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Original article

Distilled grape pomace as a functional ingredient in vegan muffins: effect on physicochemical, nutritional, rheological and sensory aspects

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Summary Wheat-based vegan muffins were formulated with increasing levels (0, 5 and 10 g per 100 g substitutes to flour) of dried grape pomace powder obtained after distillation (DGPP). The DGPP-muffins showed a greater ($P < 0.05$) total dietary fibre and a lower ($P < 0.05$) total starch content to the control. The inclusion of DGPP influenced ($P < 0.05$) both the batter's rheological properties and the baked muffins' technological characteristics, including the baking loss, the volume, the firmness, the spread ratio and the colour. The *in vitro* antioxidant activity and the phenolic content increased ($P < 0.05$) following DGPP inclusion. The untargeted phenolic profiling by UHPLC-HRMS revealed an ($P < 0.05$) increase of several phenolic classes (i.e. free and bound fractions) following the inclusion of DGPP in the recipe. Flavonoids, mainly anthocyanins, were the most abundant compounds. A quantitative descriptive sensory analysis detected the DGPP-vegan muffins showed good sensory acceptability. The vegan muffin with 5 g per 100 g of DGPP obtained the highest overall acceptability score by the panellists.

Keywords Distillery by-products, polyphenols, rheology, sensory analysis, texture.

Introduction

The global Covid-19 pandemic crisis negatively affects eating habits and lifestyle among children and adolescent, increasing on one side the intake of calorie-rich cereal-based foods and on the other side reducing the consumption of fruits and vegetables (Jia *et al.*, 2021). This may be of concern since unbalanced diets can be related to unhealthy weight gain and the incidence of childhood obesity (Malik *et al.*, 2006). In this context, the reformulation of staple cereal-based foods by selecting alternative ingredients could be a strategy to expand the market of healthy food products (Buttriss, 2020; Bianchi *et al.*, 2021). On the other hand, the use of new ingredients in cereal-based formulations could also affect the technological and sensory characteristics, thus requiring a deeper investigation (Rahman *et al.*, 2015; Nath *et al.*, 2018; Grasso *et al.*, 2020; Shih *et al.*, 2020).

Besides the nutritional amelioration of cereal-based foods, the 2030 Agenda of the United Nations aims to the sustainable use of natural resources, decoupling economic growth from environmental degradation (ONU, 2015). In the frame of sustainability, the food-industry by-products and the upcycling represent a challenge for future years. Moreover, the growing interest in plant-based vegan food products widened the research challenges of the food technologists. Accordingly, a cutting-edge approach might be represented by the formulation of a vegan cereal-based food using sustainable food-processing by-products rich in bioactive compounds and nutrients.

In light of these considerations, the use of several agro-industrial by-products in food matrixes such as meat, pasta and baked stuff has been reported (Bianchi *et al.*, 2021; Rainero *et al.*, 2022). Among the different by-products, grape pomace (GP) represents a low-cost, environmental-friendly and healthy ingredient characterised by several bioactive compounds, mainly polyphenols and dietary fibres (Tolve *et al.*, 2021). In particular, different epidemiological studies on human health showed the valuable role of certain phenolic compounds in preventing cardiovascular diseases,

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diabetes and certain types of cancer (Rothwell *et al.*, 2017). Similarly, a greater consumption of dietary fibre can positively affect human health by improving the bowel transit of faeces, the satiety, reducing blood cholesterol levels, and preventing obesity (Zhang *et al.*, 2018).

Different authors evaluated the effect on technological, nutritional and sensory properties of cereal-based foods fortified with powdered grape pomace (GPP) (Tolve *et al.*, 2020, 2021), and Rocchetti *et al.* (2021) investigated the effect of GPP on the phenolic bio-accessibility of fortified wheat bread, showing an increase of anthocyanins bio-accessibility and a reduction in the predicted glycaemic index of GPP-enriched breads. Little information is however available on cereal-based food fortification with distilled GP (DGP), which represents the by-product from ethanol extraction. The DGP is still underrated for food fortification and industrial purposes, even if is rich in dietary fibre and several bioactive phytochemicals useful within the food industry (Teixeira *et al.*, 2014).

Among the different cereal-based food products, muffins are a ready-to-eat food widely consumed among children and adolescent, appreciated for their spongy texture and delicious taste, but high in carbohydrates and generally low in dietary fibres (Harastani *et al.*, 2021). In this sense, the DGP could be considered a promising ingredient in vegan muffin formulation to produce novelty products, owing to its contribution in dietary fibre and bioactive compounds. However, to our knowledge, no studies regarding the addition of DGP to vegan muffin are currently available in literature.

Considering these background conditions, this work aimed to assess the rheological, technological, sensory and nutritional differences in vegan muffins formulated with increasing levels of DGP (0, 5 and 10 g per 100 g substitutes to flour) in the recipe. In addition, the total phenolic profile of DGP (both free and bound phenolic) by untargeted metabolomics, and the *in vitro* antioxidant capacity of baked products were investigated.

Materials and methods

Ingredient and vegan muffins preparation

Common white wheat flour (Barilla, Italy), potato flour, corn oil, salt, sugar and baking powder were purchased in local markets. As reported in the label, the white wheat flour composition was: total carbohydrates 71 g per 100 g, protein 11 g per 100 g, fat 1.7 g per 100 g and total dietary fibre 1.4 g per 100 g, while the reported potato flour composition was: total carbohydrates 80 g per 100 g, protein 1.4 g per 100 g, fat 0 g per 100g.

The grape pomace (*Vitis vinifera* cv. *Cabernet*) obtained after the distillation process was kindly provided by AssoDistil (Roma, Italy). The DGP was pressed and dried in a vacuum oven (VD 115 Binder GmbH, Tuttlingen, Germany: 40 °C, 30 kPa) until reaching the final moisture of 11.0 g water per 100 g of product. After seeds and stems removed, the DGP was finely grounded (GM200 Retsch, Haan, Germany; particle size < 200 μm). The DGP powder (DGPP) was stored in vacuum packaging at room temperature (RT). The proximate composition of the DGPP was: crude protein: 12.8 ± 0.3 g per 100 g dry matter (DM); total dietary fibre: 55.6 ± 1.7 g per 100 g DM; ash: 12.9 ± 0.2 g per 100 g DM.

