional ingredients [1,2]. According to recent market analyses, the global bakery products market was valued at approximately USD 497 billion in 2023 and is projected to continue growing in the coming years. Consequently, significant scientific attention has been directed toward the development of bakery products with improved nutritional profiles and enhanced functional properties [2,3].

In the last two decades, the demand for gluten-free bakery products has experienced a significant increase, fueled both by the increasingly frequent diagnosis of celiac disease and non-celiac gluten sensitivity, and by dietary trends adopted by the general population [4]. However, the elimination of gluten from traditional recipes involves major technological challenges, since gluten is responsible for the three-dimensional structure, gas retention and characteristic texture of bread and other bakery products. In its absence, the formulation must be based on a combination of gluten-free raw materials and structural agents that reproduce the functional properties of gluten [5].

Rice flour is the classic “carrier” in gluten-free baking due to its neutral taste, light color, affordable cost and predictable behavior during starch gelatinization. Its limitations, mainly to refined rice flour, are the low protein and fiber content, the amino acid profile weaker than wheat (especially in essential amino acids), and high glycemic index values, which could negatively affect metabolic health when excessively consumed [1,6]. In this context, fortification with high-biological-value protein sources represents a promising strategy for improving nutritional and functional quality [7].

The recent literature confirms the increasing interest in whey valorization and protein fortification of bakery and gluten-free products, particularly in the context of sustainable food development and circular economy approaches [8–12]. Global whey production is estimated at approximately 180–200 million tons annually, mainly as a by-product of cheese and casein manufacturing processes. Although whey production continues to increase due to the expansion of the dairy industry worldwide, only about 50–60% of the generated whey is currently valorized into value-added products, while the remaining quantities remain insufficiently utilized [13,14]. The disposal of untreated whey can generate significant environmental problems because of its high organic load and elevated biochemical oxygen demand (BOD) and chemical oxygen demand (COD) values. When released into wastewater systems or aquatic environments, whey may reduce dissolved oxygen levels, contribute to water pollution, and negatively affect aquatic ecosystems and soil quality. Therefore, the valorization of whey into food ingredients with added value is increasingly

regarded as an important sustainable strategy for reducing environmental impact in the dairy industry [13,15].

Whey proteins, obtained as a by-product of milk processing, have valuable technological characteristics—such as thermal gelation capacity, emulsifying properties and film formation—which can contribute to the creation of a protein network capable of stabilizing the structure of gluten-free dough. In addition, they have a superior essential amino acid profile, with a high content of leucine, lysine and tryptophan, thus improving the protein density and nutritional value of the final product [16]. For populations with increased protein requirements, such as elderly individuals and athletes, fortification with whey protein concentrates and isolates (WPCs/WPIs) has gained increasing interest due to their high biological value and digestibility [16]. In addition, several studies have reported that whey proteins can improve the viscoelastic behavior of gluten-free systems and enhance structural stability during baking [17–19]. Recent investigations have also employed advanced analytical techniques, including FTIR and SAXS/WAXS analyses, to better understand the structural modifications and molecular interactions occurring in fortified gluten-free matrices [20].

It was demonstrated that moderate additions of whey powder (WPC/WPI) to rice flour-based bakery products can increase the specific volume, improve the texture and elasticity of the crumb and contribute to the formation of a uniform crust, when combined with hydrocolloids or specific enzymes [17]. However, optimal formulation requires careful balancing of whey protein levels with other functional additives to avoid texture and color defects [21].

In gluten-free systems (including rice-based), WPC additions generally increase water absorption, modify storage/loss moduli (G'/G'') and may reduce stiffness if the dosage exceeds a threshold; the effect depends on the matrix (rice vs. sorghum/maize) and protein level. The study of Zhu et al. in 2023 showed increased water absorption and decreased G'/G'' with 8–12% WPC [17]. Moderate additions of WPCs/WPIs (≈ 2 –10% of flour) can improve volume, core elasticity and pore uniformity, especially when combined with hydrocolloids (HPMC/xanthan) or enzymes (e.g., transglutaminase). High dosages ($\approx \geq 30\%$) tend to deteriorate texture [22].

Whey accelerates browning (Maillard reactions) due to the lactose and available amino acids, positively influencing the crispness/crust, but also darkening the color if the dose is high. A study on crust with rice/pea/egg white/whey proteins in gluten-free breads (rice base + cornstarch) revealed improvements in crust quality with whey proteins [23]. Current trends in scientific research on rice flour-based gluten-free products fortified with whey proteins focus on aspects regarding rice matrix optimization (granulometry, whole/sprouted flours) and biological processes (selected yeast) for structure and flavor [9], multi-additive strategies (WPC + fiber/psyllium + enzymes) for volume and water retention [19], and alternative products (pasta, noodles, cakes) where whey brings nutritional benefits with specific technological constraints [22].

Although several studies have investigated whey utilization in bakery products [11,24], limited information is available regarding the integrated evaluation of acid whey powder incorporation into rice flour-based gluten-free biscuits, particularly from nutritional, structural, and technological perspectives. Moreover, the valorization of acid whey remains insufficiently explored despite its high nutritional potential and relevance within sustainable food systems.

Therefore, the aim of the present study was to develop gluten-free rice flour biscuits fortified with whey powder and to evaluate the effects of whey incorporation on proximate composition, mineral profile, structural characteristics, technological behavior, and sensory acceptability. The novelty of the study consists of the combined assessment of nutritional

enhancement and structural modifications induced by acid whey powder in gluten-free biscuit formulations.

2. Materials and Methods

2.1. Obtaining Whey Powder

Whey used for the production of the powder incorporated into the flour formulations was obtained from cow's milk and supplied by a dairy processing facility located in Mehedinți County, Romania. The liquid whey had an initial dry matter content of approximately 10% and a moisture content of 90%. The raw material consisted of acid whey (initial pH 4.5), generated as a by-product during dairy processing. This type of whey was selected because of its widespread availability and its potential as a sustainable source of proteins and other bioactive constituents for the development of value-added functional food ingredients.

Prior to powder production, the whey was subjected to thermal treatment under controlled laboratory conditions using an electrically heated device (Selecline, Shaoxing, China). The processing conditions were designed to represent a simple and cost-effective approach suitable for small-scale whey valorization while ensuring reproducible treatment parameters. Heating was carried out at 92 °C for 3 min, a condition chosen to promote the denaturation and aggregation of whey proteins, thereby improving their subsequent recovery efficiency while minimizing undesirable heat-induced deterioration of nutritional components. Following thermal treatment, the whey was rapidly cooled to 40 °C to prevent further protein denaturation and to prepare the sample for the subsequent processing stages. The treated whey was then distributed into 50 mL centrifuge tubes and processed using a Hettich Universal 320R centrifuge (Hettich Lab Technology, Tuttlingen, Baden-Württemberg, Germany) at 6000 rpm for 15 min at 4 °C. The resulting precipitate was separated and dried in a laboratory oven (Air Concept Precisa, Kruszwica, Poland) at 60 °C for 6 h.

Following the drying process, the material was ground using a grinder (model Silvercrest SKMS 150 A1, manufactured by Kompernass Handels GmbH, Bochum, Germany) to yield a fine, white whey powder [25].

2.2. Composite Flour Preparation

To utilize whey powder in biscuit-type products, three gluten-free composite flour mixtures were prepared from rice flour (RF) with whey powder (WhF).

The composition of WhRF5 is as follows: 5% whey powder and 95% rice flour.

The composition of WhRF10 is as follows: 10% whey powder and 90% rice flour.

The composition of WhRF15 is as follows: 15% whey powder and 85% rice flour.

The whey powder incorporation levels (5–15%) were selected based on preliminary technological tests and literature data, aiming to achieve nutritional enhancement while maintaining acceptable processing and sensory characteristics of the gluten-free biscuits.

2.3. The Obtaining of Biscuits

The technological process used for the production of gluten-free biscuits enriched with whey powder is illustrated in Figure 1, while the appearance of the final products is presented in Figure 2. The biscuit formulations were prepared by thoroughly blending the dry ingredients (rice flour and whey powder according to the experimental design) with sugar and baking powder, followed by the incorporation of butter and whole egg. The mixture was kneaded for approximately 5 min until a uniform and cohesive dough was obtained. No additional water was added during dough preparation, as the hydration requirements were provided by the natural moisture content of the egg and butter, together with the water-binding capacity of the flour components.

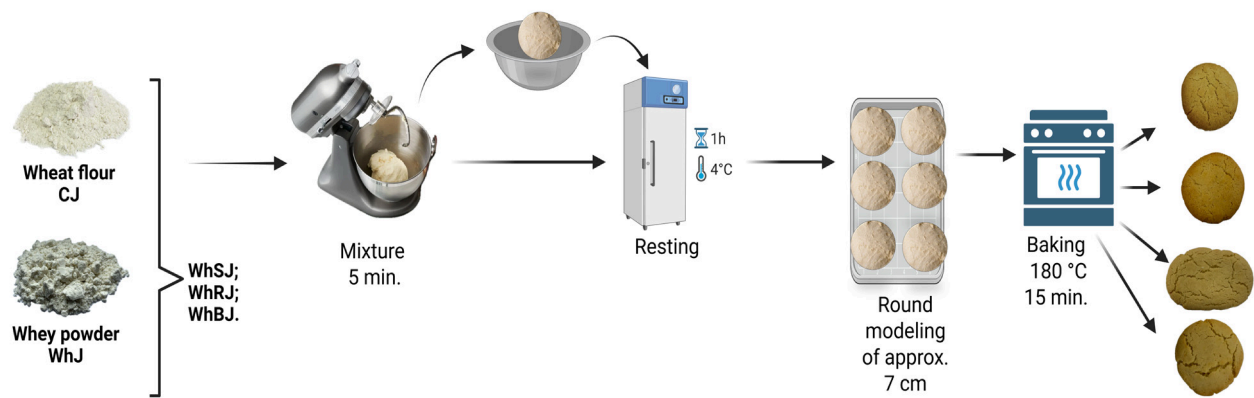


Figure 1. Technological process to obtain gluten-free biscuits with whey addition.



