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EXPLORING THE POTENTIAL OF GRAPE POMACE POWDER AS A FUNCTIONAL INGREDIENT IN YOGURT

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Abstract

Consumer demand for functional yogurts has been rising recently, particularly for those made by adding plant-based ingredients or their bioactive components. Grape, Vitis vinifera L., is abundant in antioxidant-rich phenolic compounds and dietary fiber. Although grape pomace is an agro-industrial by-product and its handling can lead to socioeconomic and environmental issues, it can be possible to valorise by extracting its bioactive components. The goal of this study was to develop functional yogurt by adding 1 and 2% of grape pomace (YGP1 and YGP2). The impact of fortification was assessed on the enhanced yogurts' physicochemical properties, total phenolic content, antioxidant activity, and sensory acceptance. Grape pomace powder had high total polyphenols contents (20.39 ± 0.23 mg GAE/g d.w.) and antioxidant activity (88.16 ± 0.45%). Values of polyphenols (8.88± 0.06 mg GAE/g d.w.) and antioxidant activity (89.47 ± 0.39%) significantly increased in fortified yogurts compared to control sample. Therefore, it is possible to conclude that grape pomace powder can be used to produce novel, functional yogurt.

***Key words**: antioxidant activity, food ingredients, functional yogurt, grape pomace, pigments.*

INTRODUCTION

Food and food selection for a healthy lifestyle have been the main concerns of human society since ancient times. Grapes (*Vitis vinifera* L.). are the most cultivated fruit worldwide, with an estimated production of more than 79 million tons in 2018 (Cardell et al., 2019). About 75% of grape production is transformed into wine production, resulting in residual by-products of 20–30% After grapes are destemming and pressed, a by-product known as grape pomace (GP) is produced (Cheng et al., 2010). The main by-products resulting from winemaking are pomace which is made up of the skin, pulp, seeds and bunches (Balbinoti et al., 2020). Because of the compositional features of GP, including its low pH and high sugar content, disposing of it is expensive and difficult. These traits present a significant environmental risk if left untreated (Ungureanu et al., 2010).
Some studies have been done on using GP for food applications because it is well-recognized that GP is a good source of fiber and antioxidants with considerable nutritional activity. For instance, grape skin flour (GSF) from GP has been used in tomato puree (Lavelli et al., 2014), baked goods (Walker et al., 2014),
while grape seed flour has been added to cereal bars, pancakes, and noodles (Rosales Soto et al., 2012) and bread (Hoye & Ross 2011). Compared to synthetic antioxidants, GP antioxidants, which comprise polyphenol components including anthocyanins, flavanols, catechins, and proanthocyanidins, can be considered fully safe (Rosales Soto et al., 2012). Due to their strong antioxidant activity, these compounds might provide disease-prevention and health-promoting benefits (Choi et al., 2010; Hogan et al., 2010). Because of this, such compounds are now being explored as novel components or food additives that can improve the nutritional value of various food products while also potentially offering a solution to the waste disposal issue (Peng et al.,

2010). Anthocyanins are widely utilized natural food colorants that exhibit pH-dependent colour gradients. They find application in various popular food items, including beverages, desserts, ice cream, and dairy goods. Anthocyanins possesses several significant health features, including its anti-cancer activity, which has been observed to exhibit chemopreventive and chemoprotective effects in both *in vivo* and *in vitro* settings across various cancer cell lines. Additionally, anthocyanins has been found to possess antioxidant and anti-inflammatory properties (Khoo et al., 2017).

Due to the presence of active probiotic bacteria, yogurt is already regarded as a nutritious diet; yet, fiber and phenolic antioxidant components are absent from it (Karaaslan et al., 2011). The information that is currently available on the addition of GP to yogurt (Tseng & Zhao 2013) is favourable in terms of the viability of utilizing GP as a novel ingredient.

The objective of this study was to assess the impact of GP on the physicochemical characteristics, phytochemical composition, antioxidant capacity, colour, and sensory aspects of yoghurts fortified with GP. Furthermore, the study sought to ascertain if powders enhanced with phenolic compounds could serve as a sustainable food ingredient in the production of foods that are higher in nutrients.