The vegan muffin preparation followed a recipe by replacing the blended flours (75 g per 100 g of wheat flour and 25 g per 100 g of potato flour) with 0, 5 and 10 g per 100 g of DGPP. Successively 80 g of tap water, 1 g of salt, 50 g of sugar, 25 g of corn oil and 2 g of baking powder were added, thus obtaining GM0, GM5 and GM10 muffin samples respectively. A commercial muffin-maker machine (Ariete Party Time, model 0188/00; Italy) was used for cooking (200 °C; 12 min) the muffins' doughs. Each formulation was performed in triplicate. After the baking process, samples were stored in airtight plastic containers at RT.

Proximate composition of vegan muffin

The DM (method 930.15), ash (method 942.05), total sugars (sum of D-glucose, D-fructose and sucrose; Megazyme cat. no. K-SUFRG) crude protein (method 976.05), crude lipid (method 954.02) and total starch (method 996.11) were measured. The total, soluble and insoluble dietary fibre contents (TDF, SDF, IDF, respectively) were measured through the method 991.43. (AOAC, 2007). Analyses were conducted in triplicate on each formulation.

Technological properties

A Hygropalm HC2-AW-meter (Rotronic Italia, Milano, Italy) was used for measuring the water activity (a_w) of vegan muffins at 23 °C. The moisture content was assessed following the AACC method 44-15A (AACC, 2000). The pH was measured with a pH meter (Mettler-Toledo Inc, Columbus, OH, USA) by mixing one gram of each minced muffin formulation with 4 mL of distilled water, vortexed for 10 s. The baking loss was calculated as the differences in the mass between the batter and the baked vegan muffins. The volume was assessed through the rapeseed displacement method AACC 10-05.01 (AACC, 2000). The heights and diameters of muffins were measured with a digital calliper, and the spread ratio was calculated by dividing the height by the diameter.

Specific gravimetry and rheology of batters

The specific gravimetry (SG) of each batter was determined gravimetrically by dividing the weight of a known volume of batter by the weight of an equal volume of water. A viscometer (One plus viscometer, Lamy Rheology, Champagne au Mont d'Or, France) equipped with an L-2 spindle probe (18.72 mm diameter and 6.886 mm height) was used for rheological analyses. Samples were placed in a 600-mL beaker according to the producer recommended standards ASTM/ISO 2555. The mixture rested for 5 min to allow relaxation at 25 °C. Shear stress was a function of shear rate over the range of 10–250 s^{-1}. The analyses occurred at a 25 °C constant temperature in a refrigerated bath. The results were fitted to the Power Law model eqn (1).

$$\tau = K \cdot \gamma^n \quad (1)$$

where 'τ' is shear stress (Pa), 'K' is the consistency index (Pa s^n), 'n' is the flow index. All measurements were made in triplicate.

Texture and physical characteristics of muffins

The texture of vegan muffins was performed 24 h after the baking process by using a texture analyser (TVT-6700; Perten Instruments, Stockholm, Sweden) according to the AIB standard procedure for bread firmness measurement. The maximum peak force of compression was considered as the muffin firmness (N) and a metal cylinder probe (25 mm diameter) was used. Ten measurements were done for each batch.

Muffins were then sliced transversely from the bottom to obtain a circular sheet of 1–2 mm of height and about 20 cm^2 of area. Images of the section of muffins were scanned by a scanner at 600 DPI. Scanned colour images were first converted to grey scale and binarised with ImageJ (version 1.8.0) to obtain the pore area fraction (total pore area/area of the section of muffin), the pore density (number of pores cm^{-2}) and the average pore diameter (mm). Pore diameter distributions were analysed as histograms, and all measures were subdivided into twelve intervals. All analyses were performed on five samples for each formulation.

Colour analysis

The colour was measured by a reflectance colorimeter (Illuminant D65) (Minolta Chroma meter CR-300, Osaka, Japan) based on the colour system CIE – L^* a^* b^*. Analyses were performed at five different points within the crumb and the crust area. Minolta eqns (2) and (3) were used to calculate the total colour difference (ΔE) respectively:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (2)$$

$$\Delta L = (L - L_0);\ \Delta a = (a - a_0);\ \Delta b = (b - b_0) \quad (3)$$

where L, L_0, a, a_0, b and b_0 are the measured values of muffins fortified with DGPP or the control muffin.

In vitro antioxidant activity and free and bound phenolic profiles by high-resolution mass spectrometry

The extraction for ABTS (2,20-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) and FRAP (ferric reducing ability of plasma) was performed by stirring 0.5 g of finely powdered sample with 7.5 mL of MeOH:HCl 97:3 (v/v) for 16 h in the dark at room temperature as described by Tolve *et al.* (2021). Free and bound phenolics were extracted following the protocol previously reported by Rocchetti *et al.* (2021). For free phenolics, a homogeniser-assisted extraction was used (Rocchetti *et al.*, 2021). Bound phenolics were extracted from the residual solids, according to an alkaline hydrolysis for 1 h at room temperature (Tomas *et al.*, 2020; Rocchetti *et al.*, 2021).

The untargeted phenolic profiling was done on high-resolution mass spectrometry (HRMS) performed on a Q-Exactive™ Focus Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Scientific, Waltham, MA, USA) coupled to a Vanquish ultra-high-pressure liquid chromatography (UHPLC) pump and equipped with heated electrospray ionisation (HESI)-II probe (Thermo Scientific). The chromatographic separation was achieved under a water-acetonitrile (both LC-MS grade, from Sigma-Aldrich, Milan, Italy) gradient elution (6–94% acetonitrile in 35 min, adding 0.1% formic acid to both phases), on an Agilent Zorbax Eclipse Plus C18 column (50 × 2.1 mm, 1.8 μm). The HRMS conditions were as reported by Rocchetti *et al.* (2021).

Raw data (.RAW files) were further processed using the software MS-DIAL (version 4.70) for post-acquisition data filtering (Tsugawa *et al.*, 2015) and the annotation was done via spectral matching against the databases FoodDB and Phenol-Explorer. The identification step was based on mass accuracy (setting a 5-ppm tolerance for m/z values), isotopic pattern and spectral matching. These criteria were used to calculate a total identification score, considering the most common HESI+ adducts for the chromatographic conditions adopted, thus reaching a level 2 of confidence in annotation (Salek *et al.*, 2013). Finally, the cumulative intensity values of the different phenolic classes annotated were converted into semi-quantitative data, exploiting methanolic standard solutions of pure compounds (Extrasynthese, Lyon, France) analysed under the same conditions. In this regard, a linear fitting ($R^2 > 0.99$) was built and used for quantification, and results were expressed as mg equivalents (Eq.) per kg DM.