Figure 2. Biscuits made from gluten-free composite flour based on rice flour and whey powder (A) RB—control biscuits made from rice flour, (B) WhRB5—biscuits with 5% whey powder, 95% rice flour, (C) WhRB10—biscuits with 10% whey powder, 90% rice flour, (D) WhRB15—biscuits with 15% whey powder, 85% rice flour.

The resulting dough was refrigerated at 4 °C for 1 h to allow moisture redistribution and stabilization of the dough structure. This resting period promoted the hydration of starch and protein components, improved dough handling properties, and facilitated the formation of a more homogeneous matrix. After refrigeration, the dough was sheeted and shaped into circular biscuits with a diameter of 7 cm and subsequently baked in a preheated oven at 180 °C for 15 min. Following baking, the biscuits were cooled at room temperature prior to physicochemical and sensory analyses.

The formulation used for biscuit production consisted of 100 g of a flour blend (according to the proportions of rice flour and whey powder specified for each experimental variant), 50 g butter (82% fat), 1.4 g baking powder, one whole egg, and 34 g sugar.

No additional water was added to the biscuit formulations. The required dough hydration was achieved through the intrinsic moisture and liquid content of the egg and butter, resulting in the desired dough consistency after mixing.

The absence of added water was intentional, as the liquid fraction supplied by the egg (approximately 74–76% moisture) and the residual moisture present in butter provided sufficient hydration for dough development. This formulation strategy ensured adequate dough cohesiveness while maintaining the low-moisture characteristics typically required for biscuit production.

The biscuits obtained were as follows:

- RB—Control biscuits made from rice flour;
- WhRB5—Biscuits with 5% whey powder and 95% rice flour;
- WhRB10—Biscuits with 10% whey powder and 90% rice flour;
- WhRB15—Biscuits with 15% whey powder and 85% rice flour.

2.4. Determination of Proximate Composition of Composite Flours and Biscuits

The proximate composition was established according to the standard methods outlined by AOAC International [26–28]. The analysis of ash content was conducted in accordance with the ISO method no. 2171:2007 [26]. The moisture and protein contents were determined in accordance with the procedures established by the ICC and AOAC [27]. The assessment of lipid content was conducted in accordance with the AOAC method 920.39 [28]. The carbohydrate content (g/100 g) was calculated by subtracting the amounts of fat, protein, moisture, and ash from 100.

The energy value was calculated using standard Atwater conversion factors (4 kcal/g for proteins and carbohydrates, 9 kcal/g for lipids) according to FAO recommendations [29]. Since dietary fiber content was not determined, total carbohydrates calculated by difference were considered in the energy estimation, which may slightly overestimate the caloric value.

2.5. Determination of Macro- and Microelements

The content of macro- and microelements was determined by atomic absorption spectroscopy (AAS), using the Varian 220 FAA equipment (Varian Inc., Palo Alto, CA, USA). Before AAS determination, the samples were subjected to dry ashing at 650 °C until white ash was obtained. The mineral residue was dissolved in a 20% hydrochloric acid solution and subsequently analyzed by atomic absorption spectroscopy (AAS). The working method and the actual execution were mainly carried out in accordance with the method presented in the previous study [25]. The following elements were analyzed: K, Ca, Mg, Na, Fe, Zn, Cu, Mn, Ni, and Cr. The parameters that were utilized in the analysis, in addition to the calibration curves of the elements, are presented in Table 1. The standard employed for the calibration curve is CertiPUR Merck, (Merck KGaA, Darmstadt, Germany). The results were expressed in milligrams per kilogram of dry sample. Each sample was analyzed in triplicate.

Table 1. The working parameters and calibration curves of the analyzed elements.

Element	Calibration Curve	R	Wave Length (nm)	Lamp Current (mA)	Slit Width (nm)	LOD (µg/mL)	LOQ (µg/mL)
K	$0.29392 \times C + 0.00919$	0.9991	232.0	5	0.2	0.011	0.033
Ni	$0.07405 \times C + 0.00344$	0.9993	213.9	5	1	0.038	0.114
Cd	$0.21355 \times C + 0.03441$	0.9933	404.4	5	0.5	0.018	0.054
Zn	$0.28040 \times C + 0.06621$	0.9955	422.7	10	0.5	0.008	0.024
Cu	$0.09997 \times C + 0.00531$	0.9995	285.2	4	0.5	0.015	0.045
Cr	$0.0588 \times C + 0.00598$	0.9985	279.5	5	0.2	-	-
Mn	$0.18060 \times C$	0.9996	248.3	5	0.2	0.011	0.033
Fe	$0.06288 \times C$	0.9989	324.8	4	0.5	0.042	0.126
Pb	$0.03468 \times C + 0.00157$	0.9994	357.9	7	0.2	0.048	0.144

2.6. Fourier Transform Infrared Spectroscopy (FTIR) of Composite Flours

The FTIR-ATR (Fourier Transform Infrared Spectroscopy with Attenuated Total Reflectance) technique was employed at ambient temperature, utilizing a Nicolet iS50 FTIR Spectrometer (Thermo Fisher Scientific, Madison, WI, USA). The infrared (IR) spectra were obtained within the spectral range of 4000–400 cm^{-1} , with a total of 32 scans at a resolution of 4 cm^{-1} . This spectral region allows the identification of the main functional groups associated with carbohydrates, proteins, lipids, and moisture-related compounds present in the composite flours and biscuits, including O–H stretching vibrations (~3200–3600 cm^{-1}), C–H stretching (~2800–3000 cm^{-1}), amide I and II bands of proteins (~1650 and ~1540 cm^{-1}), and carbohydrate-related C–O and C–O–C vibrations in the fingerprint region (~1200–900 cm^{-1}). The procedure started with the pre-drying of the sample

by keeping it in an oven at 40 °C overnight, in order to remove moisture that could interfere with the spectroscopic analysis. This mild drying condition was selected to minimize potential structural or thermal alterations of the analyzed components. After complete drying, the sample was placed directly on the ATR crystal of the FTIR spectrometer [25].

2.7. Small-/Wide- Angle X-Ray Scattering (SAXS/WAXS) of Composite Flours

The Xenocs Xeuss 3.0 (Xenocs SAS, Grenoble, France) was utilized for the analysis of powder samples. The device utilizes Cu (Genix 3D) as a source and a Dectris Eiger2 Si 1M detector. All analyses are performed in a vacuum at ambient temperature using Kapton film. The SAXS and WAXS measurements were conducted utilizing distances of 1800 mm and 45 mm with HFlux collimation for a duration of 300 s [25]. The obtained results were interpreted and fitted with the XSACT 2.10 program [30]. The nanoparticle standard operating procedure sheet regroups all the analysis tools based on the Porod and Guinier fits, together with the contrast calculator and the size distribution extraction tool.

The fitting for nanoparticle parameters was done in the low Guinier region between 0.01 and 0.1 Å^{−1}, taking into account that the particles are sphere and using the following equation:

$$I(q) = I(0) \exp(-qR_g^2/3).$$

$I(q)$ —scattered intensity; $I(0)$ —scattered intensity at zero angle; q —scattering variable; and R_g —radius of gyration.

The Guinier approximation was applied only in the low- q region to obtain comparative information regarding characteristic structural domains and sample heterogeneity. Although the investigated materials represent complex solid food matrices, the Guinier approach was used as an empirical approximation for comparative nanostructural evaluation.

The SAXS analysis was used mainly as a comparative tool to evaluate structural modifications induced by whey incorporation in the composite flour systems. Due to the heterogeneous nature of the analyzed matrices and the absence of sufficiently robust fitting conditions, quantitative parameters such as the radius of gyration or fractal dimensions were not calculated.

2.8. The Sensory Evaluation of the Gluten-Free Biscuits

The sensory evaluation was conducted using a consumer acceptance test performed with voluntary, non-trained panelists ($n = 20$). Participants were selected based on their availability, regular consumption of bakery products, and absence of known allergies to the ingredients used in the formulations. Before participation, all panelists were informed about the purpose of the study and signed an informed consent form.

Participation in the sensory evaluation was voluntary and based on informed consent. The study did not involve medical procedures, invasive interventions, or identifiable personal data. Therefore, it complied with institutional ethical requirements for low-risk food sensory studies. The study consisted exclusively of a non-invasive sensory assessment of conventional food products under low-risk conditions.

The samples were coded with random three-digit numbers and presented in a randomized order to minimize presentation bias. Sensory analysis was conducted under normal daylight conditions at room temperature. The provision of water for consumption was initiated between the evaluation of each sample, with the objective of mitigating the potential impact of carry-over effects.

The sensory scores thus obtained were expressed as mean $\pm$ standard deviation and statistically analyzed by one-way ANOVA followed by Tukey's HSD post hoc test, with significance established at $p < 0.05$.

The sensory attributes evaluated included appearance, color, aroma, texture, taste, and overall acceptability. The evaluation of each attribute was conducted employing a 5-point hedonic scale, where 1 represented “dislike extremely” and 5 represented “like extremely”.

