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Chemical profiling of domestic grape peel and its potential in bread quality improvement

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ABSTRACT

Background: The development of functional foods begins with selecting raw materials rich in bioactive compounds. Incorporating grape peel by-products into wheat manufacturing offers a sustainable strategy to enhance the product's nutritional value.

Objectives: This research aimed to review the health properties of grape peel-enriched bread and its functional food potential through vitamin and mineral evaluation. Furthermore, the study optimized grape skin drying conditions to preserve its chemical composition.

Methods: Grape pomace powder was produced by drying grape skins at 23°C for seven days, with regular mixing. Dried skins were then ground and sieved (420µm). Bread was produced using a one-phase method, with grape skins added at 10%, 15%, and 20% of the flour mass.

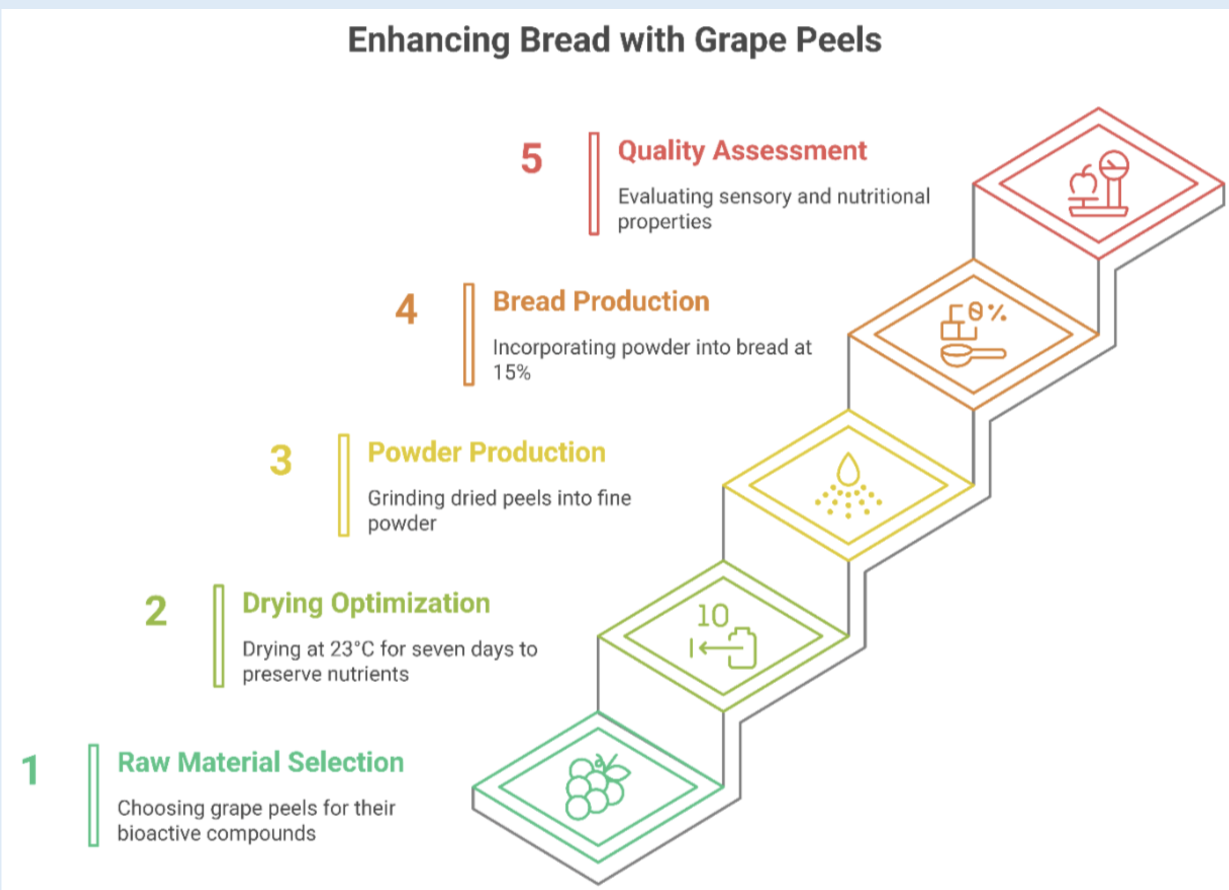
Results: Sensory and physicochemical analyses indicated that incorporating 15% grape peel produced the optimal bread quality. Despite its thermal instability, preserving vitamin C in baked products significantly contributes to functional food science.