Sensory analysis of vegan muffins

The sensory profile was evaluated by a quantitative descriptive sensory analysis (QDA) by involving a trained sensory panel of eighteen panellists (ten females and eight males; 22 and 28 years old), recruited from the staff and students of the Department of Biotechnology of the University of Verona. Panellists generated sixteen sensory terms and were qualified to recognise them. The sensory attributes were colour uniformity, porosity, cake odour, wine odour, fruity odour, global flavour, sweetness, fruitiness, acidity, bitterness, mellowness, elasticity, adhesiveness, hardness, grittiness and astringency. A hedonic 9-point scale was used (Tolve *et al.*, 2021). The muffins were placed on a covered plate, coded in a completely randomised and balanced way, and then presented to the judges for the sensory analysis session. Panellists also commented on the overall acceptability: mean scores above 5 were considered acceptable (neither like nor dislike).

Statistical analysis

All data are reported as mean values ± standard deviation of at least three measurements ($n = 3$). The analysis of variance (ANOVA) with a post-hoc Tukey test at $P < 0.05$ has been used for mean comparison. Pearson's correlation tests and statistical analyses were performed using the software XLSTAT Premium Version (2021.1.1, Addinsoft SARL, Paris, France).

Results and discussion

Proximate composition of vegan muffins

The chemical composition and the water activity of the vegan muffin formulated with increasing levels of DGPP in the recipe are reported in Table 1. The ash, TDF, IDF and SDF increased ($P < 0.05$) while the total starch decreased ($P < 0.05$) accordingly to the inclusion level of DGPP. These differences are related to the chemical composition of DGPP as well as to its level in the recipe. The increase in the ash content is related to the macro-and micro-element content of DGPP, rich in P, K, Mn, Zn and Fe (Mohamed Ahmed *et al.*, 2020). In addition, GM5 and GM10 were characterised by a greater TDF content when compared to the control (GM0), being 2.23 and 4.29 vs. 0.47 g per 100 g of product, respectively ($P < 0.05$). The rise in the TDF was mainly ascribable to the IDF fraction, which ranged from 0.38 to 3.40 g per 100 g for GM0 and GM10, respectively. In addition, the soluble/insoluble dietary fibre ratio was 0.24, 0.27, and 0.27 for GM0, GM5 and GM10, respectively. Accordingly, the GM10 can be claimed as a 'source of dietary fibre' as it contains more than 3 g per 100 g of TDF (European Parliament, 2011). Similarly, bread, breadsticks, cookies and muffins fortified with comparable levels of grape pomace were characterised by a greater TDF, IDF and SDF levels than the control (Bender *et al.*, 2017; Rainero *et al.*, 2022; Tolve *et al.*, 2021). Lastly, no differences were detected in the total sugar, protein and fat contents, being on average 17.45, 4.42 and 10.23 g per 100 g of product, respectively. In addition, the water activity was similar among the different muffin formulations with an average value of 0.876 ± 0.003. A previous study reported that bread fortified with GPP obtained similar but generally higher water activity than muffins, in which recipes enlisted ingredients like sugar and salt (Tolve *et al.*, 2021).

Technological properties of muffins

The inclusion of DGPP influenced the moistures level of vegan muffins, ranging from 23.08 to 26.11 g water per 100 g of product for GM0 and GM10, respectively ($P < 0.05$) (Table 2). Similar findings were observed in muffins fortified with brewer's spent grain, peaches fibres, linseed/flaxseed, wheatgrass powder (Sudha *et*

Table 1 Proximate composition and water activity (a_w) of vegan muffin formulated by increasing level of dried distilled grape pomace powder (DGPP)

Sample	a_w	Ash	Fat	Protein	Total starch	Total sugars	TDF	IDF	SDF
GM0	0.878 ± 0.05^a	0.87 ± 0.02^a	10.20 ± 0.11^a	4.48 ± 0.09^a	41.55 ± 0.62^a	17.05 ± 0.05^a	0.47 ± 0.06^a	0.38 ± 0.05^a	0.09 ± 0.01^a
GM5	0.879 ± 0.04^a	1.25 ± 0.02^b	10.45 ± 0.08^a	4.47 ± 0.01^a	39.53 ± 0.76^b	17.80 ± 0.54^a	2.23 ± 0.09^b	1.76 ± 0.07^b	0.47 ± 0.02^b
GM10	0.872 ± 0.06^a	1.38 ± 0.03^c	10.05 ± 0.06^a	4.32 ± 0.11^a	35.38 ± 0.42^c	17.49 ± 0.74^a	4.29 ± 0.13^c	3.40 ± 0.10^c	0.89 ± 0.02^c

Data are expressed as g per 100 g of food product. Values with different superscripts within the same column are significantly different for $P < 0.05$.

GM0, vegan muffin formulated with 0 g per 100 g of DGPP in the recipe; GM10, vegan muffin formulated with 10 g per 100 g of DGPP in the recipe; GM5, vegan muffin formulated with 5 g per 100 g of DGPP in the recipe; IDF, insoluble dietary fibre; SDF, soluble dietary fibre; TDF, total dietary fibre.