2.9. Statistical Analysis

It is imperative to note that all determinations were performed in triplicate. The results are presented as the mean values $\pm$ standard deviation (SD). The differences between the means were analyzed using one-way ANOVA, followed by Tukey’s HSD multiple comparison test. The level of statistical significance was established at $p < 0.05$.

3. Results and Discussion

3.1. Proximate Analysis of Gluten-Free Flours and Biscuits Fortified with Whey Powder

Figure 3 presents the results of the proximate analysis of composite flours based on rice flour and whey, respectively, and of the biscuits obtained.

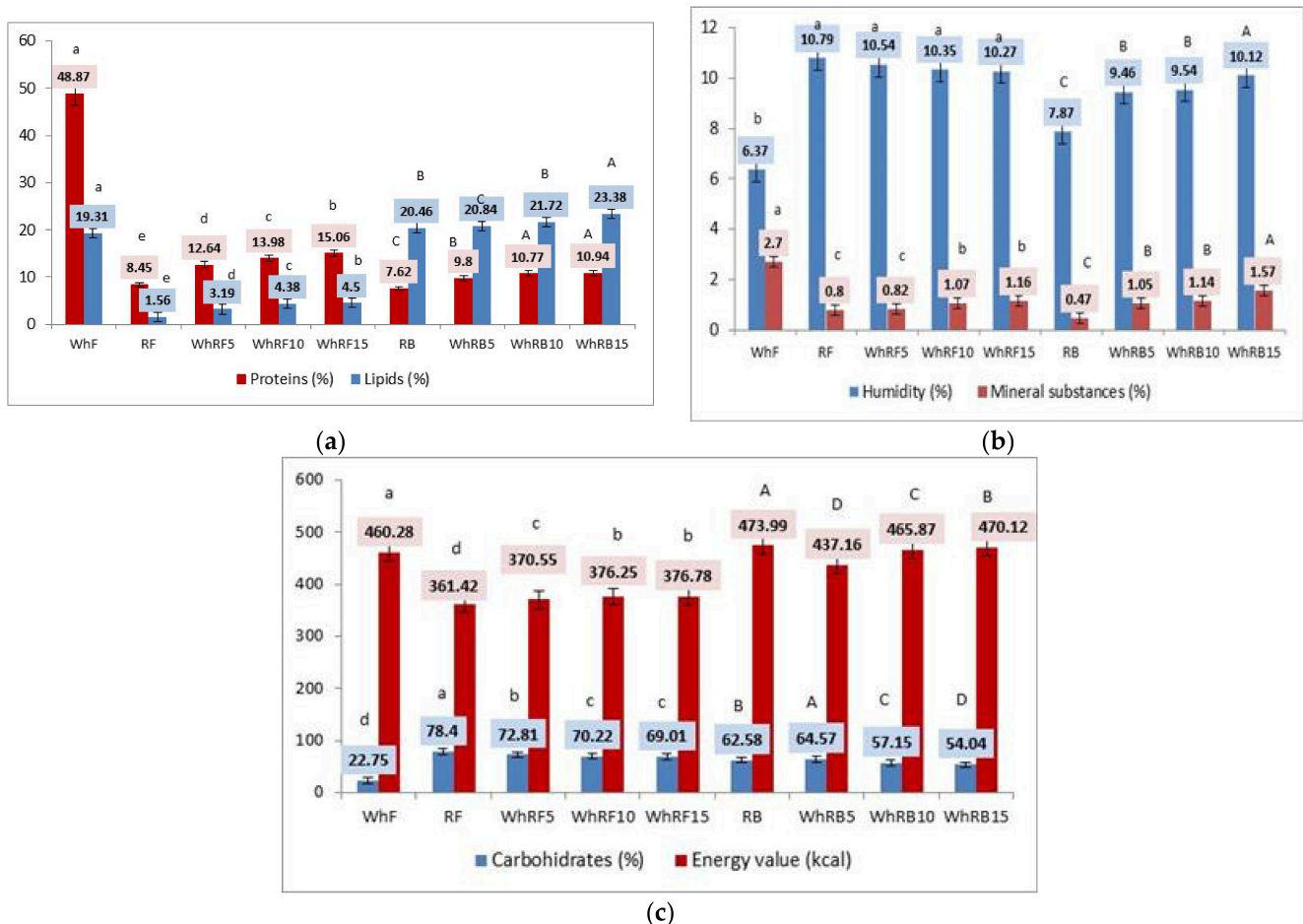


Figure 3. Proteins and lipids (%) (a), moisture content and mineral substances (%) (b), and carbohydrates (%) and energy value (kcal) (c) of composite flours and gluten-free biscuits. Different lowercase letters for the same nutrient in the case of composite flours, and different uppercase letters for the same nutrient in the case of whey-fortified biscuits, indicate significant differences ($p < 0.05$).

The compositional profile of rice flour (RF) reflects the structural organization of the rice grain, in which the endosperm is predominantly composed of starch granules that serve as the primary carbohydrate reserve. The limited proportion of protein-rich tissues, such as the aleurone layer and germ, explains the relatively low protein and lipid contents observed in RF. This imbalance between energy-providing macronutrients and nutrient-

dense components contributes to the lower nutritional quality frequently associated with rice-based gluten-free products. Moreover, the predominance of starch over protein may influence the functional behavior of the flour during processing, affecting water absorption, dough development, and the overall nutritional value of the final product [31]. In contrast, the nutritional richness of whey powder (WhF) arises from the concentration of milk solids during processing, which selectively retains whey proteins, residual lipids, and mineral constituents while removing a substantial proportion of water. The high protein content is primarily attributable to the abundance of biologically valuable whey proteins, such as β -lactoglobulin and α -lactalbumin, whereas the lipid fraction reflects the retention of residual milk fat. The elevated mineral content is associated with the presence of soluble calcium- and phosphorus-containing complexes naturally found in dairy systems. Beyond enhancing the nutritional profile of composite flours, these constituents may contribute to improved functionality through protein–starch interactions, enhanced water-binding capacity, and modifications of the structural properties of gluten-free matrices. Therefore, whey powder represents not only a source of nutrient enrichment but also a potentially active ingredient influencing the technological performance of gluten-free formulations [32].

The progressive incorporation of whey powder into rice flour altered the compositional equilibrium of the blends by replacing part of the starch-rich fraction with dairy-derived proteins and lipids. As a consequence, protein content increased from 8.45% in RF to 15.06% in WhRF15, while carbohydrate content decreased from 78.40% to 69.01%. Beyond the nutritional implications, these changes are expected to influence the functional behavior of the flour systems. The reduction in starch concentration may limit the extent of starch swelling and gelatinization during heating, whereas the increased protein fraction can modify water distribution within the matrix through the high hydration capacity of whey proteins. Such modifications may affect dough cohesiveness, thermal transitions, and the development of the biscuit structure during baking. Furthermore, whey proteins are known to exhibit emulsifying and gel-forming properties, which may contribute to improved matrix continuity and stabilization in gluten-free formulations where the structural functionality normally provided by gluten is absent [5,33,34]. The gradual increase in mineral content following whey incorporation also reflects the contribution of dairy-derived micronutrients, potentially enhancing the nutritional density of the composite flours while influencing ionic interactions within the food matrix [35].

The compositional effects observed in the flour blends remained evident after baking, suggesting that whey proteins retained their technological and nutritional functionality despite exposure to thermal treatment. Protein content increased from 7.62% in the control biscuits (RB) to 10.94% in WhRB15, demonstrating the effectiveness of whey supplementation as a strategy for improving the nutritional quality of gluten-free bakery products. In addition to increasing protein concentration, whey proteins may participate in heat-induced aggregation phenomena during baking, contributing to the formation of a more cohesive structural network capable of partially compensating for the absence of gluten. These interactions may also influence moisture retention and textural development in the final product. Similar observations have been reported in dairy-enriched gluten-free systems, where whey proteins contributed not only to enhanced protein content and amino acid quality but also to improved structural integrity and digestibility of the baked products [36,37].

The progressive reduction in carbohydrate content observed with increasing whey powder incorporation reflects the compositional displacement of starch-rich rice flour by protein- and mineral-containing whey solids. Beyond a simple dilution effect, this substitution likely alters the physicochemical behavior of the dough system by modifying starch hydration dynamics and restricting the extent of starch gelatinization during thermal processing. In gluten-free matrices, where structural organization relies predominantly

on starch–protein interactions rather than gluten network formation, such compositional redistribution may significantly affect water mobility, matrix consolidation, and the development of internal cohesion during baking. These structural modifications are likely to contribute directly to the observed changes in texture and overall biscuit architecture.

The gradual increase in lipid content with higher whey powder levels can be mechanistically associated with the incorporation of residual milk fat naturally present in whey powder, but may also reflect changes in lipid retention behavior induced by protein–lipid interactions within the developing matrix. During baking, denatured whey proteins can promote the formation of a more structured proteinaceous network capable of entrapping lipid fractions and limiting their migration or volatilization. This enhanced lipid retention may contribute to modifications in matrix plasticity, reduced structural brittleness, and altered mouthfeel characteristics, thereby influencing both the technological performance and sensory perception of the gluten-free biscuits.

The increase in ash content from 0.47% in RB to 1.57% in WhRB15 reflects the incorporation of mineral-rich whey solids into the biscuit matrix. This effect is largely attributable to the naturally high concentrations of calcium, phosphorus, potassium, and other minerals present in dairy-derived ingredients. In addition to improving micronutrient density, these constituents may influence intermolecular interactions within the dough system, affecting matrix development and the redistribution of water during thermal processing [38,39].