Novelty of the Study: This study introduces an innovative approach to enhancing bread production's nutritional value and sustainability by incorporating grape peel by-products. Furthermore, optimal drying conditions (23°C for 7 days) that preserve bioactive compounds in Areni Clone 2 grape skins have not been established before this study. Grape peel by-products have also demonstrated their potential as a functional food ingredient, rich in dietary fibers, vitamins, and minerals. Notably, maintaining vitamin C in baked goods highlights a unique contribution to functional food science that grape peel by-products could offer.

Conclusion: This study demonstrates that waste products from the wine industry are valuable functional food ingredients. Areni Clone 2 grape skins were rich in dietary fibers, vitamins (B1, B2, PP, C), and minerals (calcium, sodium). Overall, 15% grape peel incorporation was optimal for bread quality.

Keywords: Grape peel, functional foods, bioactive compounds, by-product, bread

Graphical Abstract: Chemical profiling of the domestic grape peel and its potential for bread quality improvement.



INTRODUCTION

Utilizing grape peels in wheat manufacturing enhances nutritional value and sustainability. This strategy directly uses waste from the wine industry to add antioxidants and fiber to commonly consumed foods, such as bread, appealing to health-conscious consumers [1]. Grape peels improve a product's sensory qualities and offer health benefits using natural ingredients. Research confirms that grape peels enhance color, flavor, and texture in wheat-based foods [1-2]. Improving the quality of this product appeals to both manufacturers and consumers.

This evidence supports functional food research and could offer sustainable production methods using a grape byproduct [3]. Grape peel integration addresses consumer preferences for health and sustainability [4-5]. Transparency and sustainability drive consumer decisions, prompting brands to rethink sourcing and production. Companies that invest in sustainable practices and packaging reflect a shift towards ethical and viable food business practices [3]. This is supported by consumer demand for responsibly sourced foods.

The development of functional food products begins with justifying raw material choices based on bioactive substances [6-7]. Food bioactive compounds (FBCs) are nutritive and non-nutritive compounds that promote health through eliciting a bioactive impact on the human body [5,8]. Bioactive compounds (BC) found in functional foods demonstrate beneficial biological activities, offering extra-nutritional benefits due to their antioxidant properties and disease preventative capabilities [9]. The health consequences of obesity have become so ubiquitous and serious that it is considered a global pandemic. Customers have moved to prioritize natural ingredients, which has significantly influenced the food industry [10-11].

Although functional foods are characterized by bioactive compounds, which are molecules that improve health through physiological mechanisms, the United States Food and Drug Administration (FDA) currently lacks a formal definition of functional foods. However, the Functional Food Center (FFC) has contributed to developing an improved and comprehensive definition of functional foods and a criterion for classifying a functional food. Functional foods are natural or processed foods that contain biologically active compounds that reduce disease risk by providing additional health benefits beyond their essential nutritional value. [12].

Bread is a common functional food that is a viable energy source and contains nutrients such as folate, B vitamins, zinc, and fiber [13]. White bread is widely consumed, while whole wheat and rye are associated with those with higher incomes [14]. Bakery products are ideal for fortification as individuals consume approximately 126.5 kg annually [15-16].

Dietary fiber deficits are a concern. Fibers, including cellulose and pectin, resist digestion, but ferment in the large intestine. However, the recommended daily intake for fiber varies between organizations: 20-25 grams (Methodological Guidelines), 30 grams (WHO), or 25-38 grams (USA) [17].

Wine by-products, like grape pomace, fortify foods cost-effectively. By-products in the wine industry contain diverse phenolic compound compositions with antioxidant and antimicrobial properties [18].

MATERIALS AND METHODS

This research focused on bread's health benefits and potential functional food ingredients. The study's key objective was to determine the optimal drying method for grape skins to preserve their vitamin and mineral composition without alteration.

