THE USE OF GRAPE POMACE FOR DEVELOPING AN INNOVATIVE YOGHURT WITH ENHANCED ANTIOXIDANT PROPERTIES

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Abstract

The present paper explores the use of grape pomace, a by-product of the wine industry, as a functional ingredient for enriching yoghurt with the aim of increasing its nutritional value and antioxidant capacity. Through modern ultrasound-assisted extraction techniques, bioactive compounds were recovered from the grape pomace, resulting in a high content of polyphenols, flavonoids, and anthocyanins. The study evaluated the impact of adding grape pomace powder in 1% and 2% proportions on the chemical, phytochemical, and sensory properties of the yoghurt. The chemical results showed an increase in fibre, protein, and dry matter content, while the phytochemical analysis revealed a significant rise in total anthocyanins, flavonoids, and polyphenols, along with greater antioxidant activity in the enriched yoghurts compared to the control yoghurt. Additionally, the sensory analysis demonstrated high acceptability for the yoghurt with 3% grape pomace powder, achieving a total score of 19.6, classifying it as "very good," while the 6% variant received a lower score. These findings highlight the potential of using grape pomace to create innovative dairy products with improved nutritional and functional benefits, thus contributing to the sustainable valorisation of an agro-industrial by-product.

Key words: yoghurt, by-product, grape pomace, quality.

Grape pomace, a by-product of wine and juice production, contains skins, seeds, and stems particularly bioactive compounds, in polyphenols like anthocyanins and flavonols with strong antioxidant and health-promoting properties (Averilla A. et al, 2019; Beres C. et al, 2017). Its nutritional potential has sparked interest in its application in functional foods, such as yogurt (Almanza-Oliveros A. et al, 2024). Yogurt, a probiotic-rich dairy product, is widely recognised for promoting gastrointestinal health, enhancing nutrient absorption, and supporting immune function (Gavril R.N. et al, 2024; Tikhomirova N.A. et al., 2020). The incorporation of grape pomace into yogurt may not only enhance its antioxidant capacity but also provide additional dietary fiber and nutrients, making it a promising avenue for innovation in dairy products (Constantin O. et al, 2024).

Recent studies show that incorporating horticultural by-products into yogurt can enhance its quality and consumer appeal (Belardi I. *et al*, 2024). Adding grape pomace aligns with food innovation trends, addressing sustainability and health demands (Marchiani R. *et al*, 2016). Combining grape pomace with probiotics offers

potential for a novel yogurt with improved health benefits and reduced grape processing waste (Castangia I. *et al*, 2023).

The purpose of this work is to explore the integration of grape pomace into formulations, addressing the dual objectives of promoting health benefits and enhancing sustainability. This aligns with contemporary trends in food innovation, which emphasize the development of products that support consumer well-being and reduce environmental impact. By combining the nutritional properties of grape pomace with the probiotic benefits of yogurt, this study aims to create a novel yogurt product that delivers enhanced health benefits simultaneously addressing the challenge of food waste generated during grape processing.

MATERIAL AND METHOD

The unfermented GP of the Feteasca Neagra variety came from a winery (laşi University of Life Sciences). 50 litres of cow's milk were given by the Rediu laşi Research Station of the University of Life Sciences. The skins were separated mechanically and then dried in an oven at 40±2°C for 48 hours, until they had a moisture

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content of 9.87%. With a food grinder, the GP were ground down to 0.5 mm. For 15 minutes at 121 °C, a steriliser was used to clean GP before it was used to make yoghurt.

The following chemicals were bought from DPPH (2,2-diphenyl1-Sigma Aldrich: picrylhydrazyl), gallic acid, sodium acetate, potassium chloride solution, ethanol, sodium carbonate, methanol, sodium carbonate, Folin-Ciocâlteu sodium hydroxide, reagent, aluminium chloride.

The bioactives were taken out of GP powder using the ultrasound-assisted extraction method. So, 1 gramme of GP was mixed with 10 millilitres of an 80% ethanol solution that had been acidified with citric acid (7:1, v/v). The mixture was then subjected to ultrasound for 25 minutes at 35°C and 37 kHz. The supernatant that was left over was gathered and spun at 6500 rpm for 10 minutes at 4°C. After that, the GP extract was used to study phytochemicals (including anthocvanins. flavonoids. and polyphenols) and antioxidant action. The amount of total monomeric anthocyanin was found using a modified version of the pH difference method used by Lipsa et al. (2024). We checked the absorption of diluted extracts using different buffer solutions with pH levels of 1.0 and 4.5 at 520 nm and 700 nm. The results were shown as mg cyanidin-3-glucoside (C3G)/g of dry weight (dw).