Whey supplementation was also associated with a modest increase in moisture content. This observation may result from the ability of whey proteins to retain water through hydrogen-bond-mediated interactions involving their hydrophilic functional groups. Enhanced water retention during baking may reduce dehydration of the matrix and contribute to changes in structural organization. Consequently, the final biscuits may exhibit improved cohesiveness, altered textural characteristics, and greater resistance to moisture loss during storage.

From a technological standpoint, the substitution of rice flour with whey powder modified the relative proportions of starch and protein within the formulation, which may influence dough characteristics and structural development during baking. In the absence of gluten, the functionality of gluten-free systems depends largely on the interactions established among starch, proteins, and other matrix constituents [40]. Whey proteins are known to undergo heat-induced denaturation and aggregation, processes that may contribute to matrix stabilization and improved cohesion during baking. At higher incorporation levels, however, the increased protein content may favor the formation of a more compact internal structure, potentially affecting texture by increasing firmness and reducing flexibility. Similar effects have been described in previous studies on whey-enriched gluten-free bakery products [41].

Overall, the observed compositional modifications should not be interpreted solely as nutritional improvements but also as factors capable of altering physicochemical interactions within the gluten-free matrix. The increase in protein and mineral fractions, together with the reduction in the starch-rich phase, likely influenced hydration properties, starch gelatinization behavior, and matrix organization during baking, thereby contributing to the final quality characteristics of the developed biscuits [42–45].

3.2. Macro- and Microelement Contents of Composite Flours and Biscuits Fortified with Whey Powder

Gluten-free rice flour (RF) products are often deficient in essential nutrients, including minerals such as calcium (Ca), iron (Fe), and zinc (Zn). Partial replacement of rice flour with protein- and mineral-rich ingredients such as whey powder (WhF) is an effective strategy to increase the nutritional value of these products. Whey is a concentrated source of calcium, phosphorus, magnesium, and sodium, and is frequently used to fortify foods for groups

with special nutritional needs [46,47]. Whey powder represents an important source of phosphorus and sodium, with concentrations of 912 mg/kg and 434.65 mg/kg, respectively.

The incorporation of whey powder into the composite flour formulations resulted in a progressive increase in the content of these minerals (Figure 4). These mineral enhancements may contribute positively to physiological functions associated with electrolyte balance and bone metabolism. However, sodium intake should be considered carefully within the context of overall dietary consumption due to its potential association with elevated blood pressure when consumed excessively. The elevated phosphorus content observed in whey-containing formulations is consistent with previous reports indicating that dairy-derived ingredients and whey protein supplements constitute important dietary sources of phosphorus, frequently associated with calcium-rich matrices [48,49].

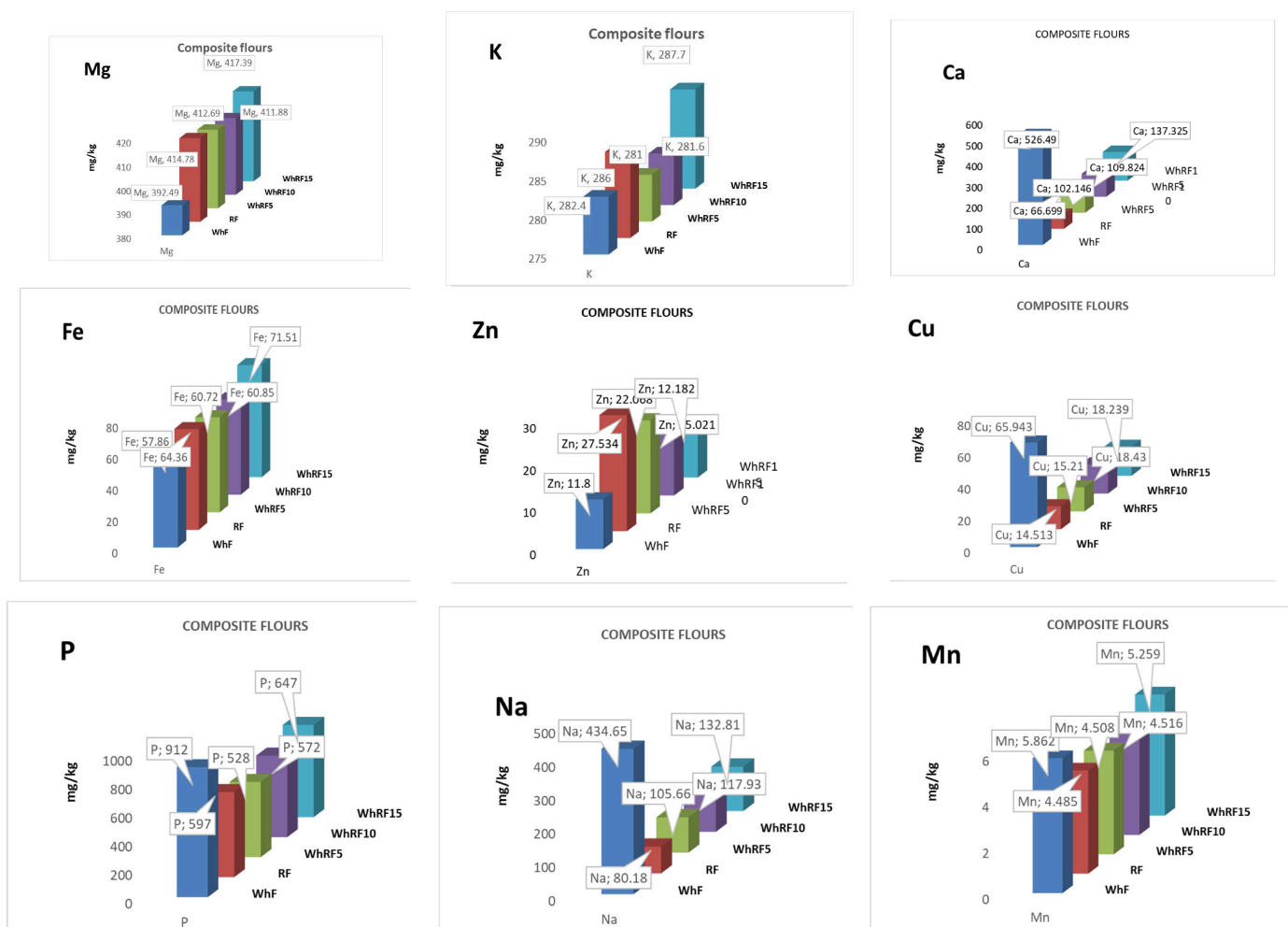


Figure 4. Macro- and micro-element contents of rice flour (RF), chestnut flour, fruit powders, WhF and composite flours.

Whey powder (WhF) exhibited a substantially higher calcium content (526.49 mg/kg) than rice flour (66.70 mg/kg). As a result, calcium concentrations increased progressively in the composite flours with increasing levels of whey powder incorporation (WhRF5 < WhRF10 < WhRF15). This enrichment is directly attributable to the mineral-rich nature of whey, in which calcium remains associated with milk proteins and soluble mineral complexes during processing. Beyond increasing the nutritional value of the formulations, the higher calcium content may also influence the functional properties of gluten-free systems by promoting interactions with proteins and other flour constituents, potentially affecting dough structure and product quality. These findings support the use

of whey-derived ingredients as effective calcium fortification agents in gluten-free products intended for individuals with gluten intolerance or celiac disease [50].

Commercial rice flour typically reports 10–150 mg/kg Ca ($\approx$ 1–15 mg/100 g), with variations depending on variety/processing; values in this range are reported for various gluten-free flours analyzed by ICP-OES/ICP-MS. Overall, they are modest sources of Ca, poorer than many gluten-containing flours. Increased calcium is beneficial, especially for individuals with celiac disease, who often have inadequate calcium intake and poor absorption, which can lead to osteoporosis. Milk/whey and derivatives are high in Ca; detailed profiles indicate Ca, P, and Na as the dominant macrominerals in milk/whey, justifying the increase observed when whey is included in the mixture [14,51].

Magnesium content remained relatively stable among the analyzed formulations, suggesting that the substitution of rice flour with whey powder did not markedly affect the availability of this mineral within the composite blends. This limited variation may result from the comparable magnesium contribution of the two ingredients and the relatively low replacement levels applied. The observed stability indicates that the mineral balance of the formulations was largely preserved despite the compositional modifications induced by whey incorporation [52].

Although the analyzed biscuits cannot be considered a sole source capable of covering the complete daily mineral requirements of a healthy adult, the obtained results demonstrate a nutritionally relevant contribution of essential minerals, particularly calcium, magnesium, iron, and zinc, due to whey powder incorporation. According to EFSA dietary reference values, the recommended daily intake for adults is approximately 950–1000 mg/day for calcium and 300–350 mg/day for magnesium, while zinc and iron requirements vary depending on sex and physiological status [53]. Therefore, the developed gluten-free biscuits may be considered a complementary dietary source of minerals within a balanced nutritional regime.

The increase in sodium content following whey supplementation likely reflects the higher concentration of soluble sodium compounds naturally present in dairy-derived ingredients compared with cereal-based raw materials [10]. The gradual rise observed in the composite flours remained proportional to the level of whey incorporation, indicating that the mineral composition of the blends was influenced by the relative contribution of the dairy fraction. In addition to nutritional considerations, changes in sodium concentration may affect the ionic environment of the dough system, potentially influencing interactions among proteins and starch components.