Table 2 Technological properties of vegan muffin formulated by increasing level of dried distilled grape pomace powder (DGPP)

Sample	Moisture content (%)	pH	Baking loss	Height (cm)	Spread ratio	Volume (cm^3)	Specific volume ($cm^3\ g^{-1}$)
GM0	23.08 ± 0.35[a]	7.75 ± 0.02[a]	32.9 ± 0.2[a]	30.04 ± 1.52[a]	1.69 ± 0.1[a]	43.2 ± 4.9[a]	1.74 ± 0.20[a]
GM5	24.71 ± 1.01[ab]	5.31 ± 0.01[b]	32.2 ± 0.2[b]	28.92 ± 0.54[a]	1.76 ± 0.5[a]	38.3 ± 3.8[a]	1.39 ± 0.07[a]
GM10	26.11 ± 1.17[b]	4.39 ± 0.02[c]	31.8 ± 0.2[b]	22.10 ± 2.66[b]	2.25 ± 0.27[c]	29.3 ± 3.7[b]	1.09 ± 0.11[b]

Data are expressed as mean ± standard deviation. Values with different superscripts within the same column are significantly different for $P < 0.05$.
GM0, vegan muffin formulated with 0 g per 100 g of DGPP in the recipe; GM5, vegan muffin formulated with 5 g per 100 g of DGPP in the recipe; GM10, vegan muffin formulated with 10 g per 100 g of DGPP in the recipe.

al., 2010; Rahman *et al.*, 2015; Shih *et al.*, 2020). This change is probably related to the addition of fibre-rich ingredients, which can raise the moisture content of the final food products (Sudha *et al.*, 2010). The pH of the muffins changed ($P < 0.05$) accordingly to the DGPP inclusion level, ranging from 7.75 to 4.39 for GM0 and GM10, respectively. This finding could be ascribed to the presence of organic acids in the selected ingredient, in line with previous findings (Rainero *et al.*, 2022; Tolve *et al.*, 2021). The baking loss of GM5 and GM10 samples decreased ($P < 0.05$) compared to GM0, with no statistical differences among the fortified vegan muffins. Bender *et al.* (2017) reported that the baking loss of muffins added with Riesling skin powder or Tannat skin powder decreased due to the hydration capacity of the fibre fractions in the fortified products. The presence of fibre components from DGPP affected the baking performances of the vegan muffins since the height, the volume and the specific volume of GM10 was lower ($P < 0.05$) while the spread ratio was higher ($P < 0.05$) than the control. Different authors reported a similar effect after the addition of several fibre-rich ingredients in cake and muffin formulations, thus concluding that the decrease in the volume can be related to the damage of the gluten network following the fibre addition, causing a decrement in the gas retention in the leavened doughs (Salehi & Aghajanzadeh, 2020; Ayoubi *et al.*, 2021). Indeed, in our study, there was a negative correlation between TDF vs. volume of muffin ($r = -0.99$; $P < 0.05$) and a positive correlation between TDF and the spread ratio ($r = 0.94$; $P < 0.05$). Moreover, Salehi & Aghajanzadeh (2020) concluded that cake fortified with fruit/vegetable powders contributed to reducing the gluten content of batters, thus lowering the volume of the final products after baking.

Table 3 Rheological properties and specific gravity of vegan muffin formulated by increasing level of dried distilled grape pomace powder (DGPP)

	Power law model			
Sample	*n*	*K*	r^2	Specific gravity
GM0	0.628 ± 0.017[a]	36.82 ± 2.46[a]	0.9975	1.25 ± 0.01[a]
GM5	0.598 ± 0.014[a]	38.90 ± 2.83[a]	0.9968	1.23 ± 0.01[a]
GM10	0.664 ± 0.009[b]	27.06 ± 2.10[b]	0.9981	1.22 ± 0.01[a]

Data are expressed as mean ± standard deviation. Values with different superscripts within the same column are significantly different for $P < 0.05$.
GM0, vegan muffin formulated with 0 g per 100 g of DGPP in the recipe; GM5, vegan muffin formulated with 5 g per 100 g of DGPP in the recipe; GM10, vegan muffin formulated with 10 g per 100 g of DGPP in the recipe.

Rheological properties of vegan muffins' batter

In the current study, the shear stress vs. shear rate recorded data of fortified batters well fitted the Power Law model equation ($r^2 > 0.9968$) (Table 3). The flow index (n) may vary from $n = 1$ (leading to the Newtonian flow) to $n < 1$ or $n > 1$ to describe a shear-thinning or shear-thickening flow behaviour, and the consistency index is an indicator of the viscous nature of the batter system (Kırbaş *et al.*, 2019). All batter formulations with different levels of DGPP showed a shear-thinning (pseudoplastic) behaviour ($n < 1$) over the studied shear rate (10–250 s^{-1}), suggesting that the apparent viscosity decreased with the increase in shear rate. Kırbaş *et al.* (2019) reported a similar behaviour in cake batters. Indeed, flow index (n) for GM10 was higher ($P < 0.05$) than GM0 and GM5, indicating the apparent viscosity of the muffin batter with 10% of DGPP decreased less noticeably when the shear rate increased. The addition of 5% of carrot, apple and orange pomace powder had, similarly, no significant effect on the flow behaviour and consistency indexes compared to control in gluten-free cake batters (Kırbaş *et al.*, 2019).

The K values decrease ($P < 0.05$) from 36.82 to 27.06 for GM0 moving to GM10. Lebesi & Tzia (2011) reported a similar trend for cupcake batters enriched with different types of bran. Instead, the addition of 5 g per 100g of DGPP did not affect the consistency index since similar K values ($P > 0.05$) were recorded between GM0 and GM5. Batter consistency affects the baked product volume thanks to its capacity to entrap air, representing an important parameter for leavening products. Indeed, if consistency is too low, cake volumes become smaller because of the batter's poor effectiveness in holding air bubbles (Marchetti *et al.*, 2018). In addition, there was a slightly positive correlation between volume of muffin vs. consistency index ($r = 0.86$) and specific volume ($r = 0.74$), while a stronger and negative correlation subsisted with the spread ratio ($r = -0.96$; $P < 0.05$).

The higher specific gravity of the batters is associated with less aeration indicating the lower capacity of the batter to retain air during beating operation and consequently lower height and volume in the baked product (Nath *et al.*, 2018). In this study, the specific gravity did not change ($P > 0.05$) with the GDPP addition, indicating the same capacity of bubble retention among the different formulations. In contrast, Nath *et al.* (2018) and An (2014) showed that batters fortified with increasing red capsicum powder or bitter melon, respectively, had a slightly higher specific gravity.