The method outlined by Dewanto et al. (2002) was used to find out how many total flavonoids were in GP extract. Soon, 2 mL of pure water were mixed with 0.25 mL of the GP extract and 0.075 mL of 5% sodium nitrite.

After 5 min., 0.15 mL of 10% aluminium chloride is added and the mixture is left to work for another 6 minutes. Finally, 0.5 mL of 1M sodium hydroxide is added. A wavelength of 510 nm was used to measure the absorption of the mixture that was made. The total amount of flavonoids is given in mg catechin equivalents/g sample (mg CE/g d.w.). To find out how much total polyphenolic content there was, the Folin-Ciocâlteu spectrophotometric method described by Dewanto et al. (2002) was used.

200 µL of the GP extract, 1 mL of the Folin-Ciocâlteu reagent, and 15.8 mL of pure water were put into a test tube. They were left alone for 10 minutes, then 3 mL of 20% sodium carbonate was added. They were then left alone in the dark at room temperature for an hour. 765 nm was used to measure the absorption. The findings were given in mg of gallic acid equivalents per gramme of sample (mg GAE/g d.w.).

To find out how much antioxidant activity there is by blocking the DPPH radical, the steps outlined by Castro-Vargas et al. (2010) were followed. In a 100 mL volumetric 560 flask, 3.8 mg of DPPH was mixed with methanol to make a stock solution. After that, 100 μ L of the sample to be tested (GP extract) and 3.9 mL of DPPH (A sample) were put into a test tube. 100 μ L of

methanol and 3.9 mL of DPPH (A control) were used for the control sample. It was read at a wavelength of 515 nm after being left alone for 90 minutes in the dark. The values were shown as μ mol of Trolox equivalents (TE)/g dry weight. We looked at how the antioxidant capacity changed by finding the inhibition (I%) for each sample. To do this, we used the formula I% = (A controlA sample)/A control x 100.

Getting unpasteurised milk, tasting it, and analysing it. 200 litres of milk were taken from the farm's storage tank and put in clean containers. It was kept at 4°C for twenty-five hours.

Once the milk was completely mixed, it was added to the lab tests for analysis. According to Ratu et al. (2024), the AOAC methods were used to find out the physicochemical parameters of milk samples. These parameters included the pH, solid non-fat content, protein content, and fat content (moisture content).

Making yoghurt that has been improved with GP.

The pasteurised milk was heated to 42°C, at which point the selected lactic cultures YF-L812, a commercial product from Chr. HANSEN, Denmark - a mix of Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus (2:1) were added. After the addition of the selected cultures (with the milk maintained at 42°C), the three batches were prepared as follows: Lc – the control batch (without added powder), LY-3 - the batch with 3% GP added, and LY-6 - the batch with 6% GP added. Once the batches were prepared, the milk was portioned into plastic cups, heat-sealed, and placed into a thermostat (figure 1). According to the Association of Official Analytical Chemists (AOAC) (Usturoi et al, 2017; Tseng & Zhao, 2013), the pH, fat, ash, moisture content, and total protein of the samples were measured.

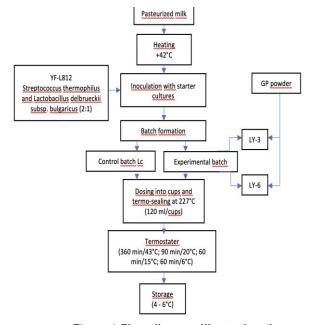


Figure 1 Flow diagram illustrating the processing step of yoghurt

Sensory Analysis

The sensory evaluation of the yoghurt samples was carried out by a panel comprising 10 individuals. A 9-point hedonic scale was employed assess the sensory attributes, with 1 representing the least pleasant/weakest and 9 representing the most pleasant/strongest. The attributes evaluated included appearance, colour, aroma, texture, taste, odour, aftertaste, and overall acceptability. The panel members were nonsmokers, aged between 24 and 40 years, all with academic backgrounds in the food industry. The yoghurt samples were presented in a randomised order. Prior to the evaluation, participants were informed about the study's purpose and the measures taken to ensure the confidentiality of their personal data.