The primary focus of the research was to identify the chemical composition of the grape derived from local cloned varieties. A significant scientific advancement in this study was the development of a clone of the Areni grape variety, known as Areni Clon 2. This clone is notable for its more prosperous chemical composition in the berry skin, making it a more valuable functional ingredient for bread production.

During grape processing, the fruit is crushed and separated from the stems. As documented in the literature, the solid parts of the grapes, such as peel, stems, and seeds, are a source of fermentable sugars. In addition, grapes are rich in valuable components, and the quantity of these components can vary depending on the variety and cultivation conditions.

To obtain grape pomace powder, the material was dried on flat mesh surfaces at a stable laboratory temperature of 23°C. During the drying process, which lasted 7 days, the mass was mixed 3-4 times daily. After drying, the material was ground and sieved through a 420mm sieve.

Determination of dietary fibers: Advanced measurement devices were employed to determine dietary fibers accurately. The properties of the dietary fibers were assessed using methods based on the oxidation, degradation, and solubility of different chemical compounds. It was found that the fibers are insoluble in the production process and are filtered and weighed. A precisely weighed 1 g sample of ground material was placed in 120 cm³ of solution, followed by 40 cm³ of acid (3.6 cm³ of pure acid, 36.4 cm³ of 80% sulfuric acid solution). The solution was sealed and kept at room temperature for 1 hour. Then, it was filtered using an N2 glass filter. The residue was washed several times with a 0.2M sodium hydroxide hot ethanol solution, then rinsed several times with distilled water and a mixture of ethanol and water. The clean, white residue was dried at 100-105 °C until a stable weight was achieved. The residue was then packaged and weighed using an analytical scale [17].

Experimental sample moisture determination: The moisture content of the resulting samples of raw soybeans, flour, and wafers was determined using a Kern DAB analyzer with an accuracy of 0.95%. The samples were dried in SOFT mode, which is designed for complete dehumidification. The drying temperature gradually increased to 120 degrees C until the samples were fully dried. The temperature was maintained at this level for the remainder of the process. The samples were placed on a drying tray with a flat, evenly distributed surface. At least 15 grams of grains were placed in the bowl [19].

Assessment of bread porosity: To assess the porosity of bread, a standard piece measuring 7 to 8 cm wide was cut from the center, and impressions were made using a cylinder from the Zhuravlyov device. The sharp edge of the cylinder was pre-greased with vegetable oil. The cylinder was then inserted into the crumb of the bread piece, in a rotating motion. The bread crumb was pushed out of the cylinder using a wooden plug and cut at the edge of the cylinder using a sharp knife. The internal diameter of the cylinder was 3cm, and the distance from the mark to the cut is 3.8cm, giving a total volume of 27 cm^3.

Three impressions were conducted to determine the porosity of the bread. Porosity was determined using the formula $X = V/(M\rho)$, where V is the total volume of the impressions in cm³, M is the mass of the impressions in grams, and ρ is the density of the non-porous crumb mass in g/cm³. The porosity of the product was calculated with an accuracy of up to 1% [19]. The prepared samples were simultaneously weighed on technical scales with an accuracy of 0.01g. The density of the non-porous crumb mass (g/cm³) from the highest and first-grade wheat flour was 1.31 g/cm³.

Determination of the specific volume of bread: The particular bread volume was determined by dividing the volume of the bread in cm³ by its mass [20].

Determination of shape stability (H: D): The diameter D and height H of round bread were determined using a ruler or a specialized device. The bread was first cut in half along the diameter, and the height and diameter of these halves were measured at their most significant cut point [20].

Determination of vitamins: In this study, B-vitamin analysis was performed using a sophisticated HPLC system to assess the final product's composition and the purity of raw materials. Samples were filtered through a 0.45 mm Millipore filter and introduced into HPLC vials. The HPLC analysis was conducted with a Shimadzu LC-2010 system.