Manganese concentrations exhibited a slight upward trend with increasing whey powder levels, suggesting a limited but measurable contribution of dairy-derived trace minerals to the composite formulations. This behavior indicates that whey incorporation may support micronutrient enrichment while maintaining the overall mineral equilibrium of the flour blends. The relatively small variation observed also suggests that manganese content remained primarily governed by the cereal fraction, with whey acting as a supplementary source of this trace element [10]. Iron concentrations showed only minor variation among the analyzed formulations, suggesting that whey powder incorporation exerted a limited influence on the iron content of the composite flours. Considering the relatively similar iron levels of the raw materials and the moderate replacement ratios employed, the preservation of this mineral fraction was expected. The observed stability indicates that the nutritional contribution of iron was maintained despite the compositional modifications induced by whey addition. The slightly elevated concentration detected in WhRF15 may reflect redistribution phenomena within the matrix associated with changes in the relative abundance of other constituents, although additional analyses would be required to confirm the mechanisms underlying this behavior [54]. In contrast, the progressive decline in zinc concentration with increasing whey powder addition can be mechanistically

explained by the absence of detectable zinc in whey powder and the resulting dilution of the zinc-rich rice flour fraction. This trend highlights the direct influence of ingredient mineral composition on the micronutrient balance of composite gluten-free systems [55]. Since zinc is closely involved in enzymatic regulation, immune competence, and cellular metabolic processes, its reduction may limit the nutritional enhancement achieved through whey supplementation. These findings suggest that while whey powder improves the protein and functional characteristics of the flour matrix, complementary fortification with zinc-rich ingredients may be required to counterbalance mineral dilution effects and achieve a more optimized micronutrient profile in future gluten-free formulations. Whey powder (WhF) exhibited a markedly higher copper concentration (65.94 mg/kg) compared to rice flour (14.51 mg/kg), indicating that whey incorporation may contribute positively to the enrichment of composite flours with this essential trace element.

The composite flour formulations exhibited a progressive increase in copper content with increasing levels of whey powder incorporation, reaching 18.24 mg/kg in the WhRF15 sample. This enrichment may be considered nutritionally advantageous, as copper is an essential trace element involved in hemoglobin synthesis, iron metabolism, and antioxidant defense mechanisms through its role as a cofactor for several oxidative-stress-related enzymes [56].

Manganese concentrations also showed a slight upward trend, increasing from 4.49 mg/kg in rice flour to 5.26 mg/kg in the WhRF15 formulation. Although moderate, this increase may contribute positively to the nutritional profile of the composite flours, given the important physiological functions of manganese in carbohydrate and lipid metabolism, enzymatic activity, and cellular antioxidant processes.

Whey-enriched biscuits may be considered a better source of calcium, contributing to a reduced risk of osteoporosis in people with celiac disease. The addition of whey causes a progressive increase in calcium content, from 152.47 mg/kg (RB) to 225.17 mg/kg (WhRB15) (Figure 5). Whey is recognized as a concentrated source of calcium and is frequently used in food fortification to combat osteoporosis and support bone health. Studies show that gluten-free products generally have a low calcium intake, which can be compensated for by the inclusion of dairy ingredients [57].

Magnesium concentrations remained relatively constant, at approximately 410 mg/kg, across all formulations, indicating that whey incorporation had a limited influence on the availability of this mineral within the biscuit matrix. This stability may be explained by the comparable contribution of the raw materials to the magnesium pool and by the relatively low substitution levels employed. The preservation of magnesium levels suggests that the mineral profile associated with this element was largely maintained despite the compositional modifications introduced by whey supplementation [58].

In contrast, phosphorus content increased following whey addition, reflecting the naturally higher concentration of phosphorus-containing compounds in dairy-derived ingredients. This behavior indicates an effective transfer of minerals from the whey fraction into the biscuit matrix. Beyond enhancing the micronutrient density of the formulations, the increased phosphorus concentration may also influence interactions among matrix components during processing, particularly in systems enriched with dairy proteins, where mineral–protein associations can contribute to structural organization [59,60].

Sodium levels also increased with increasing whey powder incorporation, a trend that can be attributed to the presence of soluble sodium salts naturally occurring in whey and subsequently concentrated during powder production. The gradual enrichment of the formulations with sodium-containing compounds may alter the ionic environment of the dough system, potentially affecting water distribution and interactions among starch and protein fractions. Such changes may contribute to modifications in hydration behavior and matrix development during baking. Nevertheless, the magnitude of the increase

remained proportional to the level of whey addition, indicating that the observed changes were primarily driven by the mineral composition of the dairy ingredient rather than by processing-related factors [61,62].

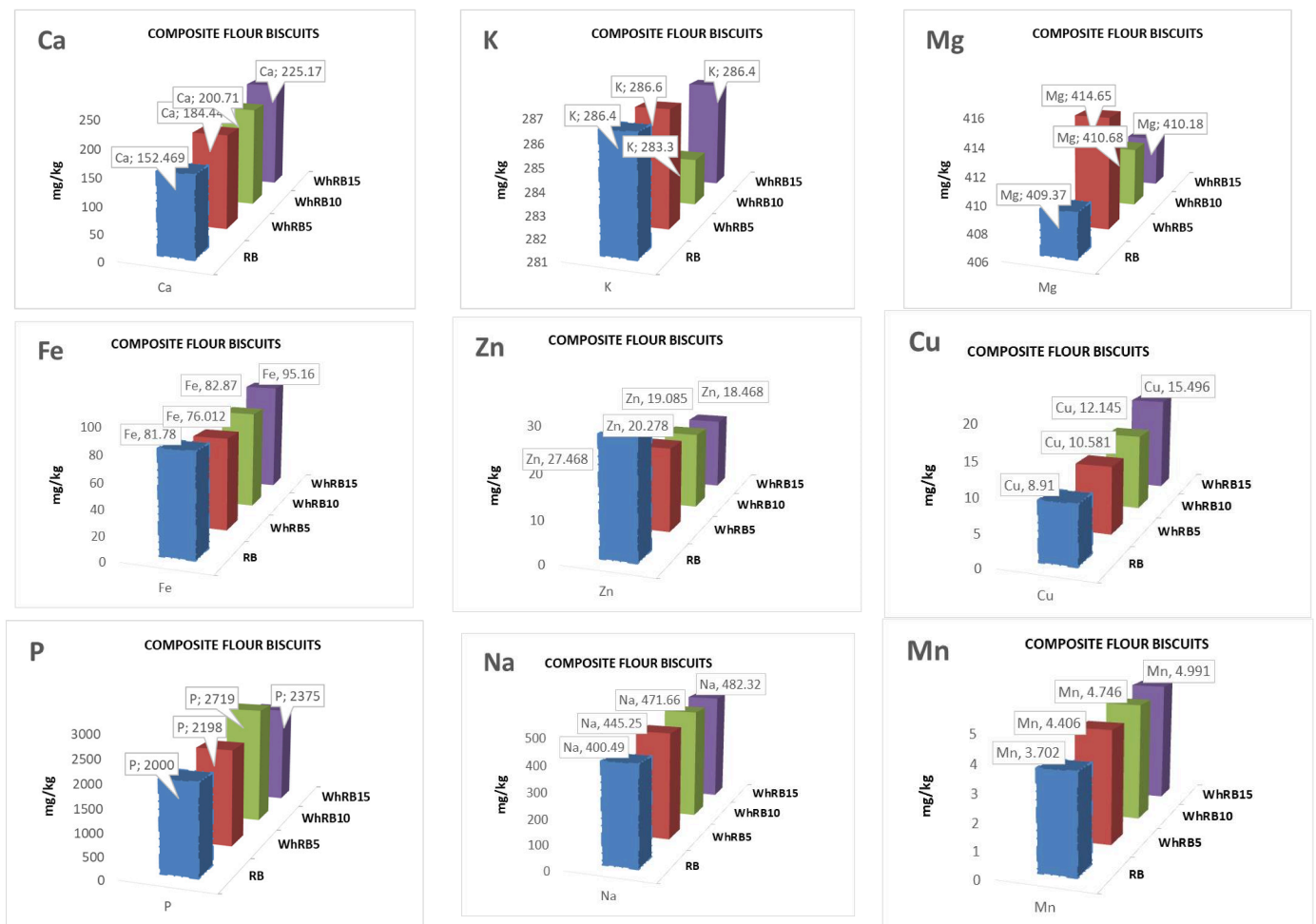


Figure 5. Macro- and microelement contents of biscuits (control samples and fortified formulations).

Iron content showed slight variations among the gluten-free biscuit formulations, ranging from 76.01 mg/kg (WhRB5) to 95.16 mg/kg (WhRB15). The literature indicates that gluten-free products are often characterized by low iron content, which may contribute to iron-deficiency anemia in individuals with celiac disease. The modest increase in iron at higher whey addition levels may be nutritionally beneficial; however, overall levels remain relatively low, suggesting that additional fortification with bioavailable iron sources may be required [63,64].

The increase in copper concentration following whey powder incorporation reflects the contribution of trace minerals naturally present in the dairy matrix and retained during whey processing. This trend is likely associated with the relatively high copper content of the whey powder used in the formulations. Copper is an essential trace element involved in several physiological processes, including hemoglobin formation and antioxidant enzyme activity; therefore, increased copper levels may enhance the nutritional value of the final products by contributing to antioxidant defense and iron metabolism [40,65].

The copper concentration measured in whey powder (65.94 mg/kg) was higher than values commonly reported for commercial whey powders, where copper levels are typically below 20 mg/kg. This difference may reflect variations in the mineral composition of the raw milk, as well as the influence of processing and concentration steps applied during

whey powder production. In addition, the concentration effect associated with water removal may have contributed to the enrichment of trace elements in the final powder. However, the potential contribution of processing equipment or technological handling procedures cannot be excluded and may partially explain the elevated copper concentration observed in the present study [66,67].