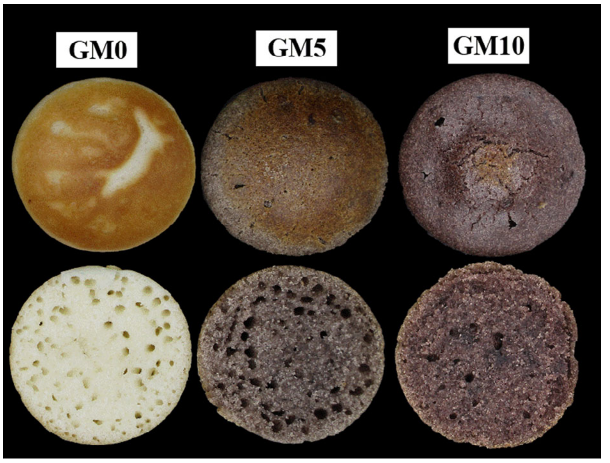

Figure 1 From top to bottom and from left to right: crusts and cut sections of vegan muffin formulated with dried distilled grape pomace powder (DGPP). GM0: muffin with 0 g per 100 g of DGPP; GM5: muffin with 5 g per 100 g of DGPP; GM10: muffin with 10 g per 100 g of DGPP.

Colour of muffins

The colour of baked products is pivotal for consumers and their willingness to buy. In this sense, consumers typically attribute darker crusts and crumbs to whole grain baked goods, rich in fibres and healthier. The addition of DGPP significantly affected ($P < 0.05$) the colour of muffins (Table 4, Fig. 1), explained by the increased concentration of pigments from the grape skins. The L and b^* parameters decreased ($P < 0.05$), both in crust and crumb, following the substitution level of the flour mix with DGPP, while a^* parameter significantly increased ($P < 0.05$) in the crumb. Acun & Gül (2014) obtained similar results in cookies fortified with grape seed flour, grape skin flour and whole grape flour, where parameters L and b^* decreased with the flour replacement. Moreover, the colour parameter of bread and cake fortified with grape pomace from winemaking showed a similar trend (Šporin *et al.*, 2018; Tolve *et al.*, 2021). All confronted samples have a colour difference ΔE greater than 3. Thus all crusts and crumbs of DGPP-fortified muffins differed in colour as compared to the control, and such differences could be considered perceivable by the human eyes (Table 4).

Physical properties of vegan muffins

As a result of the addition of DGPP in the recipe, the firmness of vegan muffins increased, ranging from 13.0 to 23.6 N for GM0 and GM10, respectively ($P < 0.05$) (Table 5). Present results are in accord with data obtained by Heo *et al.* (2019), who reported an increase in hardness for baked muffins formulated with increasing amounts of a fibre-rich ingredient derived from kimchi by-product. With the addition of DGPP, the gluten in the dough was diluted, and thereby it might cause decreased gas-capturing ability and thus

Table 4 Colour evaluation of crumb and crust of muffins

	Crumb			Crust					
Sample	L	a^*	b^*	L	a^*	b^*	Confront	Crumb	Crust
GM0	71.42 ± 2.57^a	−1.77 ± 0.11^a	15.60 ± 0.64^a	63.50 ± 5.38^a	8.25 ± 3.52^a	34.76 ± 5.07^a	GM0–GM5	36.44	34.60
GM5	36.14 ± 1.39^b	4.34 ± 0.43^c	8.83 ± 0.64^b	35.07 ± 2.47^b	10.89 ± 0.66^a	15.21 ± 1.79^b	GM0–GM10	49.58	45.42
GM10	24.15 ± 1.76^c	7.19 ± 0.28^b	3.62 ± 0.61^c	28.98 ± 1.31c	6.78 ± 0.64^b	5.27 ± 0.64c	GM5–GM10	13.38	12.36

Data are reported as mean ± standard deviation. On the right, colour differences ΔE between samples have been reported. Values with different superscripts within the same column are significantly different for $P < 0.05$.

GM0, vegan muffin formulated with 0 g per 100 g of DGPP in the recipe; GM5, vegan muffin formulated with 5 g per 100 g of DGPP in the recipe; GM10, vegan muffin formulated with 10 g per 100 g of DGPP in the recipe.

Table 5 Physical properties of vegan muffin formulated by increasing level of dried distilled grape pomace powder (DGPP)

Sample	Firmness (N)	Pore density (pore cm^{-2})	Pore area fraction (%)	Average pore diameter (mm)
GM0	13.0 ± 1.93^a	7.8 ± 0.8^a	9.4 ± 1.2^a	0.98 ± 0.01^a
GM5	16.9 ± 1.81^b	6.1 ± 1.1^a	11.0 ± 1.0^a	0.97 ± 0.02^a
GM10	23.6 ± 3.21^c	9.5 ± 0.8^b	9.6 ± 0.4^a	0.84 ± 0.01^b

Data are expressed as mean ± standard deviation. Values with different superscripts within the same column are significantly different for $P < 0.05$.

GM0, vegan muffin formulated with 0 g per 100 g of DGPP in the recipe; GM5, vegan muffin formulated with 5 g per 100 g of DGPP in the recipe; GM10, vegan muffin formulated with 10 g per 100 g of DGPP in the recipe.

increased the density of the vegan muffins. Moreover, Kamel & Rasper (1988) pointed out that the firmness of muffins was indirectly related to the volume of the baked products, thus corroborating current findings.

The image analyses showed that control and GM5 vegan muffins had the lowest pore density of GM10 (Table 5). These data are in line with the previous studies reporting that muffins fortified with defatted sunflower seed flour and pecan nut expeller had higher pore density than the control (Marchetti *et al.*, 2018; Grasso *et al.*, 2020). Marchetti *et al.* (2018) debated that a higher proportion of pecan nut by-product led to muffins with greater pore density, greater heights and substantially better gas retention capacity. Conversely, in this study, there was a negative correlation between heights and pore density ($r = -0.80$; $P < 0.05$), a positive correlation between average pore diameter vs. volume ($r = 0.96$; $P < 0.05$), and a negative correlation with spread ratio ($r = -0.99$; $P < 0.05$). Thus, in agreement with Marchetti *et al.* (2018), the height of muffins does not just depend on the number of pores but may rely on the pore size distribution. Moreover, the relative frequencies of pore diameter under 0.63 mm (lower bound of the third interval) increased following the DGPP inclusion level: the cumulative frequencies were 0.43, 0.50, 0.53 for GM0, GM5 and GM10, respectively (Fig. 2). Grasso *et al.* (2020) found a similar increment in the range of 0.2–0.5 mm diameter in muffins fortified with defatted sunflower seed flour. Therefore, the average pore diameter of GM10 was lower than the control ($P < 0.05$). Conversely, Aranibar *et al.* (2019) reported that partially deoiled chia flour improved the number of greater pores. In terms of pore area fraction, no differences were recorded in this study. Grasso *et al.* (2020) obtained similar results when defatted sunflower seed flour substitution did not exceed the threshold of 15% in muffin formulation. In contrast, Aranibar *et al.* (2019) reported an increment in pore area fraction in muffins with partially deoiled chia flour.