MATERIALS AND METHODS

A winery (Iaşi University of Life Sciences) supplied the nonfermented GP of the Feteasca Neagra varietal. The Rediu Iași Research Station of the University of Life Sciences contributed 200 liters of cattle milk. The skins underwent mechanical separation, then were dried in an oven for 48 h at 40±2°C, reaching a moisture content of 9.80%. The GP were ground (0.5 mm) using a kitchen grinder. GP was sanitized in an autoclave for 15 minutes at 121 °C before being used to make yogurt.

DPPH (2,2-diphenyl1-picrylhydrazyl), gallic acid, sodium acetate, potassium chloride solution, ethanol, sodium carbonate, methanol, sodium carbonate, Folin-Ciocâlteu reagent, sodium hydroxide, aluminium chloride, were acquired from Sigma Aldrich (Schnelldorf, Germany).

The ultrasound-assisted extraction method was utilized to extract the bioactives from GP powder. Therefore, 1.0 g of GP was mixed with 10 mL of an 80% ethanol solution acidified with citric acid (ratio 7:1, v/v) and then treated with ultrasound for 25 minutes at 35°C and a frequency of 37 kHz. The resulting supernatant was collected and centrifuged for 10 minutes at 6500 rpm at 4°C. Thereafter, the GP extract was used for phytochemicals (anthocyanins, flavonoids, and polyphenols) and antioxidant activity analysis.

A modified pH differential method employed by Lipșa et al. (2024) was utilized to determine the total monomeric anthocyanin content. At 520 nm and 700 nm, the absorbance of diluted extracts using various buffer solutions at pH 1.0 and 4.5 was determined. The findings were exhibited as mg cyanidin-3-glucoside (C3G)/g of dry weight (dw).

The content of total flavonoids in GP extract was measured by the technique described by Dewanto et al. (2002). Shortly, 0.25 mL of the GP extract and 0.075 mL of 5% sodium nitrite were combined with 2 mL of distilled water. The mixture is left to act for 5 minutes, after which 0.15 mL of 10% aluminium chloride is added and left to act again for 6 minutes, then 0.5 mL of 1M sodium hydroxide is added. The absorbance of the resulting mixture was read at a wavelength of 510 nm. The total flavonoid content is expressed as mg catechin equivalents/g sample (mg CE/g d.w.).

The Folin-Ciocâlteu spectrophotometric method described by Dewanto et al. (2002) was used to measure the total polyphenolic content. In a test tube, 200 µL of the GP extract was added, followed by 1 mL of the Folin-Ciocâlteu reagent, and 15.8 mL of distilled water. They were left to stand for 10 minutes, after which 3 mL of 20% sodium carbonate was added and left to stand for one hour at room temperature in the dark. The absorbance was read at a wavelength of 765 nm. The results obtained were expressed as mg gallic acid equivalents/g sample (mg GAE/g d.w.).

The method for determining the antioxidant activity by neutralizing the DPPH radical was carried out according to the protocol described by Castro-Vargas et al. (2010). DPPH stock solution was obtained by mixing 3.8 mg of DPPH with methanol, in a 100 mL volumetric flask. Afterward, 100 µL of the sample to be analyzed (GP extract) was added to a test tube, along with 3.9 mL of DPPH (A sample). For the control sample, 100 µL of methanol was used, and 3.9 mL of DPPH (A control). The absorbance was read after 90 minutes of rest in the dark at a wavelength of 515 nm. The results were represented as µmol of Trolox equivalents (TE)/g d.w. The variation of the antioxidant capacity was studied by determining the inhibition (I%) for each sample to be analyzed, using the following equation: I% = (A control-A sample)/A control x 100.

Collection, sampling, and analysis of unpasteurized milk. 200 litres of milk were removed in clean containers from the farm's storage tank. It was kept in a 4°C for 25 hours. After that, milk was thoroughly homogenized and added to the analytical tests in the laboratory. The AOAC procedures were utilized to determine the physicochemical parameters of milk samples, which included moisture content, total solid, pH, solid non-fat content, protein content, and fat content (Rațu et al., 2024).

Preparation of yogurt enhanced with GP. Full-fat milk was utilized in the technological process; it was not normalized milk. After 30 minutes of pasteurization at 60°C, the milk was chilled to 36°C and then specific lactic bacteria were introduced. Starter culture YF-L812 (commercial product, Chr. HANSEN, Denmark) comprising a blend of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (2:1) was added to milk when the temperature reached 42°C.