For thiamine(B1) analysis, a standard stock solution was prepared by dissolving 26.7 mg of thiamine hydrochloride in 25 mL of double-distilled water. Similarly, for riboflavin (B2), 6.9mg of riboflavin was dissolved in 100 mL of extraction solution, with a maximum solubility of 7mg riboflavin. The buffer solution was made by dissolving 1.36 g of potassium dihydrogen phosphate and 1.08 g of hexane sulfonic acid sodium salt in 940 mL of HPLC-grade water, adding 5 mL of triethylamine, and adjusting the pH to 3.0 with orthophosphoric acid. The extraction solution was prepared by mixing 50 mL of acetonitrile with 10 mL of glacial acetic acid, bringing the final volume to 1000 mL with double-distilled water.

For sample preparation, 10 g of each sample was homogenized, transferred to conical flasks, and mixed with 25 mL of extraction solution. The mixture was shaken in a water bath at 70°C for 40 minutes. The mixture was then cooled, filtered, and diluted to 50 mL with the extraction solution. An autosampler injected the filtered samples (20 µl aliquots) into the HPLC system. A Waters Symmetry C18 column (4.6×150 mm, 5 mm) was employed with a buffer composition of menthol (96:4), a flow rate of 1 mL/min, and a pressure of 2300 psi. Detection was performed using a UV detector at 210nm, with a 5nm bandwidth. Solutions were degassed by sonication before injection.

Standard solutions at concentrations of 5 mg/mL, 10 mg/mL, 20 µg/mL, and 40 mg/mL were prepared and injected into the HPLC to validate this method. The analyses were conducted in triplicate, using calibration curves to determine the coefficient of correlation, slope, and intercept values. The concentration of B-vitamins in the samples was calculated using the equation $y = mx + c$, where y represents the peak area, and x represents the vitamin content. The results were then adjusted by the dilution factor [17].

Determination of alkalinity by titration: To determine alkalinity by titration, the alkaline substances present in flour confectionery products were neutralized using acid in the presence of a bromothymol blue indicator, until a yellow color appeared. To begin, 25 g of the crushed sample, which was selected according to section 8.2 and weighed to the second decimal place, was placed in a 500 cm³ conical flask. Then, 250 ± 1 cm³ of distilled water was added, measured using a graduated cylinder. The mixture was thoroughly mixed, and a stopper closed the flask. The mixture was left to stand for 30 minutes, shaken every 10 minutes. After the 30-minute period, the contents of the flask were filtered through a filter paper into a dry flask or glass. Next, 50±0.5 cm^3 of the filtrate, measured with a graduated cylinder, was transferred into a 250 cm^3 conical flask. Two to three drops of bromothymol blue solution were added, and the mixture was titrated with a sulfuric acid solution at a concentration of 0.1 mol/dm^3 (1/2 H_2SO_4) or hydrochloric acid with a concentration of 0.1 mol/dm^3 (HCl) until a yellow coloration appeared. The results were then processed according to the formula provided in the standard [20-21].

Statistical analysis: The data were collected using scientific databases, such as ISI Web of Knowledge, ResearchGate, Elsevier, and Scopus, as well as traditional texts and books. Field experiments were replicated threetimes. All analyses were carried out in four copies, and the data were expressed as means ± standard deviation.

A comparative analysis was done using parametric ANOVA, with a Bonferroni correction applied for multiple comparisons. The Mann-Whitney test was used for data that did not follow normal distribution. Statistical calculations were performed using SPSS Version 16.

RESULTS AND DISCUSSION

Grapevine by-products, produced during the winemaking process, are often considered unsuitable secondary raw materials [22-23]. However, they contain valuable functional components. Figure 1 shows the technological stages involved in obtaining these components. The drying process was conducted under natural conditions, without washing and exposure to moisture, to preserve the maximum amount of beneficial ingredients. The process of peeling the grape is depicted in Fig. 1.

