



# Grape skin and seed flours as functional ingredients of pizza: Potential and drawbacks related to nutritional, physicochemical and sensory attributes

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## ABSTRACT

As a consequence of the increase in consumer demand for added-value foods, the purpose of this study was to develop pizza bases, exploiting the techno-functional properties of oenological flours. Fortified pizza bases were obtained by replacing wheat flour with 15, 20, and 25% of skin flour (GS) and skin/seed flour (GM). The replacement with GS and GM allowed to increase the content of anthocyanins and phenolic compounds and the antioxidant activity, especially when the mix of grape skin and seeds was used. The addition of GS and GM at the highest percentages, allowed to obtain “high fiber content” pizza bases by reaching more than 6 g of dietary fiber/100 g. At the highest substitution levels, significant differences were found depending on GM and GS addition both for hardness (13.93 vs 22.75) and chewiness (9.73 vs 18.19). The analysis of volatile compounds (VOCs), instead, showed that the addition of grape flours reduced the presence of pyrazine and increased the concentration of esters, acids, ketones, and aldehydes, known for their significant sensory impact. The sensory evaluation was in line with the results of VOCs, highlighting the perception of must, acid, and pungent, as well as with the physicochemical analysis.

## 1. Introduction

In recent years, the nutritional aspects of food products have become key parameters for consumers. This led to increased demand for functional foods with beneficial effects on consumer's health. Consequently, the development of innovative high nutritional value products is becoming increasingly importance. The use of innovative flours, such as those obtained from agro-industrial by-products, could provide the necessary nutrients for the development of this type of foods, increasing their nutritional value.

Huge quantities of waste and by-products are produced daily during all stages of production in the food industries, representing a serious environmental problem. Winemaking generates a large amount of by-products corresponding to 30% w/w of starting grape, among which grape pomace (consisting mainly of grape skin and seeds), represent the most abundant (Bordiga, Montella, Travaglia, Arlorio, & Coisson, 2019; Bordiga, Travaglia, Locatelli, Arlorio, & Coisson, 2015; Muhlack,

Potumarthi, & Jeffery, 2018).

Grape skin has been recently proposed as food ingredient owing to its nutritional value and functional properties due to the presence of high levels of dietary fiber, phenols, flavonoids, and other antioxidant substances (Beres et al., 2016; Bordiga, Travaglia, & Locatelli, 2019; Mattos, Tonon, Furtado, & Cabral, 2017). Grape seeds, instead, represent 2–5% of the weight of whole grapes and constitute 40–50% of the solid waste of wine industries (Bordiga, Montella, Travaglia, Arlorio, & Coisson, 2019; Libera, Latoch, & Wójciak, 2020). From the nutritional point of view, this by-product is valuable due to the presence of antioxidant compounds, tocopherol, vitamin E, dietary fiber, and proteins (Barba, Zhu, Koubaa, Sant'Ana, & Orlie, 2016; Troilo, Difonzo, Paradiso, Summo, & Caponio, 2021). Grape seeds are typically reused for the extraction of the oil, which is characterized by an acidic profile rich in polyunsaturated/monounsaturated fatty acids and poor in saturated ones (Bordiga, Travaglia, & Locatelli, 2019). Among other functional molecules,  $\beta$ -sitosterol and  $\alpha$ -tocopherol reach values of about 70% of

*Abbreviations:* GS, grape skin flour; GM, mix of grape skin/seed flour; ABTS, 2,20-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl; TE, trolox equivalents; TPC, total phenols content; GAE, gallic acid equivalents; TAC, total anthocyanins content; Cyn 3-glu, cyanidin 3-O-glucoside; TDF, total dietary fiber; TPA, textural profile analysis; VOCs, volatile organic compounds.

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the unsaponifiable fraction (Bordiga, Travaglia, & Locatelli, 2019; Fiori et al., 2014).

Many studies focus on the enrichment of numerous types of foods such as bakery products (bread, muffins, cookies, breadsticks, pancakes), pasta, cheese, or meat with oenological by-products both in form of powder and extracts (Acun & Gül, 2014; Altinok et al., 2022; Bianchi et al., 2022; Bianchi, Lomuscio, Rizzi, & Simonato, 2021; Gaglio et al., 2021; Hoye & Ross, 2011; Libera et al., 2020; Lou, Zhou, Li & Nataliya, 2021; Nakov et al., 2020; Troilo, Difonzo, Paradiso, Pasqualone, & Caponio, 2022).

Among bakery products, pizza is one of the most appreciated and consumed foods in the world due to its cheap price, quick preparation and high versatility, being topped with a wide choice of ingredients (Helstosky, 2008). In 2020, the two dominant pizza markets were Western Europe (\$ 49.3 billion) and North America (\$ 48.6 billion). The market has also witnessed year-on-year growth of 4.84% in 2021 and will record a compound annual growth rate (CAGR) of 6.11% during the period 2022–2026, growing by 51.38 million dollars in the forecast period, due to the increase in urban population and to the acceleration of the fast food pizza market (Global Pizza Market).

The main ingredient used to prepare pizza bases is wheat flour, usually refined, consisting mainly of carbohydrates (especially starch) and a low amount of dietary fiber, vitamins, and minerals. It follows that the nutritional intake of the latter is very limited, hence pizza consumption contributes to the increase in blood glucose (Riccardi, Clemente, & Giacco, 2003; Della-Corte et al., 2020).

In this context, the addition of grape skin and grape seed flours to the pizza formulation, could make up for the poor properties of refined flours, resulting in an improvement in the nutritional quality and functional properties of the final product. In fact, previous studies highlighted the quality improvement of pizza, following the addition of innovative ingredient, such as pseudocereals flour, jujube powder, teragon extract, soya protein isolate, fenugreek leaves and lotus stem or legumes (Bharath, Kathalsar, Chandrashekhar, & Prabhasankar, 2021; Falciano, Sorrentino, Masi, & Di Pierro, 2022; Gupta, Milind, Jeyarani, & Rajiv, 2015; Kanauiya, 2017; Pasqualone et al., 2019, 2022; Ribeiro et al., 2016). However, the effect of the addition of grape pomace to pizza bases has not studied so far.

For this reason, the aim of the present investigation was to exploit grape skin and skin/seed flours in reformulating pizza bases. The effect of the partial wheat flour substitution with these oenological flours was evaluated by investigating the nutritional composition, the phenolic profile, the physicochemical and sensory attributes of the fortified pizza bases.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Methanol HPLC grade and ethanol absolute anhydrous were purchased from Carlo Erba (Milan, Italy); sodium carbonate from Honeywell (Seelze, Germany); formic acid, Folin-Ciocalteu reagent, ABTS (2,20-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt) and DPPH (2,2-diphenyl-1-picrylhydrazyl) were purchased from Sigma-Aldrich (St. Louis, USA).

### 2.2. Grape flours preparation

Red grape pomace (*Vitis vinifera* L.) was kindly provided by a winery in Santeramo in Colle (Apulia, southern Italy), following a maceration phase of seven days, and collected after pressing. Grape pomace was dried at 120 °C for 60 min in a ventilated oven (Argo-lab-TCF120, Carpi, Italy) as described by Troilo et al. (2022).

Then, the grape skin was separated from the grape seeds and residual stalks by a 5 mm sieve (Endecotts test sieve, London, England). Grape skins and seeds were ground separately by an electric mill equipped with

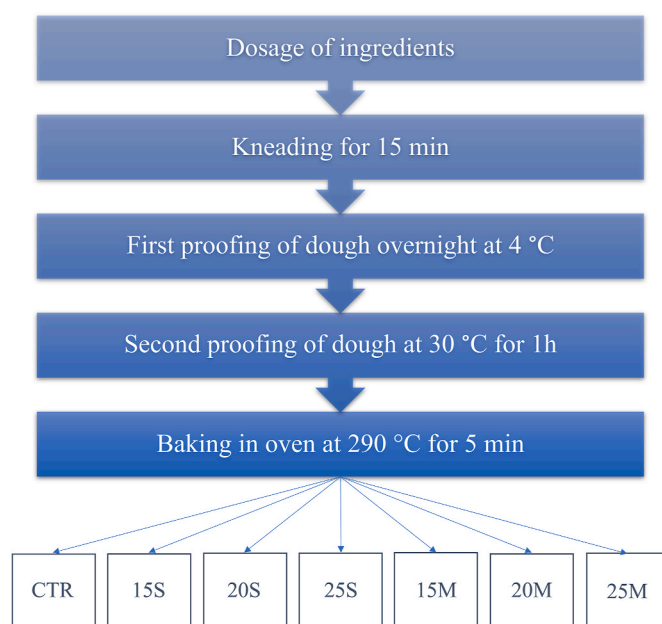
a sieve of 0.6 mm (ETA-Vercella, Turin, Italy), and further then sieved by stainless steel sieves with 300 µm mesh (Giuliani Tecnologie srl, Turin, Italy). The flour obtained from grape skins was indicated with GS; while GM indicated the flour prepared from a mix of grape skin/seed (70:30 w/w).

### 2.3. Pizza bases preparation

The production of enriched pizza bases was performed replacing the refined wheat flour (Caputo, Naples, Italy) with grape by-products flour. In particular, GS (grape skin flour) and GM (mix of skin/seed flour 70:30 w/w) were used replacing 15, 20, and 25% of wheat flour, respectively. Pizza base was prepared as follows: 700 g of refined wheat flour (or wheat flour partly substituted with GS or GM) were kneaded (Bomann Kneading machine, Kempen, Germany) with 2.5 g of yeast (Lievital, Lesaffre Treccasali, Italy), tap water, 19 g of salt (Italkali, Palermo, Italy) and 7 g of extra virgin olive oil (Olearia Desantis S.p.A, Bitonto, Italy) for about 15 min. The dough (Fig. 1) was then kept overnight at 4 °C and then put into a proofing cell (Memmert proofer, EN.CO. Srl, Spinea, Italy) at 30 °C for 1 h. Finally, the dough was manually flattened and shaped as discs having 30 cm diameter which were cooked (Oem Ali Group Srl, Bozzolo, Italy) at 290 °C for about 5 min. Seven types of pizza bases were prepared: CTR (control made of refined wheat flour only), 15S (added of 15% of grape skin flour), 20S (added of 20% of grape skin flour), 25S (added of 25% of grape skin flour), 15M (added of 15% of skin/seed flour), 20M (added of 20% of skin/seed flour), and 25M (added of 25% of skin/seed flour).