The inoculated milk was fermented for around 6.5 hours at 42°C, or until a final pH of 4.8 was reached. At this stage, yogurt and sterile GP powder were combined at a concentration of 60 g/kg and then divided into pots. Analyses were carried out on the samples as soon as they were produced and stored at 4°C. There were two distinct yogurt products made. Each production batch of yogurt was split into three batches: one batch (control, YC) had no GP, while the other two batches included enhanced yogurt (YGP1-1% GP, YGP2-2% GP).

The samples' pH, fat, ash, moisture content, and total protein were measured using the techniques recommended by the Association of Official Analytical Chemists (AOAC) (Usturoi et al., 2017; Tseng & Zhao, 2013).

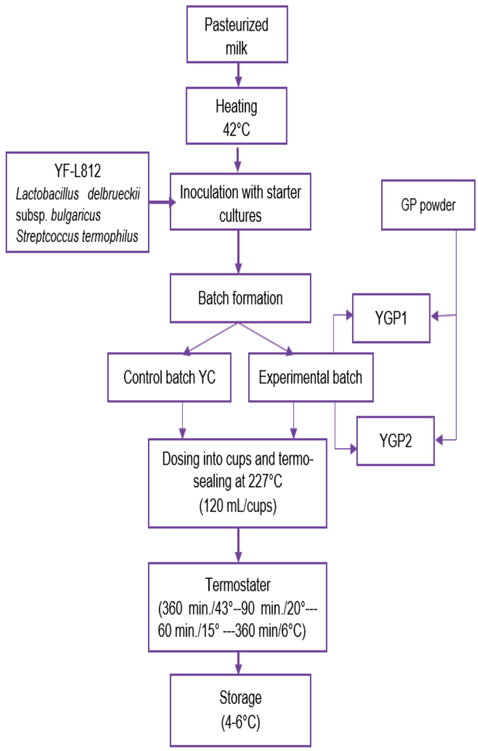

Figure 1. Flow diagram illustrating the processing steps of a functional yoghurt prototype

Colour analysis. The colour of the control and value-added yogurt were examined using the portable colorimeter with illuminator C-MINOLTA ChromaMeter CR-410 (Konica Minolta, Osaka, Japan). The color parameters determined were L*, a* and b*. Three different replicates of each sample were available.

Sensorial analysis. A panel of 16 different panellists conducted the sensory assessment of yogurt samples. A hedonic scale from 1-9 was used to evaluate the sensory attributes, where 1 is the weakest/unpleasant and 9 the strongest/very pleasant. Appearance, colour, aroma, texture, taste, odour, aftertaste, and overall acceptability are the evaluated attributes. Panel members are non-smokers aged 24-40 years and they have studies in the field of the food industry. The samples were arranged in a random configuration. The participants were provided with details regarding the study's overarching objective and the procedures for handling personal data.

Statistical Analysis. Statistical analysis of the data was performed using the data analysis tools package of Microsoft Excel software and using the statistical processing software Minitab 19. Standard deviations were calculated from triplicate experiments.

RESULTS AND DISCUSSIONS

The polyphenolic, flavonoid, and anthocyanin contents in ethanolic extracts from GP extract were measured using spectrophotometric methods (table 1). The total polyphenolic content was 20.39±0.23 mg GAE/g d.w., while the total flavonoid content was 8.28±0.78 mg CE/g d.w. Additionally, a good concentration of total anthocyanin content was observed, measuring 1.56±0.22 mg C3G/g d.w. and also a remarkable antioxidant activity of 24.15±0.12 µmol TE/g d.w. Serea et al. (2021) extracted bioactive compounds from red grapes peel from the Băbească neagră variety using ethanol 96% acidified with 0.1 N HCl on ultrasound assisted extraction and obtained higher anthocyanin values (4.29±0.04 mg C3G/g) similar values for phenolic compounds of 22.28 ± 2.96 mg GAE /g and a smaller antioxidant activity (14.07±1.03 mM Trolox/g). Rockenbach et al., (2011), reported total anthocyanin content values of 1.84-11.22 mg G3G/g d.w, in the extracts of four red grape pomace samples (Bordeaux, Cabernet Sauvignon, Isabel, and Merlot varieties) grown in Brazil. The authors determined also the degree of inhibition of the DPPH free radical (using the ß-carotene/linoleic acid method) of 41.13%, for the extracts obtained from grapes of the Bordeaux variety. Novak et al. (2008) reported a total flavonoid yield of 4.99 ± 0.07 mg CE/g d.w., using ethanol acidified with 1% hydrochloric acid, from freeze-dried grape skin of Alfrocheiro (*Vitis vinifera* L.). González-Centeno et al. (2014) stated that ultrasound treatment for 30 minutes led to the extraction of polyphenols with yields of 5.37 - 31.87 mg GAE /100 g fresh grapes.