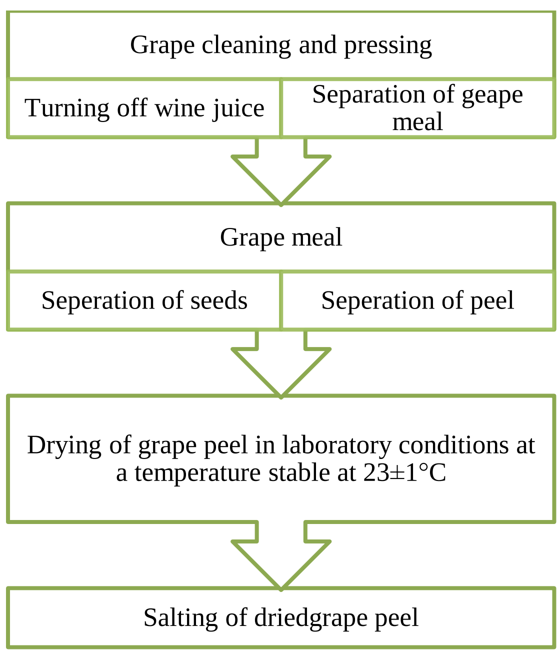


Fig 1. Technological sequence of obtaining grape mint powder

The selection of grape variety was based on the widespread use of the Black Areni variety as a primary raw material for winemaking in the Republic of Armenia. The Areni clone two variety was developed through previous research, exhibiting distinct chemical properties and unique mechanical composition compared to the traditional Areni variety. In this study, a comparative analysis between Black Areni and Areni Clone 2 was conducted to highlight the advantages of the new array as a functional food ingredient. Table 1 shows the chemical composition of the two grape varieties.

Table 1. Chemical composition of grape peel

The name of the indicators	Content, %	
	Black Areni	Areni clon 2
Dietary fibers	4,1±0.1	5,6±0.1
Ash	13,3±0.1	13,4±0.1
Mass fraction of sucrose	9,2±0.1	12,6±0.1
Flavanoid content	2,4±0.1	6,9±0.1

$P<0.5$

Trial baking tests were conducted to study the influence of grape peel on bakery products' organoleptic and physicochemical quality indicators.

Breads made from premium wheat flour or rye were produced using a one-phase method following standard recipes and technological protocols. Grape skins were added at 10%, 15%, and 20% of the total mass of the flour. The grape skins were pre-mixed with the flour before dough preparation. The established technological sequence prepared wheat and rye bread without leavening.

The bread yield is based on 100 kg of flour, the basis for calculating the recipe. For each batch, the corresponding amount of flour was adjusted to account for the grape flour addition. For example, in a formulation using 1 kg of flour, the ingredients included 900 g of wheat flour, 50 g of grape flour, 10 g of table salt, 20 g of dry yeast, and 400-450 g of water. The grape flour was thoroughly mixed with the wheat flour before further processing.

The fermentation time varied depending on the flour type, with wheat dough fermenting for 15 minutes and rye dough fermenting for 25 minutes. The extended fermentation time is explained by the presence of acetic, lactic, tartaric, and other organic acids, which contribute to the dough's gas formation and gas separation.

The bread was prepared without the sourdough method following the appointed technological sequence. The calculation for the formula was based on the bread yield from 100 kg of flour. For each added portion of grape peel, the corresponding amount of flour was removed. For example, 1 kg of flour, 90 g of wheat flour, 10 g of grape peel, 10 g of salt, 20 g of dry yeast, and 400-450 g of water were used. The grape peel is mixed together with the flour. The fermentation duration was 15 minutes for wheat dough and 25 minutes for the rye dough. The quick fermentation process is explained by acetic acid, lactic acid, tartaric acid, and several other acids in the grape peel, which contribute to the dough's gas formation and release properties. The results are presented in Table 2.

Table 2. Studies of physicochemical and sensory indicators of the quality of bakery products

The name of the Indicators	Control variante /wheat bread/	Wheat bread made from premium flour			Control variante /rye bread/	Rey bread made		
		10	15	20		10	15	20
Moisture content, %	40,5	40,5±0.01	40,6±0.01	40,6±0.01	43,4	43,4±0.01	43,5±0.01	43,5±0. 01
The acidity, ^{0}	2,0±0.01	2,2±0.01	2,4±0.01	2,6±0.01	8,3±0.01	8,6±0.01	9,1±0.01	9,5±0. 01
Porosity, %	75±0.1	71±0.1	68±0.1	65±0.1	58±0.1	53±0.1	50±0.1	48±0. 1
Form resistance, H:D	0,7±0.01	0,7±0.01	0,8±0.01	0,8±0.01	0,6±0.01	0,6±0.01	0,7±0.01	0,8±0. 01
Total volume, cm^3	790±0.02	810±0.02	825±0.02	857±0.02	762±0.02	768±0.02	772±0.02	780±0. 02
Sensory indicators								
Form	According to this product	No injuries	With noticeable cracks on the surface		According to this product	According to this product	No injuries	
Crumbs of bread	Elastic with no color difference	Elastic, with a light pink color	Elastic with no color difference		Elastic with no color difference	Elastic, with a light pink color	Good elasticity, dark pink	
Taste and smell	According to this product	Slight sour taste	Pleasant sour taste and aroma characteristic of grapes		According to this product	Slight sour taste	Pleasant sour taste and aroma characteristic of grapes	
Color	Slight brown	Slight pink color	Expressive pink color		Pink color	Dark pink color		