### 2.4. Proximate composition

The determination of total dietary fiber was carried out by the enzymatic-gravimetric procedure as described in the AOAC (method 985.29, 2006); while the lipid content was determined by a Soxhlet apparatus, using diethyl ether as an extracting solvent (AOAC, methods 945.38F, 2006). The protein content (total nitrogen × 5.7) and ash content were determined according to the AOAC methods 979.09 and 923.03, respectively (2006). The carbohydrate content was determined



**Fig. 1.** Pizza bases production process. CTR (control made of refined wheat flour only), 15S (added of 15% of grape skin flour), 20S (added of 20% of grape skin flour), 25S (added of 25% of grape skin flour), 15M (added of 15% of skin/seed flour), 20M (added of 20% of skin/seed flour), and 25M (added of 25% of skin/seed flour).

as difference subtracting the total dietary fiber, protein, ash, moisture, and lipid contents from 100.

### 2.5. Polyphenols determination and antioxidant activity evaluation

The phenolic compounds and anthocyanins of grape pomace flours and pizza bases were extracted as described by Troilo et al. (2022), with some modifications. Briefly, for polyphenols 1 g of sample was extracted with 8 mL of 80% methanol; while anthocyanins extraction was carried out adding 10 mL of methanol/water/formic acid (80:18:2, v/v/v). For both extractions, the samples were subjected to ultrasound treatment for 10 min in an ultrasound bath (CEIA international S.A., 115/230 Vac 1-50/60 Hz-400VA max, Vicinaggio, Italy), and shacked for 30 min; then the extracts were centrifuged (Thermo Fisher Scientific, Osterode am Harz, Germany) at 4 °C at 8000 g for 10 min, the supernatants were separated, and the pellet reprocessed for five time. Three replicates for each sample were analyzed.

The total phenols content (TPC) was determined according to the Folin-Ciocalteu method according to Difonzo et al. (2021), with some modifications. In particular, to 980 µL of deionized water were added 20 µL of filtered extracts and 100 µL of Folin-Ciocalteu reagent. After 3 min, 800 µL of 7.5% Na<sub>2</sub>CO<sub>3</sub> was added, and then incubated at room temperature for 60 min. The absorbance was read at 720 nm using a Cary 60 spectrophotometer (Cernusco, Milan, Italy), and the TPC was obtained by performing a calibration curve with gallic acid ( $y = 0.018x + 0.023$ ;  $R^2 = 0.998$ ). The results were expressed as mg of gallic acid equivalents (GAE)/g of sample. Each sample was analyzed in triplicate.

Total anthocyanins content (TAC) was determined by UV-vis spectrophotometry as described by Troilo et al. (2022). The extracts were filtered with 0.45 µm nylon filter and analyzed by reading the absorbance at 535 nm by Cary 60 UV-vis spectrophotometer (Agilent Technologies). A calibration curve was performed by using cyanidin 3-O-glucoside (Cyn 3-glu) as standard ( $y = 0.037x + 0.019$ ;  $R^2 = 0.997$ ). The results were expressed in mg Cyn 3-glu/g of each sample. Each sample was analyzed in triplicate.

Extracts were analyzed for the evaluation of antioxidant activity with DPPH and ABTS assay, as described by Caponio, Noviello, et al. (2022). The DPPH assay was carried out in cuvettes for spectrophotometry adding 950 µL of DPPH solution to 50 µL of sample. After 30 min of incubation, the absorbance was read at 517 nm using Cary 60 spectrophotometer. While, for the ABTS assay, was generated an ABTS<sup>+</sup> radical by reaction with potassium persulfate (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>), adding 25 mL of ABTS (7 mM in H<sub>2</sub>O) to 800 µL of K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>, and incubated in the dark for 16 h. The reaction for evaluate the antioxidant activity was carried out in cuvettes for spectrophotometry placing 50 µL of each sample and 950 µL of ABTS<sup>+</sup> solution. After 8 min, the absorbance was reading at 734 nm. A calibration curve was performed with Trolox for each reported method (DPPH,  $y = -0.001x + 0.790$ ,  $R^2 = 0.999$ ; ABTS,  $y = -0.002x + 0.744$ ,  $R^2 = 0.999$ ). The results were expressed in µmol Trolox equivalents (TE)/g of sample.

Each sample was analyzed in triplicate.

### 2.6. Texture profile analysis

The texture profile analysis (TPA) of each sample was carried out according to Pasqualone et al. (2019), with some modifications. The analysis was performed on pizza bases (4 × 4 cm) using a texture analyzer Z1.0 TN (Zwick Roell, Ulm, Germany), equipped with a stainless-steel cylindrical probe (36 mm diameter) and a 50 N load cell. Data were acquired by the TestXPERTII version 3.41 software (Zwick Roell, Ulm, Germany). Two compressive cycles were performed at 1 mm/s probe compression rate and 40% sample deformation in both the compression, with 5 s pause before second compression. At the end of compression, hardness, springiness, chewiness and cohesiveness were evaluated. The analyses were carried out on twenty-eight pizza bases (four per type, in triplicate).

### 2.7. Color analysis

The colorimetric determination was carried out on crust and crumb of pizza bases using a colorimeter CM-600d (Konica Minolta, Tokyo, Japan) supported by the software Spectramagic NX (Konica Minolta, Tokyo, Japan). Lightness ( $L^*$ ), redness ( $a^*$ , red-green), and yellowness ( $b^*$ , yellow-blue) were determined as color coordinates. The parameters were measured at several points internally and externally of samples.

### 2.8. Scanning electron microscopy (SEM)

The microstructure of pizza bases was studied with a Zeiss Sigma 300 VP (Carl Zeiss NTS GmbH, Germany) field-emission gun scanning electron microscope (FEG-SEM) equipped with a secondary electrons detector (SE). The analyses were done under vacuum ( $<10^{-4}$  Pa), using an accelerating voltage of 20 kV, an aperture of 30 µA, working distance between 4 and 5 mm and a magnification of 200 ×. Fragments of the different pizza bases samples were glued onto an aluminum stub with carbon tape. Before the analysis, all the samples were carbon-coated in order to make the surface of the specimen conductive.

### 2.9. Determination of volatile organic compounds of pizza bases

Volatile organic compounds (VOCs) were determined through headspace solid phase micro-extraction (SPME) coupled to gas-chromatography/mass spectrometry (GC/MS) as reported by Caponio, Difonzo, et al. (2022). In particular, 0.5 g of pizza bases were weighed in 12 mL vials and 150 µL of 1-propanol was added as internal standard plus 4 mL of NaCl (20% w/v aqueous solution). Vials were sealed by butyl rubber septa and aluminum crimp caps. Before extraction of VOCs, vials were shaken for 2 min with a laboratory vortex to promote samples homogenization. The extraction of volatile compounds was carried out by exposing a 75 µm carboxen/polydimethylsiloxane (CAR/PDMS) SPME fiber (Supelco, Bellefonte, PA, USA) in the headspace of the vials at 40 °C for 50 min; then the fiber was desorbed for 6 min in the injection port of gas-chromatograph, operating in a split-less mode at 230 °C for 3.5 min. For the separation of volatile compounds, an Agilent 6850 gas-chromatograph equipped with an Agilent 5975 mass-spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA) and an HP-Innowax (Agilent Technologies Inc., Santa Clara, CA, USA) polar capillary column (60 m length × 0.25 mm i.d. × 0.25 µm film thickness) was used under the following conditions: injector temperature, 250 °C; flow of 1.5 mL/min; pressure of the carrier (helium) 30 kPa. The oven temperature was held for 5 min at 35 °C, then increased by 5 °C/min to 50 °C and held in isothermal conditions for 5 min, then raised to 210 °C at 5.5 °C/min, and finally held constant at 210 °C for 5 min. The mass detector was set as: interface temperature 230 °C, source temperature 230 °C, ionization energy 70 eV, and scan range 33–260 amu. Peak identification was performed by LRI and by computer matching with the reference mass spectra of National Institute of Standards and Technology (NIST) and Wiley libraries. The volatile compounds were quantified by standardizing the peak areas of compounds of interest with the peak area of internal standard (1-propanol). The analysis was carried out in triplicate.

### 2.10. Sensory analysis

A quantitative descriptive analysis (QDA) of pizza bases was performed by a sensory panel composed by nine trained judges, at the University of Bari (Italy). All the judges had neither allergies nor food intolerances and were regular consumers of bakery products. The sensory analysis followed the ethical guidelines of the laboratory and written informed consent was obtained from each panelist. The samples were labeled with an alphanumeric code and distributed in a random order. A total number of twelve sensory descriptors were considered indicating the intensity of appearance, olfactory and taste, and textural

attributes using a 9-point scale. Appearance attributes were evaluated indicating the crust and crumb color intensity (1 = beige, 10 = dark purple), thickness (1 = 0.1 mm, 10 = 1 cm); the olfactometric descriptors were evaluated indicating the intensity of must and pungent odor notes, while sweetness, acidity, bitterness, salty, and astringency was evaluated by tasting. Finally, textural attributed in terms of softness and humidity, and were determined.

### 2.11. Statistical analysis

The data were subjected to the Dunnett test, one and two-way ANOVA (analysis of variance), followed by Tukey test for multiple comparisons, with a significance level of 95%, using the Minitab Statistical Software (Minitab Inc., State College, USA). The Dunnett test was performed for multiple comparisons with the CTR, and the differences were considered statistically significant at  $p < 0.001$ . Polar heatmap with a circular dendrogram deriving and the principal component analysis (PCA) were performed using Origin 2021 (OriginLab, Northampton, MA, USA).