In contrast, zinc content decreased as the level of whey powder increased, declining from 27.47 mg/kg in the control biscuits to 18.47 mg/kg in WhRB15 ($p < 0.05$). This trend likely reflects the lower zinc concentration of whey relative to rice flour, resulting in a gradual dilution of the zinc-rich fraction within the formulation. The reduction became more pronounced at higher substitution levels, indicating that the mineral composition of the final products was strongly influenced by the relative proportions of the raw materials. These findings suggest that while whey supplementation contributed to the enrichment of certain minerals, it simultaneously modified the balance of trace elements within the biscuit matrix, emphasizing the importance of considering both nutritional enhancement and mineral redistribution when formulating gluten-free products [68].

This decrease suggests that, although whey addition enhances calcium and phosphorus levels, it may dilute the zinc contribution from rice flour. Therefore, additional supplementation with zinc-rich ingredients (e.g., seeds and nuts) is recommended to achieve a balanced nutritional profile.

3.3. Spectroscopy Analysis (FTIR) of Fortified Flours with Whey Powder

In this analysis, the FTIR spectra of five different samples were evaluated: pure rice flour (RF), pure whey powder (WhF), and three mixtures of rice flour supplemented with 5% (WhRF5), 10% (WhRF10), and 15% (WhRF15) whey powder. The main objective was to identify structural and compositional changes induced by the increasing proportion of whey proteins within the carbohydrate matrix of rice flour, based on the characteristic bands observed in the infrared spectra (Figure 6).

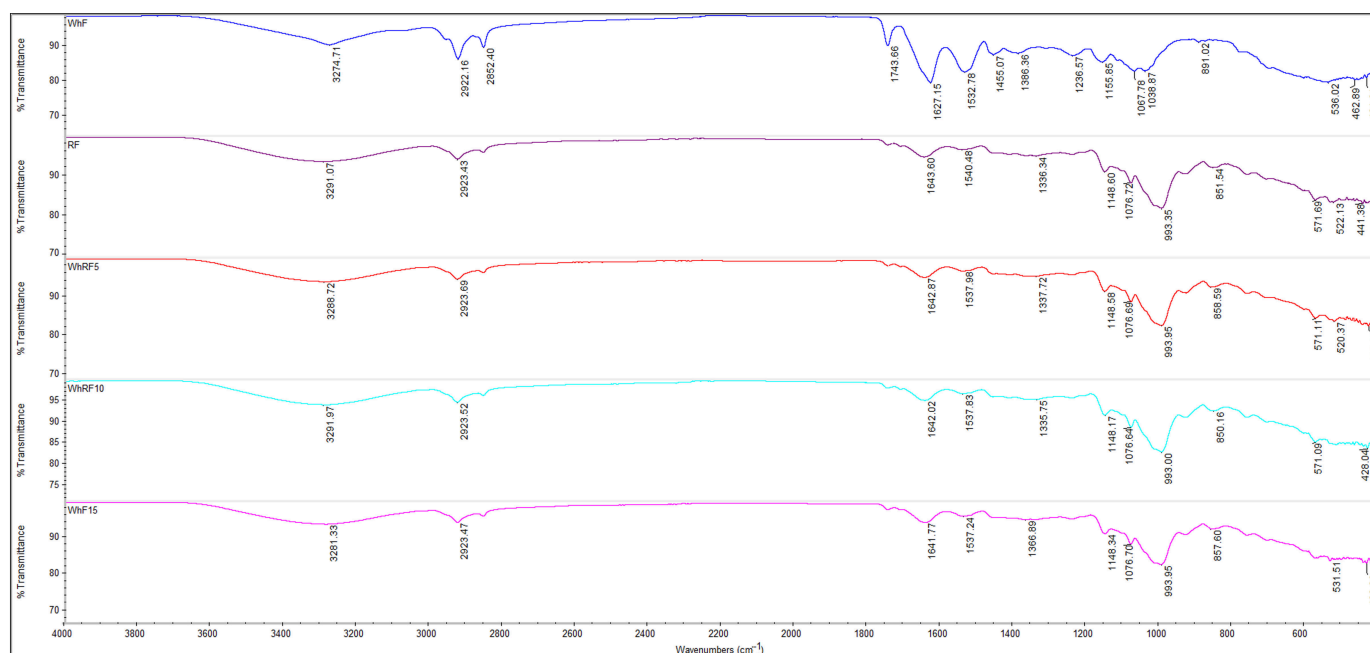


Figure 6. FTIR spectra of composite flours based on rice flour and whey powder: WhF (purple line), RF (green line), WhRF5 (red line), WhRF10 (pink line), and WhF15 (blue line). Spectral range 4000–400 cm^{-1} ; 32 scans at a resolution of 4 cm^{-1} .

The FTIR spectra revealed compositional differences between rice flour and whey powder through variations in the presence and relative intensity of characteristic absorption bands. The spectral profile of rice flour was dominated by bands commonly associated with starch and other carbohydrate-rich constituents, particularly those observed around 993 and 1076 cm^{-1} , which are generally assigned to C–O, C–C and C–O–C vibrational modes of polysaccharides. In contrast, whey powder exhibited more pronounced absorptions in the amide regions, especially near 1532 cm^{-1} and 1627 cm^{-1} . These bands are typically associated with amide II and amide I vibrations and may reflect the higher protein content of the dairy-derived material.

The observed spectral differences suggest modifications in the relative contribution of carbohydrate- and protein-associated components following whey incorporation, rather than providing direct evidence of specific molecular arrangements or structural transformations.

The absence of the band at approximately 993 cm^{-1} in whey powder further supports the interpretation that this spectral feature is predominantly associated with the starch-rich fraction present in rice flour. Similarly, the band observed around 1743 cm^{-1} exclusively in whey powder may be indicative of carbonyl stretching vibrations originating from lipid- or ester-containing constituents naturally present in dairy ingredients. However, FTIR data alone do not allow definitive identification of the molecular species responsible for this signal. The absence of this band in rice flour and the composite formulations may be related to the lower abundance of these components, signal overlap within the carbohydrate–protein matrix, or dilution effects associated with the blending process.

The spectrum of whey powder (WhF) exhibits features specific to proteinaceous compounds, with two intense bands in the amide region: amide I (~1650 cm^{-1}) and amide II (~1540 cm^{-1}). These bands are attributed to C=O stretching and N–H bending vibrations characteristic of peptide bonds. According to the literature, these regions are considered fundamental markers of whey proteins and provide information regarding their secondary structure, including the proportion of α -helix, β -sheet, and random coil conformations [45]. Additionally, a broad band around 3290–3240 cm^{-1} is observed, corresponding to O–H and N–H vibrations, indicating a high number of functional groups capable of forming hydrogen bonds, both between proteins and with water molecules [45].

In contrast, the spectrum of rice flour (RF) differs, being dominated by the “fingerprint” region (1200–900 cm^{-1}), characteristic of polysaccharides such as starch. In this region, the intense bands around 1145, 1078, and 995 cm^{-1} correspond to C–O and C–C vibrations typical of starch structures and are indicative of its degree of crystallinity. Moderate bands are also observed in the 2920–2850 cm^{-1} region, associated with CH₂ and CH₃ vibrations originating from carbohydrates and possible residual lipids [69].

The spectra of the composite formulations exhibited gradual changes as the proportion of whey powder increased. In particular, the relative intensity of the bands located in the amide I (~1650 cm^{-1}) and amide II (~1540 cm^{-1}) regions tended to increase with whey supplementation. These spectral modifications are consistent with the higher protein contribution provided by whey powder and may reflect the increasing presence of protein-associated functional groups within the composite matrix. In the formulation containing 5% whey powder (WhF5), the amide bands were discernible but relatively weak, whereas in WhF10 and especially WhF15 they became more pronounced, resulting in spectral profiles that more closely resembled that of whey powder. Similar trends have been reported in starch-based systems enriched with whey proteins, where changes in the intensity of absorption bands within the amide regions were associated with modifications in the relative contribution of protein components to the overall spectral profile [25]. However, these observations should be interpreted as indicative of compositional changes within the mixtures rather than as direct evidence of specific molecular interactions or structural

rearrangements. At the same time, the relative intensity of the starch-associated bands at 995 and 1078 cm^{-1} decreased with increasing whey addition, which may be related to the progressive dilution of the starch fraction within the composite systems. Similar trends have been described in previous studies, where reductions in the R1047/1022 ratio were associated with changes in starch structural organization and crystallinity following protein incorporation or thermal processing [46].

In addition, the broad absorption band observed around 3290 cm^{-1} showed a gradual increase in intensity as the whey concentration increased. This behavior may indicate a higher contribution of O–H and N–H functional groups involved in intermolecular interactions, possibly including hydrogen bonding. Such interactions could influence functional characteristics of the system, particularly hydration behavior and water retention capacity. Comparable spectral modifications have previously been reported in protein-enriched starch-based matrices.

A weak absorption band near 1740 cm^{-1} was also detected in some spectra and may be associated with C=O stretching vibrations originating from residual lipid fractions present in whey powder. The increased visibility of this band at higher whey concentrations may reflect the contribution of minor lipid constituents in the formulations. Similar observations have been reported in previous investigations of protein-fortified flour systems [70]. Overall, FTIR may be considered a useful analytical approach for the identification of major components and for the evaluation of potential structural modifications and intermolecular interactions within complex flour–protein systems.