P<0.05

The results showed an increase in specific volume by 3.4-13.7% and porosity by 2-8% for bread samples with added grape peel, compared to the control. The most significant improvement in quality indicators was observed by adding 15% grape peel. Increasing the mass fraction of the additive to 20% in the recipe for bakery products led to only a slight improvement in bread quality. This is likely due to decreased carbon dioxide in the dough during the proofing and baking process. The rise in acidity of the products is associated with the presence of organic acids (tartaric, malic, citric, and succinic) added by the grapes. Based on the results of the products in the trial laboratory, it was noted that using a dose of 15% was most effective in achieving the maximum improvement in the organoleptic and physicochemical quality indicators.

The main component of the peel is fiber, which is chemically linked with polyphenols, metal cations, and pectic substances. The study also examined possible changes in dietary fiber content, as shown in Figure 2.

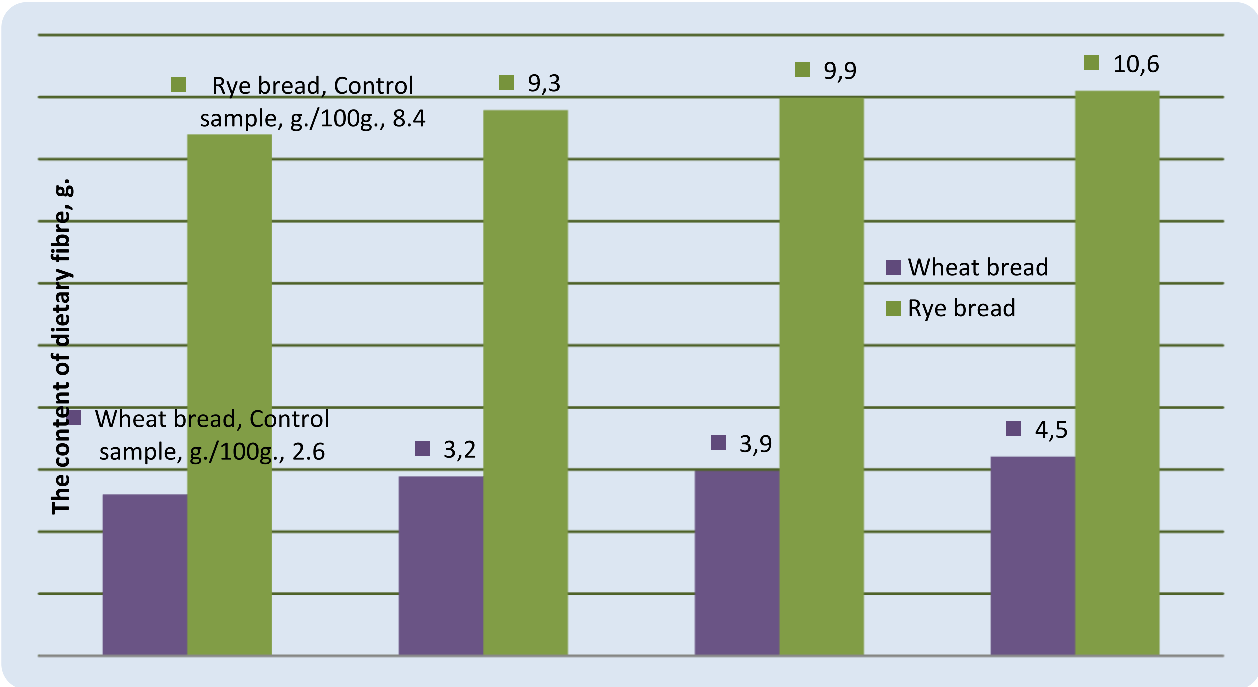


Figure 2. The change in dietary fiber content in baked products with the addition of grape peel.