## 3. Results and discussion

### 3.1. Chemical composition of flours

As shown in Table 1, the refined wheat flour used had a significant lowest content in total dietary fiber (TDF), lipids and ashes, compared to grape skin flour (GS) and the flour prepared from a mix of grape skin/seeds (GM), while the protein and carbohydrate content were higher in refined flour. Comparing the flours of the oenological by-products with each other it emerged a slightly higher lipids content in GM, due to the presence of 30% of grape seeds, known to be oil-rich as reported by Acun and Gül (2014) and Lou, Li, and Nataliya (2021). Moreover, GS had a significantly higher ash content than GM, in accordance with Kuchtová, Kohajdová, Karovičová, and Lauková (2018) due to the higher amount of minerals in grape skin (Tseng & Zhao, 2012).

The chemical composition of skin and seeds varies depending on several factor, such as the cultivar, the agronomic and climatic condition, and the wine-making technology adopted (Kuchtová et al., 2018). The protein content of grape pomace varies from 6 to 15% (Bordiga, Travaglia, & Locatelli, 2019; Nakov et al., 2020; Ortega-Heras, Gómez, de Pablos-Alcalde, & González-Sanjosé, 2019; Rainero et al., 2022). The protein content of GS flour (11.13%), in our study, was higher than GM (9.65%). Kuchtová et al. (2018), instead, reported no significant differences between grape seeds and skin, both with an amount of 8–9% protein content.

Grape polyphenols belong to different classes of compounds, among

**Table 1**  
Proximate composition, total phenol content (TPC), total anthocyanin content (TAC), ABTS and DPPH assays of flours.

Parameters	Wheat flour	GS flour	GM flour
Moisture (g/100 g)	12.23 ± 0.21a	4.75 ± 0.29b	4.91 ± 0.32b
Lipids (g/100 g)	1.35 ± 0.02c	5.97 ± 0.03b	6.79 ± 0.02a
Proteins (g/100 g)	13.75 ± 0.00a	11.13 ± 0.25b	9.65 ± 0.12c
Ashes (g/100 g)	0.68 ± 0.04c	13.78 ± 0.04a	10.43 ± 0.10b
Total dietary fiber (g/100 g)	3.30 ± 0.08c	46.56 ± 0.11a	35.03 ± 0.12b
Carbohydrates (g/100 g)	68.89 ± 0.09a	17.81 ± 0.24c	33.19 ± 0.30b
TPC (mg GAE/g)	4.21 ± 0.08c	18.53 ± 0.52b	31.37 ± 0.63a
TAC (mg Cyn 3-glu/g)	–	7.40 ± 0.05a	5.55 ± 0.05b
ABTS (μmol TE/g)	0.63 ± 0.01c	110.07 ± 0.24b	160.52 ± 1.89a
DPPH (μmol TE/g)	0.23 ± 0.01c	68.89 ± 0.23b	123.48 ± 1.86a

Different letters in the same line mean a significant difference at  $p < 0.05$ . GS, grape skin; GM, mix of skin/seeds (70:30 w/w).

which phenolic acids, anthocyanins, flavanols, and stilbenes, still persist (for, approximately, 70%) in grape pomace, after the winemaking process (Xia, Deng, Guo, & Li, 2010). Depending on the chosen oenological practice, the maceration phase differently influences the phenolic content of both wine and grape pomace. These compounds are known for their beneficial effects (e.g., anti-inflammatory, antiaging, anticancer, cardioprotective, antimicrobial, antioxidant, and anti-inflammatory properties), on human health (Caponio, Noviello, et al., 2022). In addition, TDF content was significantly higher in GS than GM, with values of about 47% and 35%, respectively, in accordance with the finding of other authors (Kuchtová et al., 2018; Nakov et al., 2020).

A significantly higher amount of TPC was observed in GM (31.37 mg/g) than GS and wheat flour, which showed a content of 18.53 and 4.21 mg/g, respectively. Similarly, the antioxidant activity evaluated with the ABTS and DPPH tests, had the same trend of TPC, with higher values in GM. Finally, the total anthocyanin content (TAC), as expected, was higher in GS and not detected in wheat flour. The obtained values are in line with those found by other authors (Llobera & Cañellas, 2007; Nakov et al., 2020; Pintač et al., 2018).

### 3.2. Characterization of pizza bases

#### 3.2.1. Proximate and chemical composition of pizza bases

Table 2 shows the proximate and chemical composition of the different experimental pizza bases. Two-way ANOVA carried out on the fortified pizza bases showed that the P (percentage) and F (oenological flour) variables had a significant effect on the composition of the experimental pizza bases irrespective of the amount and type of flour added. In fact, only the main effect of P and F for protein and total dietary fiber respectively, did not show significant differences, while the first order interaction P\*F, always showed significant differences.

The Dunnett test, carried out on the entire data set to highlight the influence of the innovative flour addition in comparison with wheat flour, showed that the fortification of pizza bases, with the exception of protein content, had a significant influence on the considered parameters. In particular, only 15S e 20S samples showed a protein content significantly lower than control ( $p < 0.05$ ). Lipids, ashes, carbohydrates, and total dietary fiber showed values significant higher in fortified pizza bases than control one – that on the contrary showed moisture content significant higher – with values directly related with the increase of the percentage of substitution of wheat flour. The obtained data were in accordance with the composition of the flours (Table 1) and with the finding of Ortega-Heras et al. (2019) who fortified muffins with 10 and 20% of grape pomace powder. The total dietary fiber content of fortified pizza bases allowed in all cases to attribute the nutritional claims established by EC Regulation 1924/2006 on the presence of dietary fiber in relevant amounts. In particular, 15S can be defined as “source of fiber” due to the dietary fiber content greater than 3 g/100 g, while the other fortified pizza bases can be labeled “high fiber content” for the presence of 6 g of dietary fiber per 100 g of product. Regarding the influence of the type of oenological flour used, the most evident differences were found in lipid content, with values significantly higher for GM than GS due to the presence of 30% of grape seeds. Finally, the differences in moisture content could be due to the higher presence of TDF that interfered with the formation of gluten network, with a consequent loss of water during cooking.

The incorporation of different oenological flours in pizza bases modified also the TPC, TAC, and antioxidant activity. In particular, all parameters significantly raised with the increase in the replacement percentage of wheat flour, as found by other authors for different fortified foods enriched with grape pomace (Kuchtová et al., 2018; Nakov et al., 2020; Smith & Yu, 2015; Theagarajan, Malur Narayanaswamy, Dutta, Moses, & Chinnaswamy, 2019). Finally, the TAC were higher when GS was used and, on the contrary ABTS and DPPH showed values significantly higher in the pizza bases containing GM. This trend confirmed the results of chemical characterization of starting flours.

**Table 2**

Results of Dunnett test and of two-way ANOVA followed by Tukey's test for multiple comparison of the proximate composition, total phenol content (TPC), total anthocyanin content (TAC), ABTS and DPPH assays of pizza bases.

Parameters	CTR	15S	20S	25S	15M	20M	25M	p-value P*F	p-value P	p-value F
Moisture (g/100 g)	25.31 ± 0.65	24.30 ± 0.41 ab*	22.79 ± 0.50cd*	22.51 ± 0.29d*	24.92 ± 0.11a*	23.73 ± 0.56bc*	22.44 ± 0.30d*	p < 0.001	p < 0.05	p < 0.05
Lipids (g/100 g)	0.81 ± 0.01	1.11 ± 0.02d*	1.44 ± 0.01b*	1.50 ± 0.01b*	1.28 ± 0.03c*	1.42 ± 0.02b*	1.88 ± 0.02a*	p < 0.001	p < 0.001	p < 0.001
Proteins (g/100 g)	10.25 ± 0.19	9.66 ± 0.26b*	9.89 ± 0.27b*	10.10 ± 0.01a	10.35 ± 0.13a	10.09 ± 0.23a	10.16 ± 0.04a	p < 0.01	ns	p < 0.05
Ashes (g/100 g)	2.28 ± 0.09	3.75 ± 0.00c*	4.29 ± 0.06b*	4.81 ± 0.13a*	3.28 ± 0.13d*	3.90 ± 0.06c*	4.34 ± 0.03a*	p < 0.001	p < 0.001	p < 0.001
Carbohydrates (g/100 g)	60.96 ± 0.67	55.40 ± 0.10 ab*	54.30 ± 0.73bc*	51.89 ± 0.51d*	54.12 ± 0.19a*	53.74 ± 0.84c*	52.21 ± 0.54e*	p < 0.001	p < 0.001	p < 0.01
Total dietary fiber (g/100 g)	0.39 ± 0.11	5.78 ± 0.01d*	7.29 ± 0.10b*	9.19 ± 0.12a*	6.05 ± 0.10c*	7.12 ± 0.10b*	8.97 ± 0.12a*	p < 0.001	p < 0.001	ns
TPC (mg GAE/g)	0.25 ± 0.00	1.70 ± 0.01e*	2.44 ± 0.10c*	3.10 ± 0.06a*	2.02 ± 0.04d*	2.66 ± 0.07b*	3.15 ± 0.01a*	p < 0.001	p < 0.001	p < 0.001
TAC (mg Cyn 3-glu/g)	–	0.45 ± 0.02c*	0.54 ± 0.01b*	0.72 ± 0.03a*	0.31 ± 0.00e*	0.39 ± 0.00d*	0.51 ± 0.01b*	p < 0.001	p < 0.001	p < 0.001
ABTS (μmol TE/g)	0.71 ± 0.01	6.83 ± 0.01e*	8.88 ± 0.10de*	9.88 ± 0.06d*	8.02 ± 0.06c*	10.32 ± 0.31b*	12.87 ± 0.60a*	p < 0.001	p < 0.001	p < 0.001
DPPH (μmol TE/g)	0.34 ± 0.02	5.98 ± 0.06e*	7.72 ± 0.22c*	8.22 ± 0.15a*	6.76 ± 0.32d*	8.35 ± 0.20b*	9.99 ± 0.06a*	p < 0.001	p < 0.001	p < 0.001

\* Significant difference with  $p < 0.001$  of fortified pizza bases than control ones. Different letters in the same row for the fortified pizza bases mean a significant difference at  $p < 0.05$ . CTR (control made of refined wheat flour only), 15S (added of 15% of grape skin flour), 20S (added of 20% of grape skin flour), 25S (added of 25% of grape skin flour), 15M (added of 15% of skin/seed flour), 20M (added of 20% of skin/seed flour), and 25M (added of 25% of skin/seed flour). P, percentage of oenological flour substitute; F, type of oenological flour used; ns, not significant.