Phenolic profile and in vitro antioxidant activity values

The results of the different *in vitro* assays are reported in Table 6. The antioxidant activity of DGPP was 526.0 ± 33.1 and 342.4 ± 21.2 μM TE per g DM evaluated by FRAP and ABTS, respectively. The *in vitro* antioxidant activities of vegan muffins significantly increased and were positively related to the amount of DGPP in the recipe ($r = 0.976$ FRAP, $r = 0.997$ ABTS). A similar trend was detected in bread, breadsticks and pasta formulated with comparable grape pomace levels in the recipe (Tolve *et al.*, 2020, 2021; Rainero *et al.*, 2022).

The untargeted phenolic profiling of DGPP by UHPLC-HRMS allowed the putative annotation of 205 polyphenols, with a great abundance of flavonoids (107 compounds), followed by fifty-one phenolic acids, forty-one other phenolics and six stilbenes. A comprehensive dataset containing all the phenolics identified together with their mass spectra and relative abundance values can be found in the Supporting Information. The subclass of anthocyanins consisted of eighteen compounds, with a great abundance of cyanidin mainly in the bound phenolic fraction of the DGPP. In this regard, the semi-quantitative analysis based on the phenolic classes annotated revealed a total phenolic content of 609.77 mg Eq. per kg DM, with a great abundance of phenolic acids (284.42 mg Eq. per kg DM), followed by flavonoids (162.42 mg Eq. per kg DM) and other phenolics (154.76 mg Eq. per kg DM). Interestingly, the DGPP consisted of 39.3% of bound phenolics (mainly phenolic acids) and 60.7% of free phenolics. Regarding anthocyanins, a similar content was found in both free (i.e. 2.96 mg Eq. per kg DM) and bound (2.45 mg Eq. per kg DM) phenolic fraction of the DGPP under investigation. The detailed phenolic content, considering each subclass annotated, of the DGPP is reported in the Supporting Information.

As the next step, we evaluated the impact of DGPP addition on the phenolic profile of the formulated vegan muffin samples. The semi-quantitative analysis (Table 7) following the UHPLC-HRMS profiling revealed a significant increase of several phenolic classes (in both free and bound fractions) following the inclusion of increasing levels of DGPP (*i.e.* 5% and 10%). Firstly, an increase of the total cumulative phenolic content was observed when moving from GM0 (100.22 mg Eq. per kg DM) up to GM10 (154.71 mg Eq. per kg DM) (Supporting Information). Overall, flavonoids were the most affected compounds by the inclusion of different DGPP levels; in particular, anthocyanins accounted for a cumulative

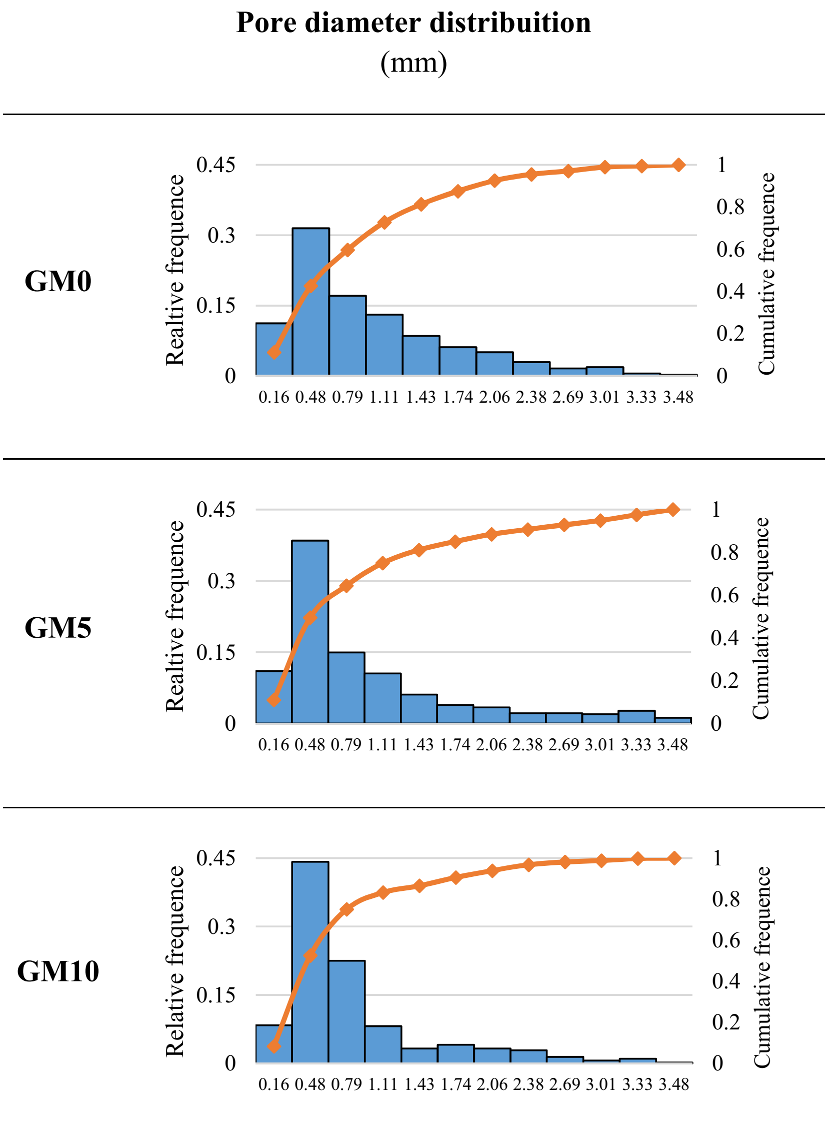

Figure 2 Pore diameter distribution of vegan muffins formulated with dried distilled grape pomace powder (DGPP). Values in the x-axis are the average diameter of pores (mm), the right y-axis is the relative frequency and the right axis is the cumulative frequency which is plotted in orange line. GM0: muffin with 0 g per 100 g of DGPP; GM5: muffin with 5 g per 100 g of DGPP; GM10: muffin with 10 g per 100 g of DGPP.