These variations could be caused by the origin material's phytochemical variability and the kind of solvent combination utilized during the extraction process.

Table 1. Phytochemical characterizations and colorimetric parameters of the GP powder

Parameters	GP powder
Total anthocyanin content (mg C3G/g d.w.)	1.56±0.22
Total flavonoid content (mg CE/g d.w.)	8.28±0.78
Total polyphenol content (mg GAE/g d.w.)	20.39± 0.23
Antioxidant activity (DPPH, µmol TE/g d.w.)	24.15±0.12
Inhibition %	88.16± 0.45
L*	23.36±0.16
a*	10.37±0.05
b*	3.32±0.02

Based on the colour indices, the GP powder was found in quadrant I (+a*, +b*).

Based on colour characteristics, values for L*, a*, and b* parameters were estimated at 23.36, 10.37, and 3.32. Regarding the parameter a*, which characterizes the tendency towards red, it was seen that GP powder exhibited a high a* value attributed to its elevated concentration of anthocyanins.

The primary chemical quality indices were identified to establish the raw material milk's quality parameters. Table 2 presents the chemical composition values of samples of cow's milk.

The water content of the raw milk was 87.07±0.05% and that of total solids was 12.93±0.07%. Milk's solid components, primarily fat and protein, contribute to its significant economic and nutritional value. The mean fat content was 3.99±0.03%, resulting in a mean solid-non fat content of 8.94±0.06%.

Table 2. Chemical contents values of raw milk

Parameters	Mean
Water (%)	87.07±0.05
Fat (%)	3.99±0.03
Protein (%)	3.37±0.03
Total Solids (%)	12.93±0.07
Solid-non fat (%)	8.94 ±0.06
pH	6.57±0.01

The average value of the protein level was 3.37±0.03%. The findings suggest that all indicators of raw milk quality meet the criteria for assessing the overall quality of the milk.

Phytochemical profile and the DPPH free radical scavenging capacity of control and supplemented yogurt are displayed in Table 3.

The products obtained were analysed to determine their overall phytochemical composition. The inclusion of GP powder in yoghurt products resulted in a dose-dependent increase in phytochemicals (such as anthocyanins, flavonoids, and polyphenols) as well as enhanced free radical scavenging capabilities, as compared to the control group. Thus, the GP-supplemented yogurt variants presented an anthocyanin content with values between 10.22±0.88 mg C3G/100 g d.w. (YGP1) and 21.09±0.97 mg C3G/100 g d.w. (YGP2). As for the antioxidant capacity, it has increased from 24.35±1.17 µmol TE/g d.w. to 35.53±1.19 µmol TE/g d.w. Therefore, the GP powder addition in the composition of the yogurts led to elevated levels of total polyphenolic compounds, total flavonoids, total anthocyanins content, and higher DPPH free radical scavenging capacity. Also, the bioactive components derived from GP had a favorable impact on the antioxidant activity of the enhanced yogurts, resulting in higher values compared to the control samples. By raising the amount of total anthocyanins and antioxidant activity, respectively, in yogurt samples manufactured with GP powder, the results shown in Table 3 validate their additional value. Our results comply with Marchiani et al. (2016) who added grape pomace of Chardonnay, Moscato and Pinot noir types as natural sources of polyphenolic compounds in yogurt formulation products. The yogurt composition with grape skin flour exhibited a significant increase in total polyphenolic contents (+55%) and antioxidant capacity (+80%) compared to the reference sample.