Although baked products contain dietary fibers, their quantities can vary significantly depending on the type of flour used. For example, high-quality wheat flour has a relatively low content of this component because bran and fiber are completely removed during the refining process. However, whole grain flour contains more fiber as it is not subjected to refinement. As seen in Figure 2, when up to 20% grape peels were used, the content of dietary fibers in 100g of wheat bread increased to 4.5g, while in rye bread, it rose to 10.6g. This shows considerable content improvement, demonstrating that grape peels could produce functional foods. The human body requires 20-25 grams of dietary fiber per day. The body can only obtain this from plant-based foods; however, the current amount consumed is roughly 15 grams [17]. Bioactive compounds affect the function of organs and systems within the human body. These ingredients control cellular metabolism and create homeostasis at different periods of life. However, the mechanism of the effect of plant compounds on the lymphatic system remains unknown [24].

During the study, attention was drawn to the change in vitamin and mineral composition within the baked goods. The study was prompted by the high content of vitamins and minerals in grape peels. Therefore, the study offered the potential for bread products to contain these components. The results of the study are presented in Table 3.

Table 3. Comparative indicators of vitamin and mineral composition of taste samples of bakery products and experimental samples

The name of Indicators	Control variante /wheat bread/	Wheat bread made from premium flour, per 100 g of product			Control variante /rye bread/	Ray bread made from premium flour, per 100 g of product		
		10	15	20		10	15	20
Vitamins, mg								
Vitamin B1	0,5±0.02	0,9±0.02	1,6±0.02	2,2±0.02	0,7±0.02	1,1±0.02	1,6±0.02	1,9±0.02
VitaminB2	0,4±0.02	0,8±0.02	1,4 ±0.02	1,9±0.02	0,6±0.02	0,9±0.02	1,4±0.02	1,8±0.02
Vitamin B3 or PP	5,50.02	6,4±0.02	7,1±0.02	7,9±0.02	3,8±0.02	4,2±0.02	4,8±0.02	5,3±0.02
Vitamin C	Not detected	0,4±0.02	0,9±0.02	1,4±0.02	Not detected	0,5±0.02	0,6±0.02	0,68±0.02
Minerals, mg								
Iron	3,1±0.02	3,4±0.02	3,7±0.02	4,1±0.02	2,8±0.02	3,2±0.02	3,9±0.02	4,3±0.02
Calcium	123,0±0.02	125±0.02	127,5±0.02	129,4±0.02	73,0±0.02	79,9±0.02	83,0±0.02	88,1±0.02
Sodium	471,0±0.02	482±0.02	489±0.02	492±0.02	603,5±0.02	609,8±0.02	613,2±0.02	619,1±0.02

Several studies have highlighted the potential of incorporating grape by-products into bakery items to develop food products with stronger nutrient profiles [25-27]. These studies and our findings demonstrate the feasibility of enhancing nutritional profiles by utilizing grape by-products from the wine industry. This research has shown that grape pomace significantly increased the antioxidant capacity of bread [26], which aligns with our observation of increased vitamin content in the experimental samples.

Enhancing bakery and pasta products with grape by-products has been shown to improve their nutritional profile by increasing fiber content and introducing polyphenolic compounds with antioxidant properties [4, 28]. Studies have found that grape seed extract increased the fiber content of pasta [4], mirroring the vitamin and mineral enrichment we observed in our bread samples. This suggests that grape by-products can effectively serve as a natural fortifying agent in baked goods.

These natural substances possess various biological activities, such as antioxidants and antibacterial properties. Grape peel is a rich source of polyphenolic compounds and dietary fiber [29], contributing to antioxidant and antimicrobial properties [30]. The primary antioxidant components in grape peel, including anthocyanins, catechins, and flavanols, play a crucial role in inhibiting oxidative processes in low-density lipoproteins [30]. Grapes contain various bioactive compounds, especially phenolic compounds and organic acids. Phenolic compounds and organic acids have antioxidant, anti-inflammatory, and antimicrobial properties [31]. Despite the high temperature conditions, these properties likely contributed to the retention of vitamin C observed in our baked samples.