Statistical Analysis

The statistical analysis was performed using the data analysis tools available in Microsoft Excel and the statistical software Minitab 19. Standard deviations were determined based on results obtained from triplicate experiments.

RESULTS AND DISCUSSIONS

The polyphenolic, flavonoid, and anthocyanin contents in the ethanolic extract of GP powder were determined using spectrophotometric methods (*table 1*).

Table 1

Phytochemical characterizations and colorimetric
parameters of the GP powder

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Parameters	GP powder		
Total anthocyanin content	1.60±0.23		
(mg C3G/g d.w.)	1.0010.23		
Total flavonoid content (mg CE/g d.w.)	8.90±0.69		
Total polyphenol content	21.06±0.69		
(mg GAE/g d.w.)	21.00±0.09		
Antioxidant activity	25.01±0.09		
(DPPH, µmol TE/g d.w.)	20.01±0.09		
Inhibition %	89.03±0.33		

The total polyphenol content measured 21.06 ± 0.69 mg GAE/g d.w., while the total flavonoid content was 8.90 ± 0.69 mg CE/g d.w.. The anthocyanin content reached 1.60 ± 0.23 mg C3G/g d.w., accompanied by significant antioxidant activity of 25.01 ± 0.09 µmol TE/g d.w., with a high inhibition rate of $89.03\pm0.33\%$.

Comparative studies have reported variations due to differences in extraction methods and raw material. For instance, Serea et al. (2021)

observed higher anthocyanin values (4.29±0.04 mg C3G/g) but lower antioxidant activity (14.07±1.03 mM Trolox/g) in red grape peel extracts, while Rockenbach *et al.* (2011) reported anthocyanin ranges of 1.84-11.22 mg C3G/g d.w. in pomace extracts. Variability in phytochemical content can be attributed to the plant source, extraction techniques, and solvent composition.

The principal chemical indices were evaluated to determine the quality parameters of raw cow's milk. *Table 2* summarises the chemical composition of the analysed samples. The water content averaged $87.13\pm0.08\%$, while total solids constituted $12.87\pm0.12\%$. Notably, the fat content was measured at $3.93\pm0.11\%$, contributing to a solid non-fat content of $8.94\pm0.16\%$. The mean protein level was $3.39\pm0.05\%$, and the average pH value was 6.61 ± 0.04 .

Table 2 Chemical composition of raw milk

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Parameters	Mean		
Water (%)	87.13±0.08		
Fat (%)	3.93±0.11		
Protein (%)	3.39±0.05		
Total Solids (%)	12.87±0.12		
Solid non fat (%)	8.94±0.16		
pH	6,61±0.04		

These results highlight that milk's solid components—primarily fat and protein—are essential for its economic and nutritional significance. Overall, the findings confirm that the assessed parameters align with established quality criteria for raw milk.

The phytochemical composition and DPPH free radical scavenging activity of control and supplemented yoghurt samples are summarised in Table 3. The results indicate a dose-dependent increase in bioactive compounds and antioxidant capacity with the addition of grape pomace (GP) powder. The control yoghurt (LC) showed no detectable anthocyanin content, while LY-3 and LY-6 exhibited significantly higher values (15.33±0.07 mg C3G/100 g d.w. and 43.27±0.06 mg C3G/100 g d.w., respectively). A similar trend was observed for flavonoid content, increasing from 1.28±0.02 mg CE/g d.w. (LC) to 4.72±0.03 mg CE/g d.w. (LY-3) and 10.69±0.11 mg CE/g d.w. (LY-6).

Table 3
The content of phytochemical compounds and the antioxidant activity of added-value yogurt samples

Parameters	Batch		
Parameters	LC	LY-3	LY-6
Total anthocyanin content (mg C3G /100 g d.w.)	0.0	15.33±0.07 ^b	43.27±0.06 ^a
Total flavonoid content (mg CE/g d.w.)	1.28±0.02 ^c	4.72±0.03 ^b	10.69±0.11a
Total polyphenol content (mg GAE/g d.w.)	2.58±0.01c	7.47±0.04 ^b	16.64±0.09a
Antioxidant activity (µmol TE/g d.w.)	13.45±0.06 ^c	36.53±0.03 ^b	61.59±0.14 ^a

The presence of distinct letters within rows denotes statistically significant variations between the samples (p < 0.05).