### 3.2.2. Texture profile

Table 3 reports the data related to texture analysis of pizza bases. All attributed detected showed significant differences between the fortified pizza bases and the control. The addition of GS and GM affected hardness and chewiness more than other parameters. This trend could be related to richness in dietary fiber and absence of gluten in oenological flours, factors that probably have interfered with the formation of gluten network. According to Glicerina, Balestra, Capozzi, Dalla Rosa, and Romani (2018), the fiber promotes a structuring effect that involves an increase in the rigidity of samples, as also found by Karnopp et al. (2015)

**Table 3**

Results of Dunnett test and of two-way ANOVA followed by Tukey's test for multiple comparison of the hardness, chewiness, springiness, and cohesiveness of different pizza bases.

Samples	Hardness (N)	Chewiness (N)	Springiness	Cohesiveness
CTR	5.89 ± 0.21	4.67 ± 0.26	0.82 ± 0.03	0.70 ± 0.01
15S	8.84 ± 0.43d*	6.45 ± 0.57b*	0.89 ± 0.01a*	0.75 ± 0.01a*
20S	22.57 ± 0.42a*	17.20 ± 0.30a*	0.90 ± 0.00a*	0.76 ± 0.03a*
25S	22.73 ± 0.80a*	18.19 ± 0.84a*	0.89 ± 0.00a*	0.76 ± 0.01a*
15M	10.67 ± 0.58c*	8.48 ± 0.41b*	0.88 ± 0.00 ab*	0.66 ± 0.03b*
20M	10.72 ± 0.16c*	9.58 ± 0.45b*	0.87 ± 0.00b*	0.65 ± 0.02b*
25M	13.93 ± 0.67b*	9.73 ± 0.78b*	0.87 ± 0.01b*	0.67 ± 0.02b*
p-value (P*F)	p < 0.001	p < 0.001	ns	ns
p-value (P)	p < 0.001	p < 0.001	ns	ns
p-value (F)	p < 0.001	p < 0.001	p < 0.05	p < 0.001

\* Significant difference with  $p < 0.001$  of fortified pizza bases than control ones. Different letters in the same column for the fortified pizza bases mean a significant difference at  $p < 0.05$ . CTR (control made of refined wheat flour only), 15S (added of 15% of grape skin flour), 20S (added of 20% of grape skin flour), 25S (added of 25% of grape skin flour), 15M (added of 15% of skin/seed flour), 20M (added of 20% of skin/seed flour), and 25M (added of 25% of skin/seed flour). P, percentage; F, oenological flour; ns, not significant.

who associated the addition of grape pomace with the increase of hardness in biscuits. Other authors associated the increase in hardness with the increase of dough density and a reduced incorporation of air during mixing (Aghamirzaei, Peighambaroudost, Azadmard-Damirchi, & Majzoobi, 2015; Bender et al., 2017; Ortega-Heras et al., 2019).

The results are in line with those reported by Falciano et al. (2022) and Sagar and Pareek (2020) following the fortification of pizza bases with jujube and onion powder, respectively. The hardness behavior, in fact, appears directly proportional to the increase in the substitution of wheat flour, and significantly higher for GS addition ( $p < 0.05$ ). Lower values in correspondence of samples formulated with the addition of 25% of grape seeds, could be linked to a greater lipid and moisture content (as shown in Table 2), which consequently made the pizza bases less hard, even at the highest percentage added.

The chewiness, moreover, was influenced by both the percentage and the type of flours used; grape skin seems to impact more on this parameter, especially at the highest quantity, as reported by Ortega-Heras et al. (2019). As regard springiness, the trend appears to be inversely proportional to the percentage added, especially when GM was used, due to the higher level of total dietary fiber which have perhaps increased the density of samples and consequently reduced this parameter at the highest percentages, as highlighted by Kuchtová et al. (2018) and Ortega-Heras et al. (2019). The high fiber content has also affected cohesiveness; the values, in fact, decreased after the use of mix of grape skin and seeds.

### 3.2.3. Color analysis

The colorimetric parameters are the main characteristics that influence the acceptability of consumers; for this reason, the crust and the crumb of fortified pizza bases were analyzed and the CIELab results obtained in terms of lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ) are reported in Table 4.

The crust and crumb showed  $L^*$  and  $b^*$  values significantly lower in fortified samples than control, with values that decreasing as the percentages of oenological flour in the mix increased. These finding confirmed the trend reported by other authors (Lou, Zhou, Li, & Nataliya, 2022; Najjaa, Arfa, Elfalleh, Zouari, & Neffati, 2020; Ortega-Heras et al., 2019; Rainero et al., 2022; Tolve et al., 2021), some of which linked the decrease of lightness to the increase of total dietary fiber.

**Table 4**

Mean values, standard deviation, and results of statistical analysis (two-way ANOVA followed by Tukey's test for multiple comparison) of colorimetric parameters of crust and crumb of pizza bases.

Samples	$L^*$	$a^*$	$b^*$	$\Delta E$
<b>Crust</b>				
CTR	73.40 ± 0.16	1.73 ± 0.02	25.14 ± 0.20	–
15S	41.04 ± 0.24b	2.32 ± 0.06b	3.36 ± 0.23cd	39.01 ± 0.02c
20S	38.74 ± 0.25c	2.55 ± 0.02a	3.18 ± 0.03d	41.03 ± 0.44b
25S	35.05 ± 0.24d	2.58 ± 0.08a	2.60 ± 0.03e	44.49 ± 0.35a
15M	42.31 ± 0.32a	2.47 ± 0.03a	4.35 ± 0.28a	37.40 ± 0.02c
20M	42.25 ± 0.11a	2.48 ± 0.04a	3.95 ± 0.05ab	37.68 ± 0.25d
25M	41.03 ± 0.28b	2.53 ± 0.05a	3.74 ± 0.02bc	38.80 ± 0.06d
<i>p</i> -value (P*F)	<i>p</i> < 0.001	<i>p</i> < 0.001	<i>p</i> < 0.001	<i>p</i> < 0.001
<i>p</i> -value (P)	<i>p</i> < 0.001	<i>p</i> < 0.001	<i>p</i> < 0.001	<i>p</i> < 0.001
<i>p</i> -value (F)	<i>p</i> < 0.001	ns	<i>p</i> < 0.001	<i>p</i> < 0.001
<b>Crumb</b>				
CTR	74.30 ± 0.19	2.71 ± 0.02	20.46 ± 0.09	–
15S	21.31 ± 0.57ab	4.79 ± 0.11bc	1.82 ± 0.06c	56.18 ± 0.36c
20S	21.28 ± 0.66ab	4.89 ± 0.08b	1.68 ± 0.04d	56.26 ± 0.50c
25S	18.10 ± 0.25d	5.42 ± 0.05a	1.37 ± 0.03e	59.38 ± 0.15a
15M	21.74 ± 0.20a	3.53 ± 0.26e	2.38 ± 0.04a	55.62 ± 0.17c
20M	20.26 ± 0.08bc	4.08 ± 0.01d	2.15 ± 0.03b	57.10 ± 0.15b
25M	19.82 ± 0.15c	4.55 ± 0.08c	2.07 ± 0.02b	57.56 ± 0.15b
<i>p</i> -value (P*F)	<i>p</i> < 0.001	<i>p</i> < 0.001	<i>p</i> < 0.001	<i>p</i> < 0.001
<i>p</i> -value (P)	<i>p</i> < 0.001	<i>p</i> < 0.001	<i>p</i> < 0.001	<i>p</i> < 0.001
<i>p</i> -value (F)	ns	<i>p</i> < 0.001	<i>p</i> < 0.001	<i>p</i> < 0.001

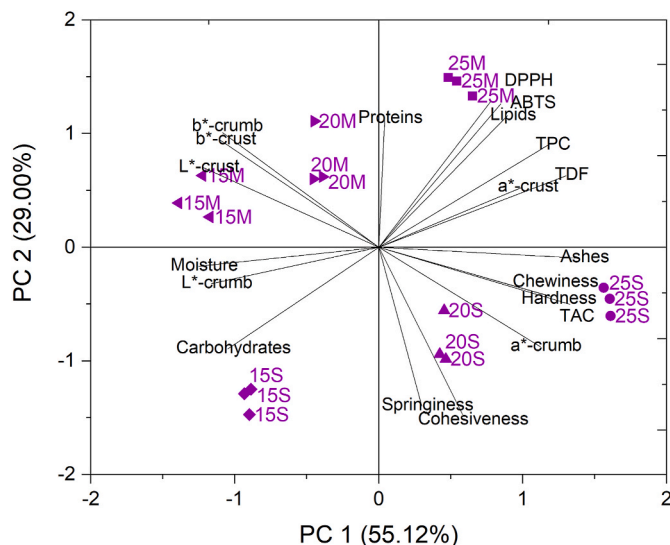
Different letters in the same column mean a significant difference at  $p < 0.05$ . CTR (control made of refined wheat flour only), 15S (added of 15% of grape skin flour), 20S (added of 20% of grape skin flour), 25S (added of 25% of grape skin flour), 15M (added of 15% of skin/seed flour), 20M (added of 20% of skin/seed flour), and 25M (added of 25% of skin/seed flour). P, percentage; F, oenological flour; ns, not significant.

Moreover, the reduction of this parameter, is correlated to the baking process and to the Maillard and caramelization reaction between reducing sugars and amino acids abundant in grape pomace (Troilo et al., 2022). The  $a^*$  value, instead, was significantly higher in fortified pizza bases than control, both for crust and for crumb and with higher values for the latter. While crust generally showed no differences between the fortified samples, crumb redness seemed to be influenced both by the added percentages and by type of oenological flour used. The data increased with the increase of wheat flour substitution, in agreement with TAC values.

The total color differences ( $\Delta E$ ) between samples and control were very high and always greater of five, indicating significant color differences between samples (Mokrzycki & Tatol, 2011). Chromatic differences were observed both externally and internally, with greater differences when higher GS concentrations were used. This may be associated with an increase in redness and a decrease in lightness.

