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Grape pomace flour incorporation for quality and sensory improvement of plant-based white chocolate alternatives

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ABSTRACT

Bioactive compounds in milled grape pomace (*Vitis vinifera* L.) are related to health benefits. The growing accessibility of these flours has increased their use in food production. This study evaluated biological properties such as antioxidant activity and total phenol content in white chocolates fortified with white (Fiano) and red (Negroamaro) grape pomace flour, at two concentrations (5 and 10%), and two particle size (≤200 µm and ≤500 µm) compared with a control white chocolate. Samples enriched with Fiano pomace showed higher antioxidant activity than those containing Negroamaro pomace. The control white chocolate showed 25-fold lower total phenol content and 35-fold lower antioxidant activity (TEAC assay) than the sample fortified with 10% of Fiano pomace (200 µm). Volatile organic compounds were also analyzed, through solid phase microextraction coupled with gas chromatography-mass spectrometry. The addition of Negroamaro pomace flour provided a larger amount of aromatic compounds than addition of Fiano pomace flour. The sensory profiles were defined by a trained panel through descriptive analysis. Consumers were involved in a co-creation task by expressing their preferences of a virtual 'Ideal Enriched Chocolate', propaedeutic to real prototypes evaluation. Chocolate and cocoa aroma emerged as the main drivers of consumer preference. However, several enriched samples received positive evaluations, suggesting potential consumer appreciation for these prototypes.

1. Introduction

Modern concepts of nutrition highlight the pivotal role of functional foods, because they provide many physiological benefits and reduce the risk of chronic diseases. Functional foods either naturally contain or are fortified with bioactive ingredients such as polyphenols, fibers and fatty acids, or are formulated by reducing or eliminating a component associated with adverse health effects (FAO).

The importance of polyphenols as agent for preventing and treating human oxidative stress-related diseases, makes the study of agri-food by-products of interest for the functional food and nutraceutical industries. Nowadays, there has been a growing interest in the exploitation of by-products from the winemaking industry, to produce extracts and novel ingredients to promote human health (Boff et al., 2022; Kulkarni et al., 2011; Nakov et al., 2020; Perumalla & Hettiarachchy, 2011). The extraction of polyphenols from waste material derived from industrial practices represents a sustainable and cost-effective source of these high-value natural bioactive, which could be incorporated into foods. In particular, polyphenols can be used to improve the quality of plant-based alternatives to white chocolate (PbA_WCh), which represent an option for vegan consumers or those who are lactose intolerant (Indiarto et al., 2025). Due to the increasing demand for nutraceutical and antioxidant compounds, the study of grape pomace (GP) may be useful for food industry purposes as source of bioactive compounds

Every year the wine industry produces approximately 14 million tons of pomace, most of which is disposed of in the fields (Wang et al., 2024). Although GP is a potential value-added ingredient for food products, it remains underutilized after winemaking. The Negroamaro and Fiano grape varieties (*Vitis vinifera* L.) are widely grown in the southern Italy. Negroamaro and Fiano GPs, which consists of skin and seed, is a winery by-product and accounts for approximately 20–25 % of the grape weight (Dwyer et al., 2014). Previous studies have reported that GP contains

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diverse and high concentrations of polyphenolic compounds such as phenolic acid, procyanidins, flavonols, stilbenes and anthocyanins (Dwyer et al., 2014; Hogervorst et al., 2017; Kurek et al., 2019). These bioactive constituents have been reported to possess potent antioxidant, antimicrobic, anticancer, antimutagenic, and anti-inflammatory properties (Calabrisoet al., 2016; Gerardi et al., 2016, 2022; Rana et al., 2022). In addition, GP is a good source of dietary fiber since it contains 50- 60 % total dietary fiber on a dry weight basis. Dietary fibers particularly soluble fibers such as inulin, pectins, and β-glucans have been identified as fat replacers in various food products (Kurćubić et al., 2024). The antimicrobial (Silva et al., 2021), antioxidant, and anticancer activities of GP (Calabrisoet al., 2022; Choleva et al., 2022; Giovinazzo & Grieco, 2015; Karantonis et al., 2023) have been investigated in many previous studies. Previous studies demonstrate that GP derived from different grape cultivars have phenolic content and antioxidant activity completely different from each other, probably due to the different metabolomic profile of the starting grapes (Onache et al., 2022; Ruberto et al., 2007).