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

Parameters	**Type of yogurts**		
	YC	YGP1	YGP2
Total anthocyanin content (mg C3G /100 g d.w.)	-	10.22±0.88^{b}	21.09±0.97^{a}
Total flavonoid content (mg CE/g d.w.)	1.28±0.20^{c}	3.15±1.03^{b}	6.23±1.04^{a}
Total polyphenol content (mg GAE/g d.w.)	2.58±0.29^{c}	4.98±0.20^{b}	8.88± 0.16^{a}
Antioxidant activity (µmol TE/g d.w.)	13.45±1.33^{c}	24.35±1.17^{b}	35.53±1.19^{a}

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

Chemical composition of control and value-added yogurts (YGP1, YGP2). The results of chemical composition of analysed samples are presented in Table 4.

The analysis of the obtained data indicates differences between the yogurts with de addition of powder obtained from grape pomace for protein and total solid ($p < 0.05$).

The results presented in Table 4 showed that the added-value yogurts with GP powder are characterized by a significantly higher protein content than the control one ($p<0.05$). In contrast, the fats contents of the value-added yogurts are similar to the control. In another study yogurt containing grape skin flour presented significantly lower pH and fat content (−20%) than the control (Marchiani et al., 2016). With the increasing concentration of GP powder, the ash content showed a small increase from 0.86% in the case of the YGP1 sample with an addition of 1% GP to 0.96% in the case of the YGP2 sample with an addition of 2% GP.

Colour evaluation of value-added yogurts samples. Colour is an important sensory characteristic that influences consumer acceptability of foods (Spence, 2015). The yogurts were also examined using CIELAB colorimetric parameters. The results of measurements of colour parameters L*, a*, and b* of the control and GP-supplemented yogurts were presented in table 5. The bioactive chemicals present in the GP powder have a tendency to turn red, as evidenced by the significant rise in the a* value with increasing concentration of the GP powder ($p<0.05$). Additionally, according to the L* and b* values the added-value yogurts show low levels of lightness and yellowness, when the GP powder concentration increases.

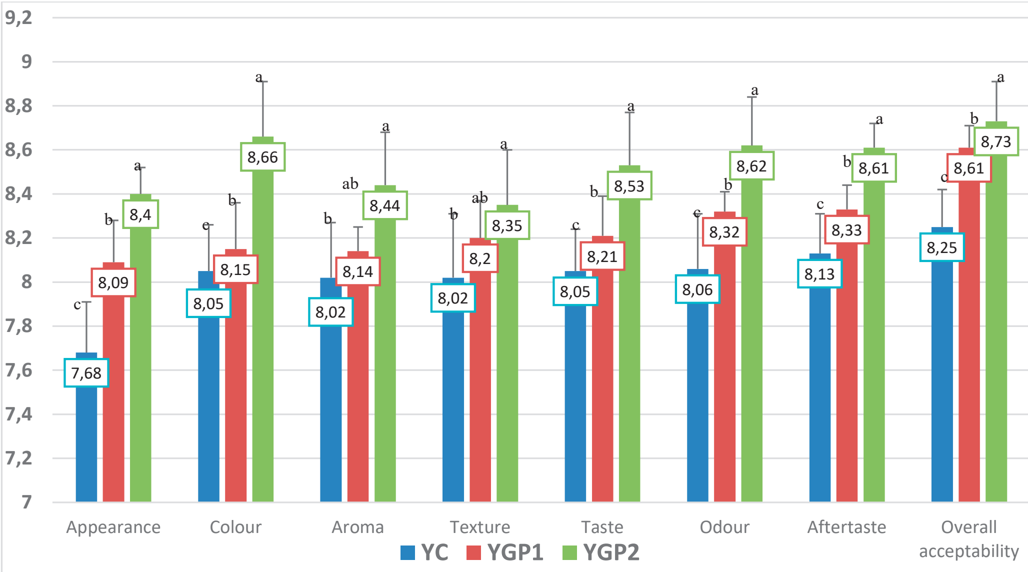

Figure 2. Sensory evaluation values of control and added-value yogurts (YGP1, YGP2) (the presence of distinct letters "a", "b", and "c" in the columns show statistically significant differences ($p < 0.05$))