### 3.2.4. Principal component analysis (PCA)

A multivariate analysis was performed by PCA from proximate, physical and chemical analysis performed on the enriched samples with grape flours (Fig. 2). The first two components PC 1 and PC 2 explain over 70% of the total variability. The combination of data sets resulted in a clear-cut differentiation of the pizza enriched with grape skin flour (15S, 20S, 25S) and those with the mix of grape skin/seeds (15M, 20M,



**Fig. 2.** Biplot from principal component analysis. 15S (added of 15% of grape skin flour), 20S (added of 20% of grape skin flour), 25S (added of 25% of grape skin flour), 15M (added of 15% of skin/seed flour), 20M (added of 20% of skin/seed flour), and 25M (added of 25% of skin/seed flour). Abbreviations: TPC, total phenol content; TAC, total anthocyanin content; TDF, total dietary fiber.

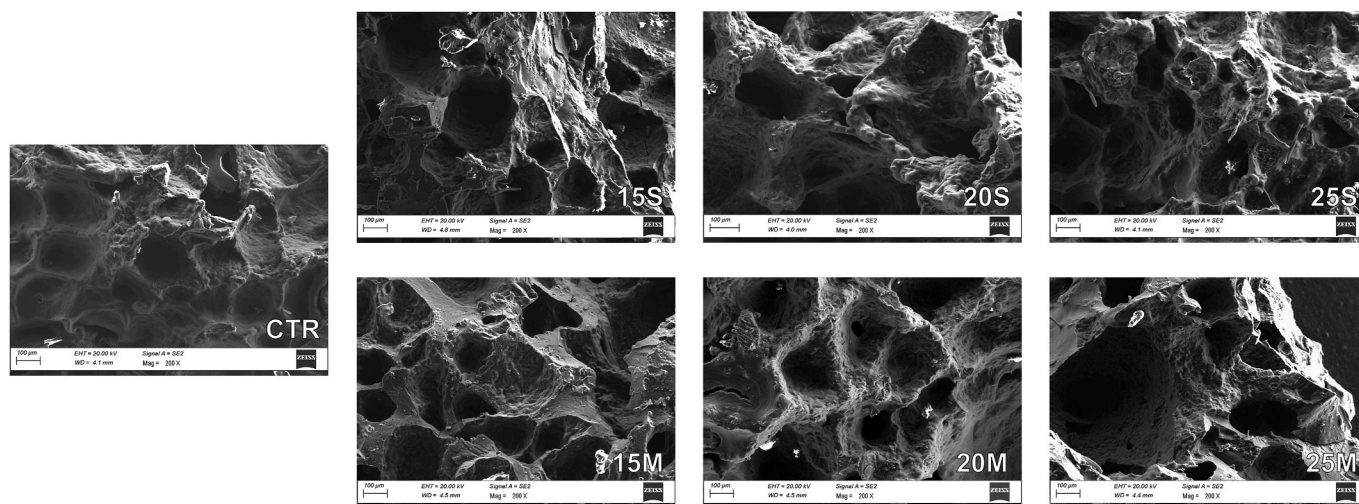
25M), mainly explained by PC2. Moreover, a clear separation has been observed especially between the samples added with 15% and those with 25% of grape flours, explained by PC 1. The main parameters related with 25M samples were the lipid content, the antioxidant activity, the TPC, the TDF and the redness index of the crust, according to the results previously reported. Springiness, cohesiveness, chewiness and hardness were mostly related with the samples 20S and 25S, in fact, as previously reported, the textural parameters were negatively affected especially by the addition of GS flours.

### 3.2.5. Microstructure analysis (SEM)

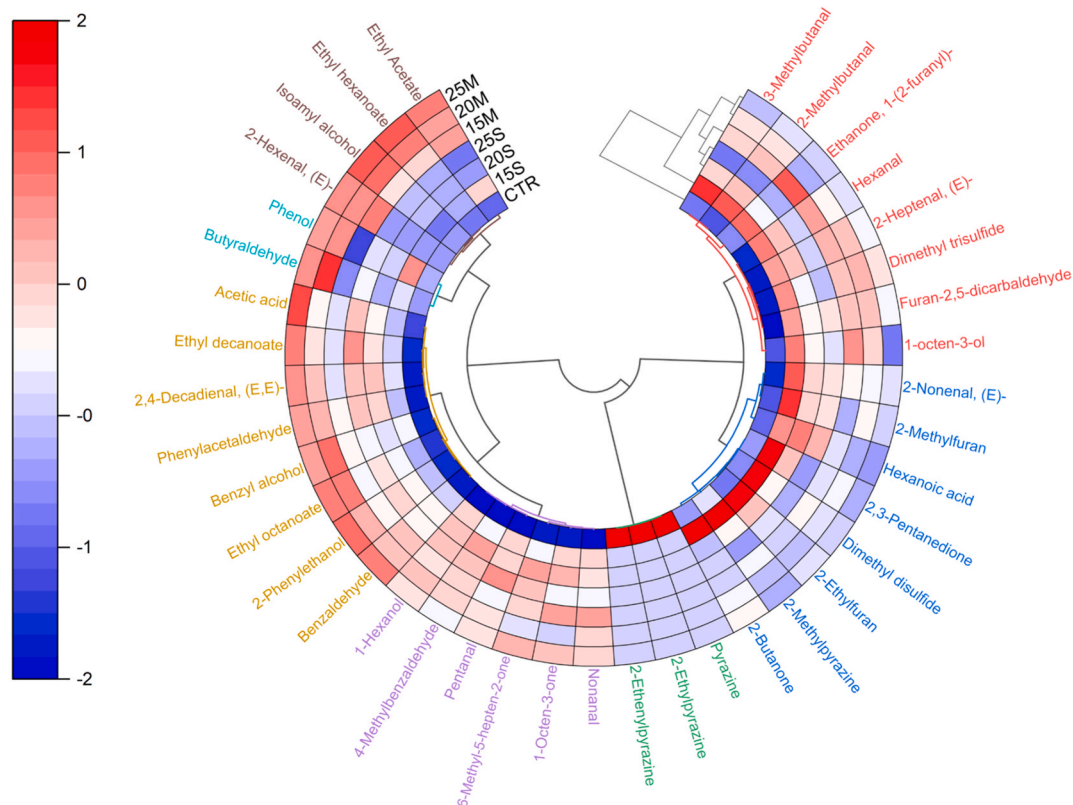
In order to determine the effect of the addition of oenological tested flours on the development of gluten network in pizza bases, a study of scanning electron microscopy (SEM) was carried out (Fig. 3). As can be seen, the CTR presented a structure with gas cells that appear to be more homogeneous in both shape and distribution, than the enriched samples; furthermore, the starch particles were wrapped and evenly distributed in a complete and continuous gluten network in CTR, as also reported by Lou et al. (2021) and Mildner-Szkudlarz et al. (2016). The integrity and continuity of the structure of protein network appears to be compromised especially when GS was added, as shown by the presence of larger and more irregular cells with jagged edges. In fact, 15S, 20S, and 25S showed more fibrous structures that led to the interruption and destruction of gluten network, as also observed by Liu, Chen, Zheng, Lu, and Chen (2020) and Chen et al. (2021) in noodles with matcha powder and grape seed flour, respectively. On the whole, the changes in the microstructure of pizza bases obtained with more than 20% of GS and GM in the formulation could be also due to the interaction between gluten and polyphenols by broking the intrinsic molecular hydrogen bonds (Liu et al., 2020). Lou et al. (2021) and Struck, Straube, Zahn, and Rohm (2018) highlighted a reduction in the elasticity of the dough, and a consequent decrease in gas retention, and thus a reduction in the volume of products when fiber and polyphenols are added in the dough.

### 3.2.6. Volatile organic compounds (VOCs)

Fig. 4 shows a total 39 VOCs grouped into 7 clusters. In particular, the most abundant volatile compounds obtained belonged to the following chemical classes: aldehydes, ketones, alcohols, esters, furans, carboxylic acids, pyrazines, and sulfur compounds. Some of these derive from cooking, others from fermentation, oxidation, or raw material.



**Fig. 3.** Scanning electron microscopy of pizza bases. CTR (control made of refined wheat flour only), 15S (added of 15% of grape skin flour), 20S (added of 20% of grape skin flour), 25S (added of 25% of grape skin flour), 15M (added of 15% of skin/seed flour), 20M (added of 20% of skin/seed flour), and 25M (added of 25% of skin/seed flour).



**Fig. 4.** Polar heatmap of volatile compounds of pizza bases fortified. Data are represented as means  $\pm$  SD of three replicates. CTR (control made of refined wheat flour only), 15S (added of 15% of grape skin flour), 20S (added of 20% of grape skin flour), 25S (added of 25% of grape skin flour), 15M (added of 15% of skin/seed flour), 20M (added of 20% of skin/seeds flour), and 25M (added of 25% of skin/seed flour).

Almost all clusters were characterized by a greater presence of VOCs in pizza bases fortified with oenological flours, unlike the fifth cluster in which the presence of pyrazine emerged exclusively in CTR samples.

The first and third clusters (analyzing clockwise the polar heatmap), for example, grouped some alcohols and aldehydes the concentrations of which have not been identified in the control samples, and increase with the increasing of replacement percentage.

As reported in Fig. 3, the concentration of ethyl hexanoate, ethyl

octanoate, ethyl decanoate, differentiate the samples obtained with oenological flours, as also observed by [Mildner-Szkudlarz, Zawirska-Wojtasiak, Szewiel, and Pacynski \(2011\)](#) in bread samples and by [Pasqualone et al. \(2014\)](#) in biscuits enriched with grape pomace extract, and especially when the highest quantities were used (25S and 25M). These compounds are typical products of fermentation and are responsible for the fruity and sweet notes ([Cardinale et al., 2021](#)) characteristics of grape pomace.

Likewise, the presence of ethyl acetate (first cluster), due to the fermentation processes carried out by the yeasts present in the dough and in grape pomace, increased in samples formulated with innovative flours and mainly in samples obtained with the mix of grape seeds and skin, due to a greater richness of the starting flour.

The third cluster differentiated the experimental samples than the control for the presence of alcohols (benzyl alcohol and 2-phenylethanol) and aldehydes. Among these there were the compounds derived from phenylalanine metabolism (benzaldehyde and phenylacetaldehyde) that, as reported by Pasqualone et al. (2014) impact on the sensorial level. These were brought by grape pomace in relation to the fermentation activities of winemaking, especially following the use of GM, due to a greater presence of these compounds in flours.