In order to provide a more quantitative interpretation of the FTIR data, spectral parameters (peak position, intensity, and band area) and an additional peak area ratio analysis were considered (Table 2). Specifically, ratios between the protein-associated amide bands (approximately 1540 and 1640 cm^{-1}) and the starch-related band ($\sim 1076 \text{ cm}^{-1}$) were evaluated to assess structural modifications induced by whey powder incorporation. The increase in the A1540/A1076 ratio with increasing whey concentration suggested an enhanced contribution of protein-related structures relative to starch components, confirming the incorporation of whey proteins into the composite matrix. The obtained trends supported the qualitative FTIR observations regarding increased protein contribution and modifications in intermolecular interactions following whey addition.

Table 2. FTIR spectral parameters (peak position, intensity, and band area) and calculated peak area ratios used for the structural evaluation of rice flour–whey composite systems.

No.	RF			Whf			WhRF5			WhRF10			WhRF15		
	Peak	Intensity	Area	Peak	Intensity	Area	Peak	Intensity	Area	Peak	Intensity	Area	Peak	Intensity	Area
1	993.35	81.32	571.69	-	-	-	993.95	82.06	554.95	993	82.50	531.52	993.95	82.05	531.9
2	1076.72	87.71	17.37	1067.78	82.40	40.11	1076.69	88.26	28.31	1076.64	88.34	26.97	1076.7	87.69	23.81
3	1540.48	96.08	40.56	1532.78	82.08	439.53	1537.98	96.15	49.76	1537.83	96.27	47.68	1537.24	95.33	67.8
4	1643.6	94.27	158.16	1627.15	79.06	727.38	1642.87	94.50	161.63	1642.02	94.67	154.88	1641.77	93.64	194.29
5	-	-	-	1743.66	89.72	91.97	-	-	-	-	-	-	-	-	-
6	3291.07	93.11	1688.53	3274.71	89.96	1200.69	3288.72	93.39	1614.62	3291.97	93.63	1524.93	3281.33	93.10	1619.78
A1540/A1076			2.335	10.958			1.758			1.768			2.848		

3.4. X-Ray Diffraction (XRD) Analysis of Fortified Flours with Whey Powder: Small–Wide-Angle X-Ray Scattering (SAXS/WAXS) of Fortified Flours with Whey Powder

The study of the structure of food materials at the nanoscale is an essential research direction for understanding the interactions between components and for optimizing the functional properties of the final products. In this context, the Small-Angle X-ray Scattering (SAXS) technique is particularly useful, as it allows the characterization of the internal

organization and dimensions of the structures of complex materials, such as mixtures of carbohydrates and proteins, without destroying the sample.

Whey, a by-product of the dairy industry, is an important source of proteins with high biological value, being rich in β -lactoglobulin, α -lactalbumin, and immunoglobulins. Its addition to rice flour-based products aims to improve the nutritional profile and functional properties, but also involves significant structural modifications at the micro- and nanometric levels. Rice flour is mainly composed of starch, with granules organized in semi-crystalline structures, and interactions between starch and proteins can influence both the degree of aggregation and the physicochemical properties of the system [71].

The results obtained from the SAXS analysis of the studied samples—pure rice flour (RF) and mixtures with 5% (WhRF5), 10% (WhRF10) and 15% whey (WhRF15)—indicate progressive changes in the internal structure as the proportion of protein increases (Table 3).

Table 3. Nanoparticle parameters extracted from SAXS data.

Name	Median of the Distribution, nm	Surface Equivalent Mean Size, nm	Goodness of Fit
RF	823.02	31.77	1.35
WhRF5	850.73	41.60	1.08
WhRF10	864.63	48.90	1.03
WhRF15	863.05	47.62	1.06

The SAXS analysis (Figure 7) revealed differences in the scattering profiles of the investigated samples, with WhRF10 exhibiting the highest median particle size (864 nm). This observation may be indicative of changes in the spatial arrangement of matrix components following whey incorporation, although SAXS data alone do not allow definitive identification of the underlying interactions. The RF sample displayed a scattering pattern typically associated with starch-rich systems, whereas the incorporation of whey powder was accompanied by gradual modifications in the shape of the SAXS curves. These changes may reflect alterations in the organization of structural domains within the matrix and are consistent with the increasing contribution of whey-derived constituents.

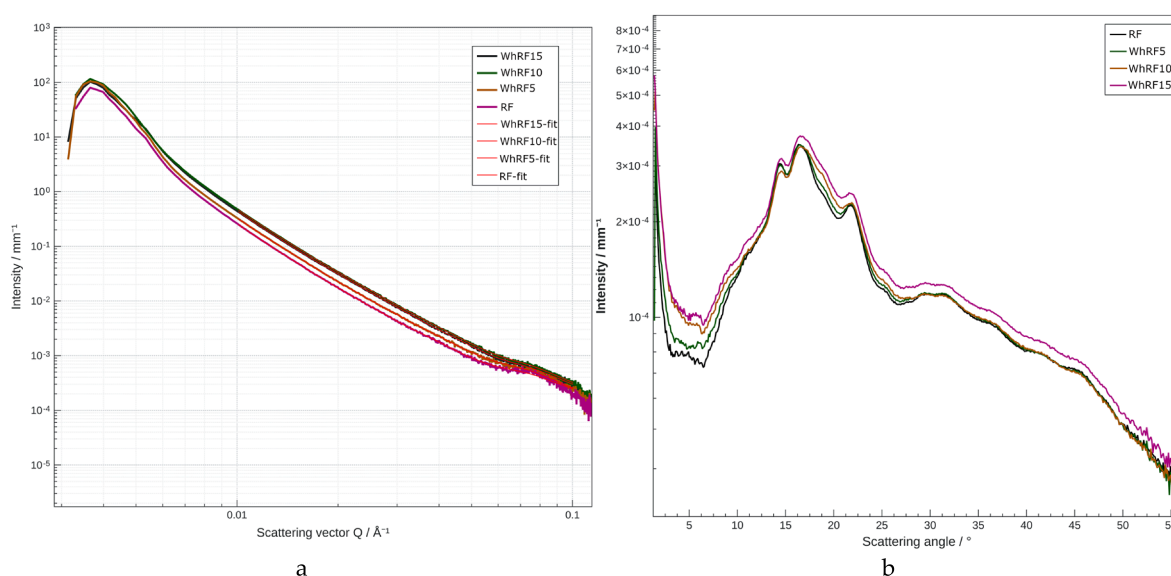


Figure 7. (a,b) Small-angle and wide-angle X-ray diffraction (SAXS/WAXS) analysis of RF (black line), WhF (red line), WhRF5 (green line), WhRF10 (orange line), and WhRF15 (blue line).

Previous studies have suggested that milk proteins can interact with starch components through non-covalent associations, including hydrogen bonding, potentially influencing the arrangement of starch-rich regions and their aggregation behavior [72]. However, the present SAXS results should be interpreted as providing indirect evidence of possible modifications in matrix organization rather than confirming specific molecular interactions or structural mechanisms.

In the high-Q region corresponding to the WAXS domain, the diffraction patterns of the samples showed a substantial degree of overlap, suggesting that whey incorporation did not induce major detectable changes in the short-range molecular ordering probed by this technique. Consequently, the differences observed in the SAXS region may be associated with modifications occurring at larger length scales, while the WAXS data do not provide evidence of pronounced alterations in the crystalline organization of the investigated systems.

A comparative evaluation of the formulations showed that the addition of 5% whey induced only limited structural modifications, suggesting the onset of interactions within the system. At 10% whey incorporation, the SAXS profiles indicated a more heterogeneous structural organization, which may reflect increased interactions between proteins and starch constituents. For the formulation containing 15% whey powder, the scattering profile suggested additional changes in the relative organization of the matrix, possibly associated with modifications in starch aggregation and protein–starch association patterns.

From a technological perspective, these structural variations may contribute to differences in dough behavior and final product characteristics. Moreover, the incorporation of whey into starch-based systems represents a sustainable strategy for the valorization of dairy by-products, supporting waste reduction and circular economy approaches in the food industry [73].

3.5. Sensory Evaluation of Gluten-Free Biscuits Fortified with Whey

Figures 8 and 9 highlight consumer preferences for gluten-free biscuit products based on composite rice flour/whey powder. The sensory evaluation of the samples aimed to assess consumer acceptance based on characteristics such as appearance, aroma, texture, taste/consistency, and overall acceptability.

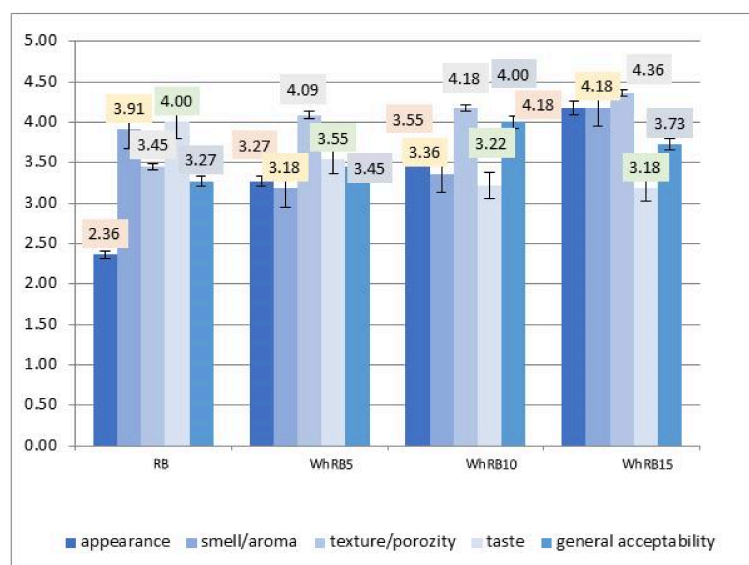


Figure 8. Average values of sensory evaluation of biscuits made from gluten-free composite flour based on rice flour and whey powder (RB—control biscuits made from rice flour; WhRB5—biscuits with 5% whey powder, 95% rice flour; WhRB10—biscuits with 10% whey powder, 90% rice flour; WhRF15—biscuits with 15% whey powder, 85% rice flour) using a 5-point hedonic scale (n = 20).