Chocolate is one of the most popular sweets worldwide (Tan et al., 2021). Different types of chocolate (*e.g.*, dark, milk and white) are produced, usually made by combining cocoa powder, cocoa butter and/or cocoa liquor, with sugar and other additives in different amount. The most desirable is dark chocolate because it contains more bioactive polyphenols than milk chocolate. White chocolate and plant-based alternative to white chocolate are seen as unhealthier products than dark chocolate because they do not contain cocoa mass (Muhammad et al., 2020). The improvement in antioxidant properties during chocolate production, especially in white and milk chocolate types, has been investigated by many researchers. Some researchers have successfully introduced matcha and moringa flours into white chocolate products (Poliński et al., 2022).

The biological properties such as antioxidant and anticancer activities of cocoa and dark chocolates, have also been widely studied (Samanta et al., 2022). The sensory attributes, mechanical properties, and digestibility of chocolates enriched with GPs were reported in some previous studies (Altınok et al., 2022). However, the bioactive molecules of GP-enriched chocolates and their antioxidant activity, especially white grape cv Fiano and red grape cv Negroamaro pomace-enriched chocolates, were barely investigated. The grape variety and the processing of GP can significantly influence the bioactive compounds, such as polyphenolic compounds, as well as the lipid fraction. In chocolate, fatty acids are mainly present as triacylglycerols, predominantly composed of palmitic and stearic acids (Segall et al., 2005), whose structure directly affects chocolate behavior during manufacturing and the characteristics of the final product, including texture, viscosity, melting behavior, and flavor (Afoakwa et al., 2007). To the best of our knowledge, there are no scientific studies investigating GP flour as a source of polyphenols to enhance the antioxidant activity of PbA_WCh. Therefore, the present study aimed to first evaluate the influence of GP flour addition on the polyphenol profile, fatty acid profile, antioxidant properties of a PbA_WCh. Subsequently, the effects on physical properties, volatile compounds and sensory characteristics of enriched samples were investigated. The study also takes into account the influence of the grape variety from which the flour is derived and the effect of particle size on the content of bioactive compounds.

2. Material and methods

2.1. Chemicals

Reagents were acquired from various suppliers: standards of oenin (Malvidin-3-O-glucoside), chlorogenic acid (5-caffeoylquinic acid) and rutin (quercetin 3-O-rutinoside), from Extrasynthèse (Genay, France); caffeic acid, gallic acid, coutaric acid, caftaric acid, catechin, epicatechin, quercetin-3-glucoside, Folin-Ciocalteu phenol reagent, Trolox [(*S*)-(−)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid], n-hexane, ethanol, methanol, acetonitrile, formic acid, sodium hydroxide, sodium chloride and boron trifluoride, from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Raw materials and grape pomace flour preparation

Whole (skins and seeds) grape pomace of two grape (*Vitis vinifera*) cultivars (2024 campaign), Fiano (white grape) and Negroamaro (red grape), were obtained from a winery located in Salento (Apulia Region, Southern Italy). GP was stabilized by drying in an oven (J.P. selecta s.a., Spain) at 50 °C with forced air circulation, in the dark, until obtaining a constant weight (48 h).

Grape pomace flours (GPF), for both cultivars, were obtained using a laboratory sample mill (FOSS, Hilleros, Denmark). Milled GP was sieved to obtain a powder of particle size ≤200 µm and ≤500 µm. The powders thus obtained were stored in the dark at 4 °C for 1 month (Gerardi et al., 2022).

2.3. Samples preparation

Samples of plant-based alternative white chocolate (PbA_WCh) were produced under industrial conditions by a local company. PbA_WCh samples were enriched with two different amount (5 % and 10 %) of GPF at two different granulometry (≤200 µm and ≤500 µm) obtained from Fiano and Negoramaro grape cultivars. Samples were prepared according to the formulation of the company with the following ingredients: cocoa butter, rice drink powder, pure toasted almond paste, sunflower lecithin, salt, natural vanilla. The samples preparation took place in small-scale conching machine (2 Kg total of final product) for two hours at 45 °*C* ± 1 °C. Then, the obtained mixture was tempered for 10 min at 29–30 °C. Lastly, the tempered samples were placed at 4 °C for 30 min to crystallize. For GPF added samples, the flour was added during conching steps. The control sample was PbA_WCh, and other 8 samples were differentiated by grape cultivar (Fiano and Negroamaro), amount (5 and 10 %) and granulometry (200, 500 µm) (Fig. 1a). Each formulation was prepared once and measured in triplicate.

2.4. Phenols extraction

Phenols were extracted from GPF (1 g) with 10 ml of an extraction solution of methanol/water/acetic acid (70:29.5:0.5) at 30 °C for 15 min in the ultrasonic bath and then centrifuged at 3000xgfor 10 min. The supernatants were collected and stored at −20 °C until analysis.