1.10 mg Eq. per kg DM in the GM10 sample, whilst a proportional increase of total flavonols was observed when moving from GM5 (4.58 mg Eq. per kg DM) to GM10 (9.40 mg Eq. per kg DM) vegan muffin samples (Table 7). The increase in anthocyanins (*i.e.* those polyphenols responsible for the colour-related attributes of the products) is coherent with the results presented in Table 4. The free phenolic fraction characterising the class of other phenolics (quantified as oleuropein equivalents) was not affected by the increasing level of DGPP. The inclusion of 5 and 10 g per 100 g of DGPP provided similar results for the bound phenolic fraction of other phenolics (on average: 25.28 mg Eq. per kg DM) and the free phenolic fraction of stilbenes (on average: 0.98 mg Eq. per kg DM). The increase of the bound phenolic content due to the increasing levels of DGPP fortification represents another interesting result. In particular, bound phenolics have been related to several health benefits that are governed by their content in the food matrix and their bioaccessibility and bioavailability (Acosta-Estrada *et al.*, 2014). Besides, recent studies have indicated that insoluble-bound phenolics can have a role in modulating gut microbiota and intestinal immune response as a dietary component (Zhang *et al.*, 2020). Therefore, our findings demonstrated that the inclusion of DGPP in vegan muffins can significantly improve the bound phenolic profile and their potential

Table 6 FRAP and ABTS values of vegan muffin formulated by increasing level of dried distilled grape pomace powder (DGPP)

Sample	FRAP (μM TE 100 g^{-1} DM)	ABTS (μM TE 100 g^{-1} DM)
GM0	189.08 ± 20.62^{a}	376.78 ± 35.52^{a}
GM5	1063.90 ± 119.21^{b}	1234.43 ± 123.70^{b}
GM10	3026.27 ± 290.62^{c}	1895.72 ± 170.63^{c}

Data are expressed as mean ± standard deviation. Values with different superscripts within the same column are significantly different for $P < 0.05$.
GM0, vegan muffin formulated with 0 g per 100 g of DGPP in the recipe; GM5, vegan muffin formulated with 5 g per 100 g of DGPP in the recipe; GM10, vegan muffin formulated with 10 g per 100 g of DGPP in the recipe.

health promoting properties once released into the large intestine. Moreover, Pearson's correlation shed light on the role which different phenols classes may have in affecting *in vitro* antioxidant activities (Table 7). ABTS assay had high correlation with free anthocyanins ($P < 0.05$), phenolic acids ($P < 0.05$) and bound flavonols ($P < 0.05$) while FRAP resulted in positive high correlation especially with bound stilbenes and flavanols ($P < 0.05$).

Sensory analysis

The substitution of common wheat and potato starch flour with DGPP significantly influenced most of the selected sensory attributes (Fig. 3). Visually, the DGPP inclusion did not alter the experimental vegan muffin's colour uniformity and porosity. On the contrary, grape pomace from winemaking significantly affected the colour uniformity and porosity of fortified bread (Tolve *et al.*, 2021). The DGPP inclusion affected all aromas' sensory attributes ($P < 0.05$). Panellists judged GM10 as having less bake cake odour than GM0 (the typical smell of even baked cake), while GM5 did not differ statistically. As expected, panellists found that the addition of DGPP significantly improved the wine and the fruity odour as a function of the inclusion level ($r = 0.99$ and $r = 0.95$, respectively; both $P < 0.05$). Indeed, the Cabernet variety is well known for its floral and fruity nuances (Allen *et al.*, 1994*)*. The DGPP inclusion also statistically affected muffins' taste as fruitiness was positively correlated with the addition of DGPP ($r = 0.99$, $P < 0.05$). The DGPP fortification did not influence elasticity, mellowness, adhesiveness and hardness. However, astringency was statistically significant

Table 7 Semi-quantitative contents of the different phenolic classes characterising vegan muffin formulated by increasing level of dried distilled grape pomace powder (DGPP) and Pearson's correlation test of different phenolic classes vs. *in vitro* antioxidant assays FRAP and ABTS

Phenolic class equivalent (Eq.)	Phenolic fraction	GM0 (mg Eq. per kg DM)	GM5 (mg Eq. per kg DM)	GM10 (mg Eq. per kg DM)	Pearson's coefficient of determination	
					FRAP	ABTS
Anthocyanins (Cyanidin Eq.)	Free	0.02 ± 0.00^{A}	0.35 ± 0.05^{B}	0.61 ± 0.05^{C}	0.920	1.000***
	Bound	<0.01^{a}	0.34 ± 0.05^{b}	0.49 ± 0.06^{c}	0.825	0.981*
Flavones (Luteolin Eq.)	Free	0.15 ± 0.04^{A}	0.69 ± 0.03^{B}	1.26 ± 0.16^{C}	0.960	0.992*
	Bound	0.11 ± 0.03^{a}	0.41 ± 0.08^{b}	0.84 ± 0.11^{c}	0.987*	0.969
Flavanols (Catechin Eq.)	Free	0.16 ± 0.10^{A}	0.70 ± 0.11^{B}	2.49 ± 0.39^{C}	0.993*	0.866
	Bound	0.40 ± 0.10^{a}	0.85 ± 0.16^{b}	1.67 ± 0.18^{c}	0.997**	0.943
Flavonols (Quercetin Eq.)	Free	0.21 ± 0.07^{A}	3.69 ± 0.03^{B}	7.81 ± 0.80^{C}	0.972	0.985*
	Bound	0.18 ± 0.03^{a}	0.88 ± 0.06^{b}	1.59 ± 0.00^{c}	0.955	0.994**
Other phenolics (Oleuropein Eq.)	Free	38.98 ± 3.68^{A}	41.88 ± 3.50^{A}	44.51 ± 4.79^{A}	0.984*	0.972
	Bound	22.84 ± 0.62^{a}	25.22 ± 0.81^{b}	25.34 ± 0.65^{b}	0.586	0.844
Phenolic acids (Ferulic acid Eq.)	Free	30.51 ± 1.15^{A}	38.86 ± 0.19^{B}	44.59 ± 0.82^{C}	0.898	0.999**
	Bound	6.07 ± 0.06^{a}	12.39 ± 0.59^{b}	22.29 ± 0.41^{c}	0.992*	0.960
Stilbenes (Resveratrol Eq.)	Free	0.49 ± 0.03^{A}	0.91 ± 0.11^{B}	1.06 ± 0.07^{B}	0.783	0.963
	Bound	0.04 ± 0.00^{a}	0.07 ± 0.01^{a}	0.13 ± 0.04^{b}	0.999**	0.932