The total polyphenol content also showed marked improvements, rising from 2.58±0.01 mg GAE/g d.w. (LC) to 7.47 ± 0.04 mg GAE/g d.w. (LY-3) and 16.64±0.09 mg GAE/g d.w. (LY-6). Antioxidant activity, as measured by DPPH scavenging capacity, increased significantly from 13.45 ± 0.06 µmol TE/g d.w. (LC) to 36.53 ± 0.03 μ mol TE/g d.w. (LY-3) and 61.59 \pm 0.14 μ mol TE/g d.w. (LY-6). These findings confirm that the incorporation of GP powder into yoghurt formulations enhances anthocyanins, flavonoids, polyphenols, and antioxidant activity in a dosedependent manner. The elevated

demonstrate the nutritional and functional benefits of GP supplementation compared to the control sample. This trend aligns with Marchiani et al. (2016), where grape pomace addition significantly improved the polyphenolic content (+55%) and yoghurt antioxidant capacity (+80%)in formulations. The chemical composition of control and value-added yoghurts (LY-3 and LY-6) is presented in table 4. The results reveal significant differences in moisture, total solids, protein, and ash content between the control yoghurt (LC) and the supplemented variants.

Table 4

Chemica	composition	of added-val	ue yogurts	samples

Downwoodowa			
Parameters	LC	LY-3	LY-6
Moisture (%)	86.51±0.12 ^a	81.22±0.09 ^b	78.09±0.11 ^c
Total solid (%)	13.49±0.12°	18.78±0.09 ^b	21.91±0.11a
Fat (%)	3.75±0.02a	4.11±0.07a	4.21±0.09a
Protein (%)	3.69±0.11ª	4.19±0.14 ^{ab}	4.43±0.12a
Ash (%)	0.71±0.02 ^b	1.07±0.06 ^a	1.25±0.07 ^a
PΗ	4.63±0.11a	4.59±0.11 ^b	4.55±0.07b

The presence of distinct letters within rows denotes statistically significant variations between the samples (p < 0.05).

The moisture decreased content progressively with the inclusion of grape pomace (GP) powder, from 86.51±0.12% in the control sample (LC) to 81.22±0.09% in LY-3 and 78.09±0.11% in LY-6. This reduction is accompanied by a corresponding increase in total solids content, which rose from 13.49±0.12% in LC to 18.78±0.09% in LY-3 and 21.91±0.11% in LY-6, indicating the concentration effect of GP powder addition. The protein content exhibited a significant increase with higher concentrations of GP powder (p < 0.05). Specifically, the control yoghurt (LC) contained 3.69±0.11%, whereas LY-3 and LY-6 showed elevated values of 4.19±0.14% and 4.43±0.12%, respectively. This rise in protein content highlights the nutritional enhancement achieved through GP supplementation.

In contrast, the fat content remained relatively stable across all samples, with values of 3.75±0.02% in LC, 4.11±0.07% in LY-3, and 4.21±0.09% in LY-6. This suggests that the addition of GP powder has minimal impact on the fat composition of yoghurt. The ash content increased progressively with the inclusion of GP powder, reflecting the mineral contribution of the added grape pomace. The control yoghurt exhibited an ash content of 0.71±0.02%, which increased to 1.07±0.06% in LY-3 and 1.25±0.07% in LY-6. This trend aligns with the dose-dependent nature of GP powder addition, enhancing the mineral composition of the final product. The pH values showed a slight but consistent decline with increasing GP powder concentrations, from 4.63 ± 0.11 in the control to 4.59 ± 0.11 in LY-3 and 4.55±0.07 in LY-6. This reduction may be attributed to the acidic nature of the grape pomace components, which can influence the overall acidity of the yoghurt. In conclusion, the incorporation of GP powder into yoghurt formulations significantly improves the protein, total solids, and ash content while slightly reducing the pH. The results confirm the nutritional and functional benefits of GP supplementation, supporting its potential application in voghurt products. These findings align with previous studies, such as Marchiani et al. (2016), which reported similar trends when grape skin flour was added to yoghurt formulations, resulting in reduced pH and increased mineral content. The sensory evaluation of the analysed voghurt samples was conducted using a 9-point hedonic scale, assessing attributes such as appearance, colour, aroma, texture, taste, odour, aftertaste, and overall acceptability. The results of the sensory analysis are summarised in Table 5.