Milled PbA_WCh and GPF added samples (1 g) were defatted twice with 10 mL of n-hexane for 10 min in an ultrasonic bath at 30 °C and then centrifuged for 10 min at 3000x*g*. Polyphenols were extracted from air-dried pellets with 10 mL of an extraction solution of methanol/water/acetic acid (70:29.5:0.5) at 30 °C for 15 min in the ultrasonic bath and then centrifuged at 3000xgfor 10 min. The supernatants were collected and stored at −20 °C until analysis.

2.5. Total phenolic content

The amount of total phenol content (TPC) was measured by the optimized Folin–Ciocalteu method (Magalhães et al., 2010). The TPC in PbA_WCh extracts was assessed in 96-well plates, using a microplate reader (Infinite 200 Pro, Tecan, Männedorf, Switzerland). Samples (50 µL) and Folin–Ciocalteu reagent (1:5 v/v, 50 µL) were placed into each well then NaOH 0.35 M, 100 µL, was added. After 5 min of incubation, the absorbance values at 760 nm were recorded. The calibration curve (2.5 to 40 mg/L) was obtained using gallic acid and results were expressed as milligram gallic acid equivalents per g of samples (mg GAEs/g). Results are expressed as mean ± SD of three independent samples, each measured in triplicate.

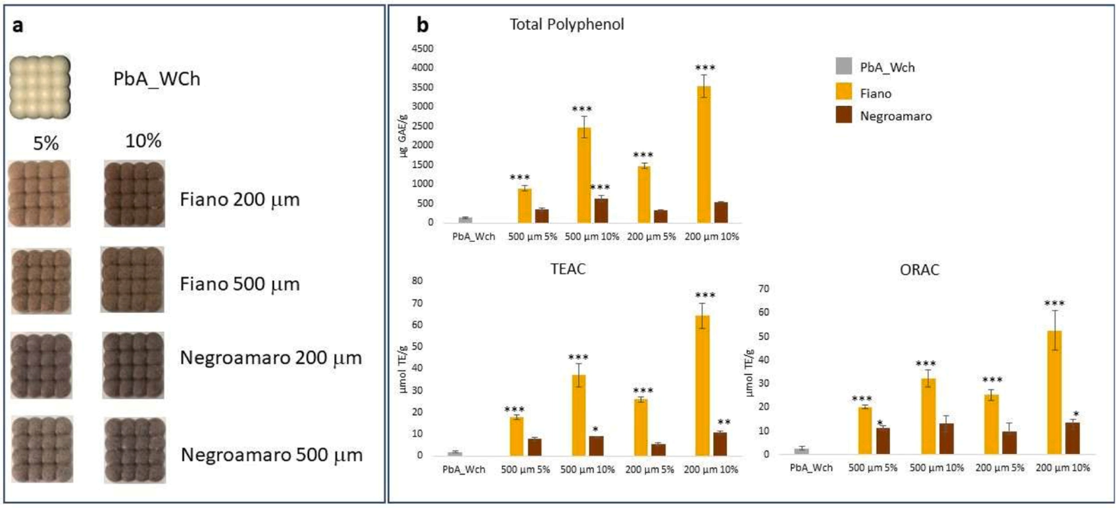

Fig. 1. a: Plant-based alternatives of white chocolate (PbA_WCh) without and with different amounts (5 and 10 %) of GPF with two granulometry (≤200 µm and ≤500 µm) obtained from vinification of two grape cultivars (Fiano and Negroamaro). b: Comparison of total polyphenol, TEAC and ORAC values in plant-based alternative of white chocolate. Data represented the mean values ± SD (n = 3). Error bars represent standard deviation. Signif. codes: '***' 0.001 '**' 0.01 '*' 0.05 relative to control (PbA_WCh) sample.

2.6. Trolox equivalent antioxidant capacity (TEAC)

The Trolox equivalent antioxidant capacity (TEAC) assay is based on scavenging ability of radical cationic of 2,2′-azinobis (3-ethyl-benzoithiazolone 6-sulphonate) ($ABTS^{\bullet+}$). The assay was performed as previously described (*Re* et al., 1999) with some modifications (Gerardi et al., 2020). Briefly, the ABTS radical cation was diluted in PBS (pH 7.4) to an absorbance of 0.40 at 734 nm. The percentage inhibition of absorbance at 734 nm was calculated and plotted as a function of the concentration of Trolox and expressed as Trolox equivalents µmolTE/g of samples using Magellan v7.2 software. For the calibration curve, the diluted $ABTS^{\bullet+}$ solution was mixed with 20 µL of Trolox standard solutions (from 0 to 0.33 mM, $R^2 \geq 0998$).