Data are expressed as mean values (mg Eq. per kg dry matter, DM) ± standard deviation ($n = 3$). Different superscript letters for free (^{A,B,C}) and bound (^{a,b,c}) phenolic classes within each row indicate significant differences ($P < 0.05$) as resulting from one-way ANOVA and Tukey post-hoc test. The test and the significance of Pearson's correlation has been performed with XLSTAT Premium Version (2021.1.1, Addinsoft SARL, Paris, France). *** for $P < 0.01$; ** for $P < 0.05$; * for $P < 0.10$.
GM0, vegan muffin formulated with 0 g per 100g of DGPP in the recipe; GM5, vegan muffin formulated with 5 g per 100 g of DGPP in the recipe; GM10, vegan muffin formulated with 10 g per 100 g of DGPP in the recipe.

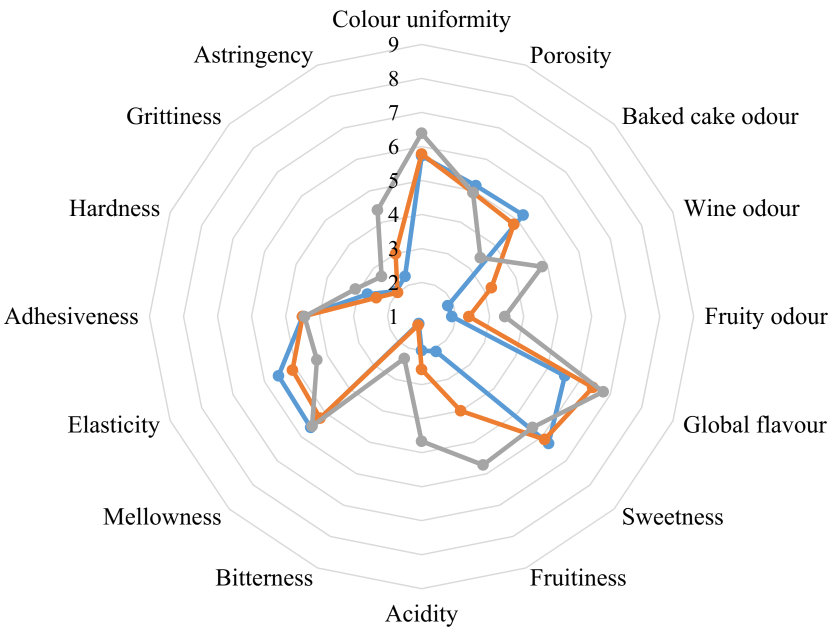

Figure 3 Sensory profile of vegan muffins formulated with dried distilled grape pomace powder in the recipe (DGPP). A 9-point hedonic scale was used. GM0: muffin with 0 g per 100 g of DGPP (blue line); GM5: muffin with 5 g per 100 g of DGPP (orange line); GM10: muffin with 10 g per 100 g of DGPP (grey line).

only in sample GM10 compared to GM0 due to the presence of tannin in grape pomace. Finally, the addition of DGPP did not significantly impact the overall acceptability of the muffins, which scores were more than 6, exceeding the threshold value of 5. The obtained scores of acceptability were 6.05 ± 1.47 for GM0, 6.88 ± 1.52 for GM5 and 6.77 ± 1.35 for GM10, respectively, with no statical difference. Likewise, Bender *et al*. (2017) reported that muffins fortified with Riesling or Tannat grape pomace flour had good sensory acceptability as well.

Conclusions

In this work, vegan muffins formulated with increasing levels of DGPP in the recipe showed higher moisture, higher firmness and greater spread ratio but a decrease in the baking loss, volume and specific volume giving an overall slight flattened aspect. The DGPP addition affected the colour of samples depending on the level of inclusion. Moreover, a higher level of DGPP favours the formation of a crumb with a greater number of smaller pores. An increase in phenolic content and *in vitro* antioxidant activity following increasing DGPP levels in the recipe was detected. The phenolic profile inspected through the UHPLC-HRMS approach revealed a significant increase in several phenolic classes (in both free and bound fractions) due to the level of inclusion of DGPP. The most affected compounds were flavonoids, especially anthocyanins. Our finding demonstrated that the muffins' fortification with DGPP improved the bound phenolic compounds, which are described as compounds exerting the highest bioaccessibility and bioavailability in the human body. In addition, DGPP fortified muffins contained the great level of dietary fibre, allowing GM10 to benefit from the claim 'source of fibre'. Vegan muffins boasted good sensory acceptability, GM5 obtaining the highest overall acceptability score. To summarise, DGPP can be considered a suitable ingredient in vegan muffin formulation, as vegan DGPP-muffins represented a baked product that can satisfy the increasing demand for foods containing several bioactive components with presumable health-promoting properties and better nutritional composition that the current market counterparts.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contribution

Federico Bianchi: Conceptualization (equal); Investigation (equal); Methodology (equal); Writing – original draft (equal); Writing – review & editing (equal). **Mariasole Cervini:** Investigation (equal); Methodology (equal). **Gianluca Giuberti:** Conceptualization (equal); Methodology (equal); Supervision (equal); Writing – original draft (equal); Writing – review & editing (equal). **Gabriele Rocchetti:** Investigation (equal); Methodology (equal); Writing – original draft (equal); Writing – review & editing (equal). **Luigi Lucini:** Writing – review & editing (equal). **Barbara Simonato:** Conceptualization (lead); Methodology (equal); Supervision (lead); Writing – review & editing (equal).

Ethical approval

Ethics approval was not required for this research.

Peer review

The peer review history for this article is available at https://publons.com/publon/10.1111/ijfs.15720.

Data availability statement

UHPLC-HRMS data are available in article Supporting Information. Dataset used in the current study is available from the corresponding author on reasonable request.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Dataset resulting from the UHPLC-HRMS analysis of the different muffin samples formulated with increasing level of dried distilled grape pomace powder (DGPP) in the recipe.