As the concentration of grape pomace (GP) powder increased in the yoghurt formulations, a noticeable enhancement in the sensory attributes was observed. Specifically, the red colour intensity became more pronounced in the yoghurts containing GP powder, attributed to the pigments present in the grape pomace, especially anthocyanins. The control yoghurt (LC) exhibited lower scores across all sensory parameters compared to the supplemented variants, LY-3 (3% GP powder) and LY-6 (6% GP powder).

Table 5

Sensory evaluation values of control and added-value yogurts (LY-3, LY-6)

Parameters	Batch			
	LC	LY-3	LY-6	
Appeance	7.70±0.12°	8.11±0.07 ^b	8.45±0.09 ^a	
Colour	8.07±0.05 ^c	8.17±0.04 ^b	8.55±0.06 ^a	
Aroma	8.04 ^b ±0.11 ^c	8.18 ^{ab} ±0.04 ^b	8.55±0.08a	
Texture	8.04 ^b ±0.09 ^a	8.22 ^{ab} ±0.06 ^b	8.38a±0.11a	
Taste	8.07°±0.03°	8.25 ^b ±0.07 ^b	8.59±0.05 ^a	
Odour	8.09°±0.02°	8.40 ^b ±0.05 ^b	8.75±0.07 ^a	
Aftertaste	8.16°±0.05°	8.45 ^b ±0.08 ^b	8.71 ^a ±0.04 ^a	
Overall acceptability	8.30°±0.08°	8.65 ^b ±0.07 ^b	8.83 ^a ±0.05 ^a	

The presence of distinct letters within rows denotes statistically significant variations between the samples (p < 0.05).

For appearance, the panel rated LY-6 the highest (8.45±0.09), reflecting its visually appealing red hue, followed by LY-3 (8.11±0.07) and LC (7.70±0.12). Similar trends were observed for colour, where LY-6 (8.55±0.06) stood out due to the enriched pigmentation, and for texture, which also improved progressively with the addition of GP powder. In terms of aroma and odour, LY-6 was again rated the highest $(8.55\pm0.08 \text{ for aroma and } 8.75\pm0.07 \text{ for odour})$ with the panellists appreciating the balanced and agreeable characteristics imparted by the GP powder. The incorporation of GP powder not only enhanced the taste but also the aftertaste, with LY-6 receiving top scores (8.59±0.05 for taste and 8.71 ± 0.04 for aftertaste). The overall acceptability of the yoghurt samples reflected a clear preference for the GP-supplemented variants. LY-6 achieved the highest overall score (8.83±0.05), highlighting its superior sensory attributes, while LY-3 (8.65 ± 0.07) also demonstrated significant improvements compared to the control (8.30 ± 0.08) . These findings are consistent with the study by Freitas-Sá et al. (2018), who reported enhanced sensory scores for yoghurts supplemented with jabuticaba peel powder. Their results indicated that the addition of natural colourants and bioactive components significantly improve sensory appeal and consumer acceptance.

CONCLUSIONS

The comprehensive characterisation of grape by-product extracts confirmed that GP extract contains a notably high anthocyanin content and exhibits strong antioxidant activity. These findings highlight the potential of GP powder as a rich source of bioactive compounds with antioxidant properties. Accordingly, we advocate for its incorporation as a natural ingredient in fortified yoghurt formulations.

Yoghurts supplemented with GP powder demonstrated elevated levels of total phenolic content and enhanced antioxidant potential compared to the plain yoghurt. Sensory evaluations revealed that the panellists appreciated the enriched colour and overall sensory characteristics of the GP-supplemented samples, deeming them acceptable and appealing.

This research underscores promising opportunities for the dairy industry to cater to the growing consumer demand for functional foods by developing innovative, nutritious, and flavourful yoghurt products. Furthermore, the findings validate the quality and bioactive potential of natural powders derived from the skin and seeds of grape berries, specifically from the Fetească Neagră variety. These ingredients hold substantial promise for application in the food industry, enabling the creation of functional products while simultaneously supporting circular economy principles by valorising agricultural by-products.

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