Table 4. Chemical composition of added-value yogurts samples

Parameters	Type of yogurts		
	YC	YGP1	YGP2
Moisture (%)	86.42±0.19^{a}	83.22±0.17^{b}	80.19±0.15^{c}
Total solid (%)	13.58±0.12^{c}	16.78±0.16^{b}	19.81±0.19^{a}
Fat (%)	3.73±0.03^{a}	4.01±0.02^{a}	4.02±0.01^{a}
Protein (%)	3.66±0.08^{a}	4.13±0.05^{ab}	4.21±0.07^{b}
Ash (%)	0.74±0.03^{b}	0.96±0.12^{a}	1.09±0.11^{a}
pH	4.65±0.10^{a}	4.54±0.04^{b}	4.46±0.03^{b}

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

Table 5. Colour data of YC, YGP1 and YGP1 yogurts

Parameters	Type of yogurts		
	YC	YGP1	YGP2
L*	93.54±0.22^{a}	88.35±0.51^{b}	84.59±0.66^{c}
a*	-2.35±0.13^{a}	4.45±0.33^{b}	8.63±0.54^{c}
b*	16.66±0.14^{a}	9.06±0.14^{b}	5.11±0.24^{c}

Averages with distinct letters indicate a comparison across colour parameters; the use of distinct letters in each row signifies significantly different final results ($p< 0.05$).

L* and b* parameters decreased together with powder concentration, but a* increased in the tested yogurt models. A similar trend was obtained by da Silva et al. (2019) who added blackberry anthocyanin-rich extract to a food system.

The sensory evaluation of the analysed yogurts was conducted using a 9-point hedonic scale. The attributes followed were appearance, colour, aroma, texture, taste, odour, aftertaste, and overall acceptability. The average results acquired from the sensory analysis are presented in Figure 2. As the GP powder concentration increased in the obtained yogurts, the red colour intensified, due to the presence of pigments in the GP powder especially anthocyanins (Figure 3). The YGP2 sample with 2% GP was acceptable and exhibited the highest scores for all nine attributes. The inclusion of GP in yogurts samples increased the red colour, so the panellists noted that the yogurts varied in colour, with YGP2 being the most reddish.

Figure 3. Pictures of the yogurt without GP, control (YC); yogurt with 1% GP (YGP1); yogurt with 2% GP (YGP2) (Own source)

YGP2 yoghurt samples yielded the highest ratings for all eight attributes at 2% GP. The GP- supplemented yogurts samples were praised for having an agreeable aroma, odour, and colour. The yogurts with added GP powder were assessed as having a balanced taste, aroma, and aftertaste. Regarding the general acceptability, it was shown that the addition of GP powder does not substantially influence the basic sensory characteristics (colour, taste, aroma). All of the proposed yogurts were positively evaluated by the panellists. The utilization of jabuticaba (*Myrciaria jaboticaba* (Vell) O. Berg) peel powder as colorant in yogurts was investigated by Freitas-Sá et al., (2018). In the sensory evaluation, yoghurts incorporated with *Jabuticaba* had the highest results in terms of appearance (6.6-6.8), flavour (6.9), and overall liking (6.8) when compared to the other flavoured yoghurts.

The obtained samples have an attractive colour with red shades, in correlation with the added GP powder concentration. Sensory analysis showed that yogurts containing GP have acceptable quality characteristics and may be well-accepted by consumers.

CONCLUSIONS

The global characterization of the grape by-products extract proved that GP extract exhibited higher anthocyanins content with good antioxidant activity. Our results underscore the significance of GP powder as an abundant reservoir of bioactive constituents exhibiting antioxidant properties. Consequently, we propose its integration as a natural constituent in fortified yoghurt.

The GP-supplemented yogurts showed higher levels of total phenolic contents and antioxidant potential compared to the plain yogurt. Sensory study revealed that panellists were pleased with the yogurts samples' increased colour. Overall, the value-added yogurts was found to be acceptable. This trend presents novel prospects for the dairy industry to address the increasing consumer desire for functional foods by developing original, nutritious, and palatable yogurt products. The obtained results certify the quality of natural ingredients with bioactive potential of powders obtained from the skin and seeds of grape berries of the Feteasca neagra variety, for use in the food industry to obtain functional products, promoting the principles of the circular economy.

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