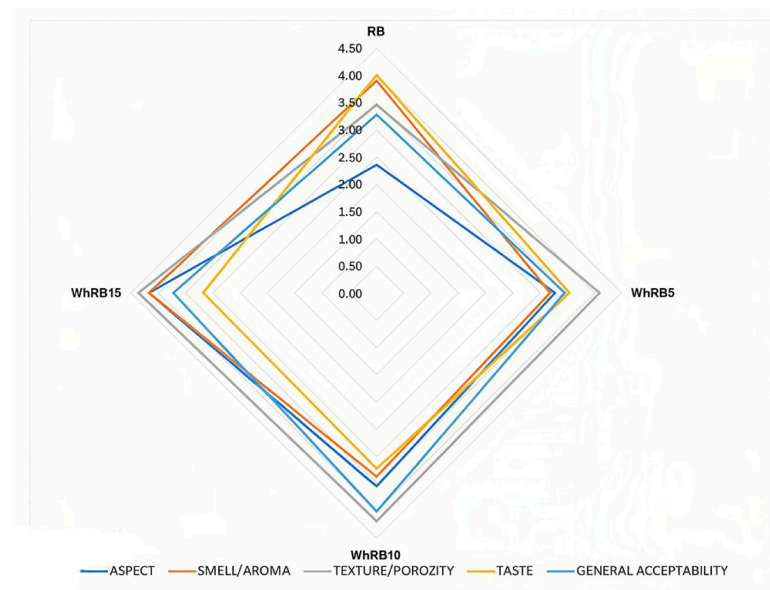


Figure 9. Sensory diagram of biscuits made from gluten-free composite flour based on rice flour and whey powder (RB—control biscuits made from rice flour; WhRB5—biscuits with 5% whey powder, 95% rice flour; WhRB10—biscuits with 10% whey powder, 90% rice flour; WhRF15—biscuits with 15% whey powder, 85% rice flour).

Sensory evaluation revealed that the control biscuits prepared exclusively from rice flour received the lowest appearance score (2.36), whereas formulations containing whey powder showed higher visual acceptability, reaching 4.18 in WhRB15. This improvement may be related to modifications in matrix development during baking, as the incorporation of whey proteins can influence the interactions between protein and starch fractions, affecting surface uniformity and structural integrity. In addition, the presence of whey-derived proteins and lactose may enhance browning reactions during thermal processing, contributing to a more attractive color development and a more homogeneous surface appearance. These combined effects may explain the increased consumer acceptance of the whey-enriched biscuits compared with the control sample.

Aroma perception exhibited only minor variations among the analyzed formulations, indicating that whey powder addition at the tested levels had a limited influence on the generation or retention of volatile compounds responsible for odor characteristics. This behavior suggests that the concentrations employed were sufficient to modify compositional and structural attributes without substantially altering the aromatic profile of the final products. Consequently, the sensory improvements observed in the enriched formulations appear to be primarily associated with visual and textural modifications rather than with changes in aroma intensity or quality.

Texture assessment revealed a clear influence of whey supplementation on the mechanical characteristics of the biscuits. The higher scores recorded for the enriched formulations, increasing from 3.45 in the control sample to 4.36 in WhRB15, may be attributed to the ability of whey proteins to interact with starch components and retain water within the matrix during baking. Heat-induced protein aggregation, together with changes in moisture distribution, may have promoted the development of a more cohesive internal structure. At higher incorporation levels, these interactions likely contributed to the formation of a denser matrix, which could explain the increased firmness perceived by the panelists and the reduced deformability commonly associated with protein-enriched gluten-free products.

Taste perception was more sensitive to whey incorporation than the other sensory attributes. The gradual decline in taste acceptability at higher supplementation levels may

be associated with the compositional characteristics of acid whey, particularly its mineral content and residual organic acids. These constituents can modify the flavor balance of the final product by introducing slightly salty and acidic notes that become more perceptible as the level of whey addition increases. The findings suggest that while whey powder contributes positively to the nutritional profile and several quality attributes of gluten-free biscuits, its sensory impact should be carefully considered when formulating sweet bakery products. In contrast, formulations intended for savory or appetizer-type applications may better accommodate the characteristic flavor profile of whey-enriched ingredients, potentially improving overall consumer acceptance. Previous studies have shown that sensory quality can be improved by optimizing whey concentration, incorporating flavoring ingredients such as cocoa or fruit powders, adjusting sweetness, or applying fermentation-based approaches to modulate flavor perception [24,74].

The overall acceptability indicates an increase in consumer preferences for biscuits fortified with whey, with a maximum attributed to the product with 10% addition, noting as a general conclusion that the superior attributes related to texture and appearance generated by the addition of whey powder are counterbalanced by its persistent salty taste.

Consumer acceptance of bakery products is influenced not only by physicochemical and sensory attributes but also by cognitive and psychological factors, including emotions, memories, familiarity, and previous food experiences. These aspects may affect the perception of texture, aroma, taste, and overall acceptability of gluten-free products, particularly when novel ingredients are incorporated into traditional formulations. Therefore, the sensory responses obtained in the present study may also reflect subjective emotional and cognitive associations related to consumer expectations and eating habits [75,76].

The sensory evaluation was performed using a relatively small panel of 20 participants and should therefore be considered a preliminary assessment of consumer acceptability. Larger-scale sensory studies would be necessary to obtain more representative consumer perception data.

4. Conclusions

The incorporation of whey powder into rice flour and gluten-free biscuits demonstrated significant potential for improving both the nutritional quality and structural characteristics of the developed products.

Whey supplementation led to a substantial increase in protein content in both composite flours and biscuits, addressing a major limitation of gluten-free products, which are typically deficient in protein. Concurrently, the enrichment resulted in higher levels of essential minerals, particularly calcium and phosphorus, contributing to improved bone health and metabolic support, especially for individuals with celiac disease who are prone to micronutrient deficiencies. The incorporation of whey powder contributed to a reduction in carbohydrate content. However, no *in vitro* or *in vivo* glycemic response evaluation was performed; therefore, potential effects on glycemic response require further investigation.

In addition, whey incorporation enhanced the content of micronutrients such as copper and maintained adequate levels of iron and magnesium. However, a consistent decrease in zinc content was observed, indicating the necessity of complementary fortification strategies. The increase in sodium content associated with whey addition highlights the importance of careful formulation to avoid excessive intake and related cardiovascular risks.

From a technological perspective, whey addition above 15% appeared to influence the sensory perception of texture, particularly through increased hardness and reduced extensibility, highlighting the importance of optimizing the incorporation level to maintain both nutritional value and overall sensory acceptability. FTIR analysis provided clear evidence of structural modifications induced by whey addition. The progressive increase

in the intensity of amide I and II bands, along with changes in the O–H/N–H region and the reduction in starch-specific bands, confirmed the integration of whey proteins into the starch matrix and the establishment of protein–starch interactions. Furthermore, SAXS analysis complemented these findings, demonstrating that whey addition also modifies the supramolecular organization of the system, with direct implications for solubility, texture, and digestibility.

The obtained results demonstrated that whey powder incorporation improved the protein content and influenced the structural characteristics of the developed products. However, higher whey addition levels were associated with certain limitations, including lower sensory acceptability due to slightly salty flavor notes and a decreasing trend in zinc content. Therefore, further optimization of formulation and incorporation levels is necessary to achieve an optimal balance between nutritional enhancement and consumer acceptability. Although the present study did not include microbiological analyses, water activity measurements, or extended shelf-life evaluation, the investigated biscuits represent low-moisture bakery products with reduced perishability and enhanced stability due to the thermal processing applied during baking. Future studies will further investigate storage stability and microbiological safety under different preservation conditions.

Additionally, the use of whey powder as a fortifying agent in rice-based gluten-free formulations contributes to the valorization of dairy by-products within a circular bioeconomy framework, enhancing both sustainability and functionality.

Finally, the development of clean-label, multifunctional gluten-free products enriched with whey-derived ingredients represents a promising direction for both academia and the food industry, aligning with current trends in functional foods and sustainable resource utilization.

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Institutional Review Board Statement: The research did not involve the collection of biological samples or any medical interventions. All tests conducted in this study adhered to rigorous data protection procedures and ethical guidelines in accordance with international standards for sensory testing with human subjects, including ISO 11136:2014 guidelines. The evaluation panel followed pre-established protocols to ensure compliance with ethical standards and data protection regulations. The sensory evaluation performed in this study involved voluntary adult participants and non-invasive testing of conventional food products. According to the Bioethics Commission of University of Life Sciences “King Mihai I” from Timisoara, this study involves low risk to human subjects (No. 691/13.04.2026).

Informed Consent Statement: All the panelists who participated in the sensory analysis gave their consent by filling in the consent form. This consent also certifies that the analyzed products do not contain ingredients that are not allowed by national/European legislation.

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

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

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