Previous research has demonstrated the potential of polyphenols and dietary fiber in enhancing food preservation and extending shelf life [32]. Grape peel contains up to 60% (dry matter) dietary fiber [33], which is essential for human health as it supports gastrointestinal function, regulates blood sugar levels, and lowers cholesterol [34]. Given the importance of dietary fiber and its current underconsumption (25–30 g) [35], incorporating grape peel into bread offers a practical approach to addressing this nutritional deficiency.

However, it has been reported that the presence of grape by-products in dough could negatively impact its rheological properties due to gluten dilution [25]. This effect can be mitigated by reducing particle size, as we ensured in our study. Additionally, higher concentrations of grape by-products significantly influence food texture, volume, and color [36]. This study found that a 15% incorporation of grape peel yielded optimal organoleptic and physicochemical quality indicators, suggesting that careful optimization is essential to balance nutritional benefits with sensory attributes.

Future studies should focus on research related to other local grape varieties that use different dosages of grape peels. Additionally, it would be valuable to create more specific functional ingredients enriched with tailored bioactive molecules for distinct population groups. This may prevent certain disorders and promote general health across diverse demographics. Furthermore, the mechanism of vitamin C retention in the baking process requires a more in-depth evaluation.

Scientific Innovation and Practical Implications: This study innovatively improves food sustainability and functional food development by integrating grape peel by-products from the Areni Clone 2 grape variety into bread formulations. While the use of food by-products in functional foods has been explored, this research uniquely focuses on optimizing drying conditions (23°C for 7 days) to preserve bioactive compounds in Areni Clone 2 grape skins, ensuring maximum retention of dietary fibers, vitamins (B1, B2, PP, C), and essential minerals (calcium, sodium).

A particularly groundbreaking finding is the presence of vitamin C in baked goods formulated with Areni Clone 2 grape skins, as this vitamin typically degrades at high temperatures. This result suggests a promising mechanism of vitamin retention, with possible protective interactions between the polyphenols within the Areni Clone 2 grape peel. Furthermore, this study systematically determines the optimal dosage of grape peel to enhance wheat and rye bread's organoleptic and physicochemical properties. This could provide a scalable model for bakery product development.

This research advances functional food science and promotes a sustainable food system by addressing the waste products in wine production from the Areni Clone 2 variety, for nutritional enhancement in wheat-based foods, which offers consumers access to nutritionally enhanced staple foods.

CONCLUSION

This research demonstrates that grape peel by-products from the Areni Clone 2 grape variety are valuable functional food ingredients. Specifically, Areni Clone 2 grape skins are rich in dietary fibers, vitamins (B1, B2, PP, C), and minerals (calcium, sodium), enhancing baked goods' nutritional profile. Notably, vitamin C was preserved in baked goods despite high-temperature baking, indicating a potential protective mechanism against vitamin degradation in grape peel. Systematizing experimental data revealed that a 15% grape peel incorporation was the optimal dosage for enhancing wheat and rye bread's organoleptic and physicochemical properties. Furthermore, the use of grape skins contributed to a reduction in dough fermentation time and increased dough volume, suggesting potential benefits in industrial baking. The scientific novelty of this research lies in the unique utilization of Areni Clone 2 grape peel, a local variety with a rich chemical composition, to produce functional bread, contributing to both nutritional enhancement and sustainable food practices.

List of abbreviations: RA: Republic of Armenia; HPLC: High-Performance Liquid Chromatography, mg: milligram, g: gram, FFC: Functional Food Center.

Authors' Contributions: All authors contributed to this article. NH conceived the research topic and served as the lead scientist. LG, GP, VG, and VA analyzed vitamin and mineral composition and performed comparative evaluations. AG provided statistical analyses. GP, KK, and AS contributed to the study of physicochemical parameters. DM provided detailed guidance and review of the article, advised on the writing process, and participated in result discussions and manuscript preparation.

Competing interest: The authors declared that there is no competing interest.

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