The increase in the percentage of substitution of refined wheat flour, on the other hand, led to a decrease in the volatile markers of the baking process. The sixth and seventh clusters, in fact, showed a decrease in furans, such as 2-ethylfuran, 2-methylfuran and furan 2,5-dicarbalddehyde, although absent in CTR. In particular, they seemed to cluster the samples with the addition of GS, due to a higher sugar content. The presence of these compounds derived from the degradation of sugars during cooking which consequently generated greater quantities of furan compounds. Strecker aldehydes, furans, pyrazines, and ketones are the main classes of volatile compounds that develop during Maillard reactions, which impart the typical aroma to bakery products such as bread, pizza, biscuits, and cakes (Pico, Bernal, & Gómez, 2015). The trend of some aldehydes such as hexanal, 3-methylbutanal and 2-methylbutanal, present in the seventh cluster, was in line with the results of Pasqualone et al. (2014), but in contrast with other authors who differently, have observed a decrease in the concentration of these compounds in fortified products (de Gennaro, Difonzo, Summo, Pasqualone, & Caponio, 2022). However, as reported by the latter, the increase in percentage of substitution caused a decrease in the quantity of aldehydes, although the addition of oenological flour gave higher initial quantities than wheat flour. This could be related to the increased antioxidant activity and phenolic compounds capable of inactivating free radicals (Gutiérrez-Del-rfo et al., 2021).

Finally, fifth cluster grouped the compounds belonging to the class of pyrazines, which were more abundant in pizza bases control and absent in fortified ones. It emerges therefore the remarkable impact of oenological flours on a wide range of these compounds (pyrazine, ethyl pyrazine and ethenyl pyrazine) present exclusively in CTR and positively influenced by the higher percentages of grape pomace flour added. Methyl pyrazine, however, present in the next cluster, clustered

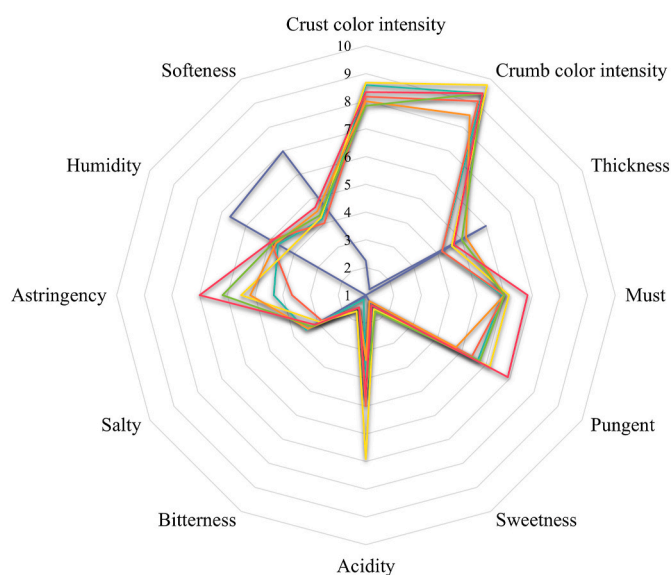
the samples in a different way; in fact, the samples containing the oenological flours were characterized by the presence of this compound, which decreased as substitution levels increased. This trend, as stated by Pasqualone et al. (2014), is probably due to the different pH of pizza bases, as values lower than 7 favor the formation of furans and their derivatives, while higher pH induces the preferential formation of pyrazine. In addition, Mildner-Szkudlarz, Róžańska, Piechowska, Waśkiewicz, and Zawirska-Wojtasiak (2019) associate the inhibitory action of phenolic compounds to the decrease in the concentration of pyrazines.

### 3.2.7. Sensory analysis

Sensory evaluation is important for the development of innovative and functional foods, due to the low acceptability that these products may have (Altinok et al., 2022). Fig. 5 summarizes the results of the sensory analysis of fortified pizza bases. Appearance descriptors showed high scores regarding the intensity of crust color in all pizza bases formulated with GS and GM, compared to the control (CTR). Crumb color intensity, as expected, exhibited differences between both CTR and other pizza bases, especially as the percentage of replacement increased. The replacement of 25% of the wheat flour determined the major intensity value ( $p < 0.05$ ), confirming the data obtained from instrumental analysis of color, according to which 25S and 25M displayed the lower and higher values of lightness and redness, respectively. A more intense color, as the quantity of grape pomace increased, was also observed in samples of muffins, bread and biscuits by Maner, Sharma, and Banerjee (2017) and Smith and Yu (2015). Thickness, instead, showed a correlation between CTR and 15M, and a decrease in this parameter directly proportional to the increase in the percentage of substitution, especially when GS was used.

As regard olfactometric evaluation, no significant differences in terms of must was highlighted, although 25S and 25M had slightly higher values, probably due to the increase in concentration of ethyl ester (Fig. 3), associated with the scent of fruity. In the same way, pungent hints were directly proportional to the wheat flour substitution level (25S and 25M). In this case, the greater perception is attributable to the increasing of concentrations of pentanal and acetic acid, as described above, associated with the smell of acid and pungent (Pico et al., 2015).

Sweetness, bitterness, and salty, evaluated with taste analysis, were not influenced by different oenological flours, illustrating comparable value between fortified samples and control. On the contrary, acidity was most perceived in the innovative pizza bases, particularly when the



**Fig. 5.** Results of the sensory analysis of the pizza bases. Data are represented as means  $\pm$  SD of three replicates. Appearance attributes (crust color intensity, crumb color intensity, thickness); olfactometric attributes (must and pungent); tasting attributes (sweetness, acidity, bitterness, salty, astringency); textural attributes (softness and humidity). CTR (control made of refined wheat flour only), 15S (added of 15% of grape skin flour), 20S (added of 20% of grape skin flour), 25S (added of 25% of grape skin flour), 15M (added of 15% of skin/seed flour), 20M (added of 20% of skin/seed flour), and 25M (added of 25% of skin/seed flour). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)



high percentage of GS was added ( $p < 0.001$ ). This hint was found also by [Gaglio et al. \(2021\)](#) in cheese samples fortified with 1% grape pomace compared to control one.

The intensity of astringency, as expected, was higher in samples formulated with GM ( $p < 0.001$ ), presumably for the presence of grape seed flour correlated to a higher content of flavan-3-ol ((+)-catechin, (–)-epicatechin, proanthocyanidins) associated to this scent ([Bianchi et al., 2022](#)). The astringency derives from the precipitation of proline-rich salivary protein in the mouth, caused by the phenolic compounds of which the grape seeds are rich ([Hoye & Ross, 2011](#); [Rosales Soto, Brown, & Ross, 2012](#)). [Kuchtová et al. \(2018\)](#) and [Silva, Domingues, and Nerín \(2018\)](#) reported that increased perception of astringency reduced the acceptability of biscuits.

Moreover, texture attributes perceived in the mouth in terms of humidity and softness, exhibited differences with fortified pizza bases and control. As regard humidity, the samples seemed to be drier than the control ( $p < 0.05$ ), as reported by instrumental analysis of moisture. Likewise, softness of CTR pizza bases displayed results statistically higher compared to those formulated with alternative flour; in addition, the use of GM made the samples softer than GS, probably due to the considerable lipid content present in grape seeds. These results were in line with the TPA analysis, according to which 20S and 25S had higher hardness. This was confirmed also by [Altinok et al. \(2022\)](#) and [Laguna, Varela, Salvador, and Fiszman \(2013\)](#), that associate the increase in lipid content to the addition of grape pomace that interrupts the gluten network, making the foods more fragile.

#### 4. Conclusions

This study examined the possibility of using the main oenological by-products, namely the grape pomace, in the form of a skin flour or a flour prepared from a mix of grape skin and seeds, for the production of innovative pizza bases, by replacing 15, 20, and 25% of wheat flour. The effects of partial substitution of refined flour on the chemical, technological, nutritional, and sensory properties were evaluated.

One of the most important aspects is the considerable increase in total dietary fiber content, greater than 3 and 6 g/100 g, which allows to label these products “source of fiber” and “high fiber content”, respectively, in line with EC Regulation 1924/2006. Moreover, the addition of these innovative flours, allowed to enrich the pizza bases in bioactive compounds, such as polyphenols and anthocyanins, with a consequent significant increase in antioxidant activity as the percentage of substitution increases, especially when GM was used.

Moreover, the oenological flours affected in different way the textural parameters, with the increasing of hardness and chewiness when highest percentages of GS were added in pizza bases formulation. In addition, GS modified the internal structure of the samples with the presence of irregular gas cells and interruptions of gluten network. The sensory analysis, instead, confirmed the results of instrumental analysis, related to the increase of crust and crumb color intensity and in the perception of pungent and must notes, as well as of acidity and astringency, caused by the presence of specific volatile compounds (such as esters, alcohols, and aldehydes) and tannin in the skin and seeds.

In conclusion, the direct use of grape skin and seed flours in the food sector allows to upcycle the main winemaking by-products, improving at the same time the nutritional and functional quality of the fortified products, obtaining pizza bases with high added value. Nevertheless, especially when the grape skin flours were used at the highest concentration different issues were found both for textural parameters and sensory aspects.

Based on the promising results, especially from nutritional point of view, future investigation could be targeted on the study of the consumer acceptance. Consequently, an industrial scale-up of these pizza bases could be carried out to meet the consumer needs and demands for alternative and functional foods, especially in the bakery sector.

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#### CRedit authorship contribution statement

**Graziana Difonzo:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **Marica Troilo:** Methodology, Writing – original draft. **Ignazio Allegretta:** Methodology, Writing – original draft. **Antonella Pasqualone:** Writing – original draft. **Francesco Caponio:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing.

#### Declaration of competing interest

The authors declare no conflict of interest.

#### Data availability

Data will be made available on request.