2.7. Oxygen radical absorbance capacity assay (ORAC)

The ORAC assay was carried out as reported by Gerardi et al. (2020), using an Infinite 200 Pro plate reader (Tecan). The antioxidant capacity of 20 µL of sample phenolic extracts was assayed by monitoring the decay curves of fluorescein (70 nM final concentration) for 80 min after the addition of a generator of radical species (2,2′-Azobis-(2-methyl-propionamidine) dihydrochloride (AAPH, 12 nM, final concentration). The fluorescence was recorded (ex 485 and em 527 nm wavelengths) every minute for 80 min. Phosphate buffer was a blank and all the reaction mixtures were prepared in triplicate. A standard curve was obtained using the antioxidant Trolox (1–6 µM, $R^2 \geq 0.998$) and final ORAC values were expressed as µmol Trolox equivalents (TE)/g of samples.

2.8. Color evaluation

Samples of plant-based alternative of white chocolate, both PbA_WCH and GPF added samples, were evaluated by Minolta CR-410 chromatometer (Konica Minolta Camera Co., Ltd., Osaka, Japan). Color measurement was repeated three times for each sample at room temperature using D65 as illuminant. The CIELAB color space was employed to evaluate the following parameters: L^* from black (0) to white (100), a^* (red (+a) to green (-a) color) and b^* (yellow (+b) to blue (-b) color).

2.9. Determination of phenolic compounds by High Performance Liquid Chromatography (HPLC)

Different phenolic compounds were separated and quali-quantitatively analyzed by RP (Reverse Phase)-HPLC technique equipped with a diode array detector as described in Gerardi et al. (2022). The mobile phases were (A) H_2O/formic acid = 95/5 and (B) acetonitrile/formic acid = 95/5. The samples were eluted following a linear gradient: 1 min of isocratic elution with 6.7 % B, 25 min of linear gradient from 6.7 to 16.7 % B, 9 min of linear gradient from 16.7 to 55.6 % B, 5 min of isocratic elution with 55.6 % B, 3 min of linear gradient from 55.6 B to 80 % B and 8 min of isocratic elution with 80 % B. Flow rate: 0.7 mL/min; total run time 51 min. A 1100 Series HPLC system (Agilent) equipped with a Luna 5µm C18(2) 100 Å column (250 × 4.6 mm) (Phenomenex, Torrance, *CA*, USA) has been used for phenol compounds quantification using wavelengths at 280, 306, 320, 370 and 520 nm. The characterization of phenolic compounds was carried out on the basis of their Retention Times (RT) and spectroscopic spectrum compared with RT and UV/vis spectra of external standards. Quantification of single phenolic compound was obtained by deduction from ten-point regression curves ($r^2 \geq 0.99$) of each standard and was expressed in µg/g of PbA_WCh and fortified samples. Quantification of total anthocyanins was performed by HPLC/DAD using a five-point regression curve ($r^2 \geq 0.99$) generated through the use of malvidin 3-O-glucoside (oenin) as a reference compound and expressed as oenin equivalents (OEs).

2.10. Lipid extraction and gas chromatography analysis

Total lipids were extracted from 0.500 g of PbA_WCh and fortified samples with 6 mL of n-hexane, overnight at 4 °C. The samples were centrifuged at 1500x*g* for 10 min at 4 °C and the supernatant was recovered and dried with stream of nitrogen. Each sample was dissolved in 3 mL of methanol containing 0.5 M of NaOH and then boiled at 100 °C for 5 min. After cooling at room temperature, boron trifluoride in methanol (2 mL) was added and incubated at 100 °C for 30 min. Successively, 1 mL of NaCl (0.6 % w/v) and 1 mL of n-hexane were added. The samples were then centrifuged, and the organic phase was recovered and analyzed by GC.

The analysis was performed by GC–MS employing the Agilent 6889–5977E Series GC/MS system, using a DB-Wax column (60 m, 0.25

mm i.d., 0.25 mm film thickness, Agilent). The following parameters were used for GC analysis: the temperature of the column was 50 °C after injection for 1 min, then programmed at 15 °C/min to 200 °C, at 3 °C/min to 230 °C and maintained at constant temperature of 230 °C for 25 min. Split injection was conducted with a split ratio of 5:1. Online published library (NIST-library spectra) and MS data were used to identify compounds. Moreover, the fatty acid standard was used to confirm MS data.