#### References

- Acun, S., & Gül, H. (2014). Effects of grape pomace and grape seed flours on cookie quality. *Quality Assurance and Safety of Crops & Foods*, 6(1), 81–88. <https://doi.org/10.3920/QAS2013.0264>
- Aghamirzaei, M., Peighambari, S. H., Azadmard-Damirchi, S., & Majzoubi, M. (2015). Effects of grape seed powder as a functional ingredient on flour physicochemical characteristics and dough rheological properties. *Journal of Agricultural Science and Technology A*, 17(2), 365–373.
- Altinok, E., Kurlutay, S., Boluk, E., Atik, D. S., Kopuk, B., Gunes, R., et al. (2022). Investigation of using possibility of grape pomace in wafer sheet for wheat flour substitution. *International Journal of Food Science and Technology*, 57(6), 3634–3642. <https://doi.org/10.1111/ijfs.15687>
- AOAC. (2006). *Official methods of analysis of AOAC international (18<sup>th</sup> ed.)*. Gaithersburg, MD, USA: AOAC International.
- Barba, F. J., Zhu, Z., Koubaa, M., Sant’Ana, A. S., & Orlie, V. (2016). Green alternative methods for the extraction of antioxidant bioactive compounds from winery wastes and by-products: A review. *Trends in Food Science & Technology*, 49, 96–109. <https://doi.org/10.1016/j.tifs.2016.01.006>
- Bender, A. B. B., Speroni, C. S., Salvador, P. R., Loureiro, B. B., Lovatto, N. M., Goulart, F. R., et al. (2017). Grape pomace skins and the effects of its inclusion in the technological properties of muffins. *Journal of Culinary Science & Technology*, 15(2), 143–157. <https://doi.org/10.1080/15428052.2016.1225535>
- Beres, C., Simas-Tosin, F. P., Cabezudo, I., Freitas, S. P., Iacomini, M., Mellinger-Silva, C., et al. (2016). Antioxidant dietary fibre recovery from Brazilian Pinot noir grape pomace. *Food Chemistry*, 201, 145–152. <https://doi.org/10.1016/j.foodchem.2016.01.039>
- Bharath, A. P., Kathalsar, A. K., Chandrashekar, S., & Prabhasankar, P. (2021). Influence of tetraploid wheat (Triticum dicoccum) on low glycaemic index pizza base processing and its starch digestibility. *International Journal of Food Science and Technology*, 56(5), 2273–2281. <https://doi.org/10.1111/ijfs.14845>
- Bianchi, F., Cervini, M., Giuberti, G., Rocchetti, G., Lucini, L., & Simonato, B. (2022). Distilled grape pomace as a functional ingredient in vegan muffins: Effect on physicochemical, nutritional, rheological and sensory aspects. *International Journal of Food Science and Technology*, 4847–4858. <https://doi.org/10.1111/ijfs.15720>
- Bianchi, F., Lomuscio, E., Rizzi, C., & Simonato, B. (2021). Predicted shelf-life, thermodynamic study and antioxidant capacity of breadsticks fortified with grape pomace powders. *Foods*, 10(11), 2815. <https://doi.org/10.3390/foods10112815>
- Bordiga, M., Montella, R., Travaglia, F., Arlorio, M., & Coisson, J. D. (2019). Characterization of polyphenolic and oligosaccharidic fractions extracted from grape seeds followed by the evaluation of prebiotic activity related to oligosaccharides. *International Journal of Food Science and Technology*, 54(4), 1283–1291. <https://doi.org/10.1111/ijfs.14109>
- Bordiga, M., Travaglia, F., & Locatelli, M. (2019). Valorisation of grape pomace: An approach that is increasingly reaching its maturity – a review. *International Journal of Food Science and Technology*, 54(4), 933–942. <https://doi.org/10.1111/ijfs.14118>
- Bordiga, M., Travaglia, F., Locatelli, M., Arlorio, M., & Coisson, J. D. (2015). Spent grape pomace as a still potential by-product. *International Journal of Food Science and Technology*, 50(9), 2022–2031. <https://doi.org/10.1111/ijfs.12853>
- Caponio, G. R., Difonzo, G., de Gennaro, G., Calasso, M., De Angelis, M., & Pasqualone, A. (2022b). Nutritional improvement of gluten-free breadsticks by olive cake addition and sourdough fermentation: How texture, sensory, and aromatic profile were affected? *Frontiers in Nutrition*, 9, Article 830932. <https://doi.org/10.3389/fnut.2022.830932>

- Caponio, G. R., Noviello, M., Calabrese, F. M., Gambacorta, G., Giannelli, G., & De Angelis, M. (2022). Effects of grape pomace polyphenols and in vitro gastrointestinal digestion on antimicrobial activity: Recovery of bioactive compounds. *Antioxidants*, *11*(3), 567. <https://doi.org/10.3390/antiox11030567>
- Cardinale, M., Trinchera, R., Natrella, G., Difonzo, G., De Benedittis, C., D'amato, I., et al. (2021). Dynamics of the fermentation process and chemical profiling of pomegranate (*Punica granatum* L.) wines obtained by different cultivar × yeast combinations. *Foods*, *10*(8), 1913. <https://doi.org/10.3390/foods10081913>
- Chen, S. X., Ni, Z. J., Thakur, K., Wang, S., Zhang, J. G., Shang, Y. F., et al. (2021). Effect of grape seed powder on the structural and physicochemical properties of wheat gluten in noodle preparation system. *Food Chemistry*, *355*, Article 129500. <https://doi.org/10.1016/j.foodchem.2021.129500>
- Della-Corte, T., Gentile, S., Di Blasi, V., Guarino, G., Corigliano, M., Cozzolino, G., et al. (2020). Is pizza suitable to type 1 diabetes? A real life identification of best compromise between taste and low glycemic index in patients on insulin pump. *Diabetes & Metabolic Syndrome: Clinical Research Reviews*, *143*(3), 225–227. <https://doi.org/10.1016/j.dsx.2020.03.003>
- Difonzo, G., Aresta, A., Cotugno, P., Ragni, R., Squeo, G., Summo, C., et al. (2021). Supercritical CO<sub>2</sub> extraction of phytochemicals from olive pomace subjected to different drying methods. *Molecules*, *26*(3), 598. <https://doi.org/10.3390/molecules26030598>
- Falciano, A., Sorrentino, A., Masi, P., & Di Pierro, P. (2022). Development of functional pizza base enriched with jujube (*Ziziphus jujuba*) powder. *Foods*, *11*(10), 1458. <https://doi.org/10.3390/foods11101458>
- Fiori, L., Lavelli, V., Duba, K. S., Sri Harsha, P. S. C., Mohamed, H. B., & Guella, G. (2014). Supercritical CO<sub>2</sub> extraction of oil from seeds of six grape cultivars: Modeling of mass transfer kinetics and evaluation of lipid profiles and tocopherols. *The Journal of Supercritical Fluids*, *94*, 71–80. <https://doi.org/10.1016/j.supflu.2014.06.021>
- Gaglio, R., Restivo, I., Barbera, M., Barbaccia, P., Ponte, M., Tesoriere, L., et al. (2021). Effect on the antioxidant, lipoperoxyl radical scavenger capacity, nutritional, sensory and microbiological traits of an ovine stretched cheese produced with grape pomace powder addition. *Antioxidants*, *10*(2), 306. <https://doi.org/10.3390/antiox10020306>
- de Gennaro, G., Difonzo, G., Summo, C., Pasqualone, A., & Caponio, F. (2022). Olive cake powder as functional ingredient to improve the quality of gluten-free breadsticks. *Foods*, *11*(4), 552. <https://doi.org/10.3390/foods11040552>
- Glicerina, V., Balestra, F., Capozzi, F., Dalla Rosa, M., & Romani, S. (2018). Influence of the addition of soy product and wheat fiber on rheological, textural, and other quality characteristics of pizza. *Journal of Texture Studies*, *49*(4), 415–423. <https://doi.org/10.1111/jtxs.12311>
- Global Pizza Market. [https://www.reportlinker.com/p04259376/Global-Pizza-Market.html?utm\\_](https://www.reportlinker.com/p04259376/Global-Pizza-Market.html?utm_) (Accessed 25 October 2022).
- Gupta, C. S., Milind Jeyarani, T., & Rajiv, J. (2015). Rheology, fatty acid profile and quality characteristics of nutrient enriched pizza base. *Journal of Food Science & Technology*, *52*(5), 2926–2933. <https://doi.org/10.1007/s13197-014-1338-2>
- Gutiérrez-Del-río, I., López-Ibáñez, S., Magadán-Corpas, P., Fernández-Calleja, L., Pérez-Valero, Á., Tuñón-Granda, M., et al. (2021). Terpenoids and polyphenols as natural antioxidant agents in food preservation. *Antioxidants*, *10*(8), 1264. <https://doi.org/10.3390/antiox10081264>
- Helstosky, C. (2008). *Pizza: a global history*. Reaktion books.
- Hoye, C., & Ross, C. F. (2011). Total phenolic content, consumer acceptance, and instrumental analysis of bread made with grape seed flour. *Journal of Food Science*, *76*(7), S428–S436. <https://doi.org/10.1111/j.1750-3841.2011.02324.x>
- Kanaujija, G. (2017). To nutritional profile of dietary fibre pizza base and sensory evaluation of develop product. *International Journal of Advance Research, Ideas and Innovations in Technology*, *3*(6), 932–936.
- Karnopp, A. R., Figueroa, A. M., Los, P. R., Teles, J. C., Simões, D. R. S., Barana, A. C., et al. (2015). Effects of whole-wheat flour and bordeaux grape pomace (*Vitis labrusca* L.) on the sensory, physicochemical and functional properties of cookies. *Food Science and Technology*, *35*(4), 750–756. <https://doi.org/10.1590/1678-457X.0010>
- Kuchtová, V., Kohajdová, Z., Karovičová, J., & Lauková, M. (2018). Physical, textural and sensory properties of cookies incorporated with grape skin and seed preparations. *Polish Journal of Food and Nutrition Sciences*, *68*(4), 309–317. <https://doi.org/10.2478/pjfn-2018-0004>
- Laguna, L., Varela, P., Salvador, A., & Fiszman, S. (2013). A new sensory tool to analyse the oral trajectory of biscuits with different fat and fibre contents. *Food Research International*, *51*(2), 544–553. <https://doi.org/10.1016/j.foodres.2013.01.003>
- Libera, J., Latoch, A., & Wójciak, K. M. (2020). Utilization of grape seed extract as a natural antioxidant in the technology of meat products inoculated with a probiotic strain of lab. *Foods*, *9*(1), 103. <https://doi.org/10.3390/foods9010103>
- Liu, Z., Chen, J., Zheng, B., Lu, Q., & Chen, L. (2020). Effects of matcha and its active components on the structure and rheological properties of gluten. *Lwt – Food Science and Technology*, *124*, Article 109197. <https://doi.org/10.1016/j.lwt.2020.109197>
- Llobera, A., & Cañellas, J. (2007). Dietary fibre content and antioxidant activity of Manto Negro red grape (*Vitis vinifera*): Pomace and stem. *Food Chemistry*, *101*(2), 659–666. <https://doi.org/10.1016/j.foodchem.2006.02.025>
- Lou, W., Li, B., & Nataliya, G. (2021). The influence of Cabernet Sauvignon wine grape pomace powder addition on the rheological and microstructural properties of wheat dough. *CyTA - Journal of Food*, *19*(1), 751–761. <https://doi.org/10.1080/19476337.2021.1981458>
- Lou, W., Zhou, H., Li, B., & Nataliya, G. (2022). Rheological, pasting and sensory properties of biscuits supplemented with grape pomace powder. *Food Science and Technology*, *42*, Article e78421. <https://doi.org/10.1590/fst.78421>
- Maner, S., Sharma, A. K., & Banerjee, K. (2017). Wheat flour replacement by wine grape pomace powder positively affects physical, functional and sensory properties of cookies. *Proceedings of the National Academy of Sciences, India - Section B: Biological Sciences*, *87*(1), 109–113. <https://doi.org/10.1007/s40011-015-0570-5>
- Mattos, G. N., Tonon, R. V., Furtado, A. A. L., & Cabral, L. M. C. (2017). Grape by-product extracts against microbial proliferation and lipid oxidation: A review. *Journal of the Science of Food and Agriculture*, *97*(4), 1055–1064. <https://doi.org/10.1002/jsfa.8062>
- Mildner-Szkudlarz, S., Bajerska, J., Górnaś, P., Segliņa, D., Pilarska, A., & Jesionowski, T. (2016). Physical and bioactive properties of muffins enriched with raspberry and cranberry pomace powder: A promising application of fruit by-products rich in biocompounds. *Plant Foods for Human Nutrition*, *71*(2), 165–173. <https://doi.org/10.1007/s11130-016-0539-4>
- Mildner-Szkudlarz, S., Różańska, M., Piechowska, P., Waśkiewicz, A., & Zawirska-Wojtasiak, R. (2019). Effects of polyphenols on volatile profile and acrylamide formation in a model wheat bread system. *Food Chemistry*, *297*, Article 125008. <https://doi.org/10.1016/j.foodchem.2019.125008>
- Mildner-Szkudlarz, S., Zawirska-Wojtasiak, R., Szwengel, A., & Pacynski, M. (2011). Use of grape by-product as a source of dietary fibre and phenolic compounds in sourdough mixed rye bread. *International Journal of Food Science and Technology*, *46*(7), 1485–1493. <https://doi.org/10.1111/j.1365-2621.2011.02643.x>
- Muhlack, R. A., Potumarthi, R., & Jeffery, D. W. (2018). Sustainable wineries through waste valorisation: A review of grape marc utilisation for value-added products. *Waste Management*, *72*, 99–118. <https://doi.org/10.1016/j.wasman.2017.11.011>
- Najjaa, H., Arfa, A. B., Elfalleh, W., Zouari, N., & Neffati, M. (2020). Jujube (*Ziziphus lotus* L.): Benefits and its effects on functional and sensory properties of sponge cake. *PLoS One*, *15*(2), Article e0227996. <https://doi.org/10.1371/journal.pone.0227996>
- Nakov, G., Brandolini, A., Hidalgo, A., Ivanova, N., Stamatovska, V., & Dimov, I. (2020). Effect of grape pomace powder addition on chemical, nutritional and technological properties of cakes. *Lwt – Food Science and Technology*, *134*, Article 109950. <https://doi.org/10.1016/j.lwt.2020.109950>
- Ortega-Heras, M., Gómez, I., de Pablos-Alcalde, S., & González-Sanjosé, M. L. (2019). Application of the just-about-right scales in the development of new healthy whole-wheat muffins by the addition of a product obtained from white and red grape pomace. *Foods*, *8*(9), 419. <https://doi.org/10.3390/foods8090419>
- Pasqualone, A., Bianco, A. M., Paradiso, V. M., Summo, C., Gambacorta, G., & Caponio, F. (2014). Physico-chemical, sensory and volatile profiles of biscuits enriched with grape marc extract. *Food Research International*, *65*, 385–393. <https://doi.org/10.1016/j.foodres.2014.07.014>
- Pasqualone, A., Costantini, M., Faccia, M., Difonzo, G., Caponio, F., & Summo, C. (2022). The effectiveness of extruded-cooked lentil flour in preparing a gluten-free pizza with improved nutritional features and a good sensory quality. *Foods*, *11*(3), 482. <https://doi.org/10.3390/foods11030482>
- Pasqualone, A., De Angelis, D., Squeo, G., Difonzo, G., Caponio, F., & Summo, C. (2019). The effect of the addition of Apulian black chickpea flour on the nutritional and qualitative properties of durum wheat-based bakery products. *Foods*, *8*(10), 504. <https://doi.org/10.3390/foods8100504>
- Pico, J., Bernal, J., & Gómez, M. (2015). Wheat bread aroma compounds in crumb and crust: A review. *Food Research International*, *75*, 200–215. <https://doi.org/10.1016/j.foodres.2015.05.051>
- Pintač, D., Majkić, T., Torović, L., Orčić, D., Beara, I., Simin, N., et al. (2018). Solvent selection for efficient extraction of bioactive compounds from grape pomace. *Industrial Crops and Products*, *111*, 379–390. <https://doi.org/10.1016/j.indcrop.2017.10.038>
- Rainero, G., Bianchi, F., Rizzi, C., Cervini, M., Giuberti, G., & Simonato, B. (2022). Breadstick fortification with red grape pomace: Effect on nutritional, technological and sensory properties. *Journal of the Science of Food and Agriculture*, *102*(6), 2545–2552. <https://doi.org/10.1002/jsfa.11596>
- Ribeiro, A., Barros, L., Calhella, R. C., Carochi, M., Ćirić, A., Sokovic, M., et al. (2016). Tarragon phenolic extract as a functional ingredient for pizza dough: Comparative performance with ascorbic acid (E300). *Journal of Functional Foods*, *26*, 268–278. <https://doi.org/10.1016/j.jff.2016.08.019>
- Riccardi, G., Clemente, G., & Giacco, R. (2003). Glycemic index of local foods and diets: The Mediterranean experience. *Nutrition Reviews*, *61*, S56–S60. <https://doi.org/10.1301/nr.2003.may.S56-S60>
- Rosales Soto, M. U., Brown, K., & Ross, C. F. (2012). Antioxidant activity and consumer acceptance of grape seed flour-containing food products. *International Journal of Food Science and Technology*, *47*(3), 592–602. <https://doi.org/10.1111/j.1365-2621.2011.02882.x>
- Sagar, N. A., & Pareek, S. (2020). Dough rheology, antioxidants, textural, physicochemical characteristics, and sensory quality of pizza base enriched with onion (*Allium cepa* L.) skin powder. *Scientific Reports*, *10*(1), Article 18669. <https://doi.org/10.1038/s41598-020-75793-0>
- Silva, F., Domingues, F. C., & Nerin, C. (2018). Control microbial growth on fresh chicken meat using pinosylvin inclusion complexes based packaging absorbent pads. *Lwt – Food Science and Technology*, *89*, 148–154. <https://doi.org/10.1016/j.lwt.2017.10.043>
- Smith, I. N., & Yu, J. (2015). Nutritional and sensory quality of bread containing different quantities of grape pomace from different grape cultivars. *EC Nutrition*, *2*(1), 291–301.
- Struck, S., Straube, D., Zahn, S., & Rohm, H. (2018). Interaction of wheat macromolecules and berry pomace in model dough: Rheology and microstructure. *Journal of Food Engineering*, *223*, 109–115. <https://doi.org/10.1016/j.jfoodeng.2017.12.011>
- Theagarajan, R., Malur Narayanaswamy, L., Dutta, S., Moses, J. A., & Chinnaswamy, A. (2019). Valorisation of grape pomace (cv. Muscat) for development of functional

- cookies. *International Journal of Food Science and Technology*, 54(4), 1299–1305. <https://doi.org/10.1111/ijfs.14119>
- Tolve, R., Simonato, B., Rainero, G., Bianchi, F., Rizzi, C., & Cervini MariasoleGiuberti, G. (2021). Wheat bread fortification by grape pomace powder: Nutritional, technological, antioxidant, and sensory properties. *Foods*, 10(1), 75. <https://doi.org/10.3390/foods10010075>
- Troilo, M., Difonzo, G., Paradiso, V. M., Pasqualone, A., & Caponio, F. (2022). Grape pomace as innovative flour for the formulation of functional muffins: How particle size affects the nutritional, textural and sensory properties. *Foods*, 11(12), 1799. <https://doi.org/10.3390/foods11121799>
- Troilo, M., Difonzo, G., Paradiso, V. M., Summo, C., & Caponio, F. (2021). Bioactive compounds from vine shoots, grape stalks, and wine lees: Their potential use in agro-food chains. *Foods*, 10(2), 342. <https://doi.org/10.3390/foods10020342>
- Tseng, A., & Zhao, Y. (2012). Effect of different drying methods and storage time on the retention of bioactive compounds and antibacterial activity of wine grape pomace (Pinot Noir and Merlot). *Journal of Food Science*, 77(9), H192–H201. <https://doi.org/10.1111/j.1750-3841.2012.02840.x>
- Xia, E. Q., Deng, G. F., Guo, Y. J., & Li, H. B. (2010). Biological activities of polyphenols from grapes. *International Journal of Molecular Sciences*, 11, 622–646. <https://doi.org/10.3390/ijms11020622>