2.11. Determination of volatile organic compounds

Extraction, identification and quantification of volatile organic compounds (VOC) from samples of alternative plant-based white chocolate were performed using the headspace solid-phase microextraction (HS-SPME) method coupled with gas chromatography-mass spectrometry (GC–MS), following the method proposed by Pott et al. (2021). The method was applied with slight modifications, specifically: samples were stored at 15 °C before being analysed; the pre-incubation and extraction temperatures were set to 60 °C; and a 1-cm SPME fiber coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PD MS, 50/30 µm film thickness; Restek S.R.L., Milan, Italy) was used to collect volatile compounds. Temperature and fiber type were selected according to the method proposed by Ducki et al. (2008), which investigates cocoa products. After extraction, fiber was inserted in the GC–MS injection port and the volatiles were analysed with a 7890A gas chromatograph linked with a 5975C mass detector (GC–MS, Agilent Technologies, Wilmington, USA). Similarly, GC temperatures program and mass spectrometry settings were fixed as reported by Pott et al., 2021, with slight modifications: volatiles were desorbed from the fiber at 250 °C in splitless mode for 5 min instead of 1 min, following recommendations from Ducki et al. (2008). An HP-5MS column ((5 %-Phenyl) -methylpolysiloxane, 60 m x 0,25 mm x 0,25 µm) from Agilent was used to separate the VOC. Finally, VOC were identified by software comparison of retention times and fragmentation pattern with the NIST 11 database of mass spectra and external reference compounds.

For VOC quantification the individual response factors were determined using the total ion count from multilevel calibration curves ($r^2 >$ 98 %) and using n-pentadecane-d32 (Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany) as internal standard (Vitalini et al., 2014). The response factors of compounds for which no commercial standard was available were derived from the most similar chemical structure compounds and normalised by molecular weight to ensure correct counting of total ion responses due to different molecular masses (Ahn et al., 2011). The concentration of each compound was calculated by dividing the concentration of the trapped volatiles by the fresh weight of the sampled chocolate (ng/g FW). The analysis was executed in triplicate for each sample.

2.12. Sensory and hedonic evaluation

2.12.1. Expert panel

Sensory analysis was performed by a trained panel. Eleven expert assessors, previously screened for anosmia and taste deficiencies using a specific screening kit (Burghart Messtechnik GmbH., Sniffin' Sticks and Taste Strips), were trained in sensory descriptive methodologies and chocolate sensory analysis (80 h of training) to get an adequate expertise to accomplish descriptive analysis tests. Standard references used for the training were retrieved from the literature (De Pelsmaeker et al., 2019) and from the standard aromas master kit "le nez du vin: master kit 54 aromas" (Editions Jean lenoir). Data acquisition was performed at IBE-CNR Sensory Lab (Bologna, Italy), using the software (FIZZ, Biosystemès, Couternon, France), inside the sensory lab booths, according to the standard protocol UNI 8589:1990 (UNI ISO, 2023). Panellists gave voluntary consent for participation, respecting privacy and current legislation (GDPR 2016/679). The samples of chocolate cubes (11 g), labelled with three-digit random numbers, were served on a tray at room temperature (20+/−2 C°) and were presented to assessors monadically, in a balanced order. Tests were carried out in duplicate. Panellists rinsed their mouths with water between samples. A specific lexicon list for descriptive analysis was created choosing twenty-three sensory attributes from the literature (Giosuè et al., 2024) or adding novel terms. Four olfactory and seven aromatic attributes were chosen: grape odour, vanilla odour, nuts odour, chocolate odour, spicey flavour, nuts flavour, chocolate flavour, raisin flavour and vanilla flavour, vegetable drink flavour, winy flavour. Besides other terms were analysed, four gustatory and eight texture attributes: sweet, acid, bitter, salty; crunchiness, firmness, pungent, astringent, flouriness, granulosity, meltiness, and adhesiveness. The sensory terms were rated on a 9-point scale from "no perception" to the "highest intensity perceivable".

2.12.2. Consumer test

Consumer tests studied: the main hedonic reasons for chocolate consumption and the liking of functional chocolate prototypes deriving from grape waste recovery. Data were gathered through two online surveys designed on the LimeSurvey platform (LimeSurvey—Free Online Survey Tool, 2023). Consumer tests were performed in autumn 2023 during public events at the CNR in Bologna, Lecce and Imola. Respondents were screened based on their age only participants older than eighteen took part to the survey. The participation was voluntary, before the test, consumers signed an informed consent for their data to be used and for the chocolate tasting experience (ethical clearence number 447122 del 20241119, 2024-CNR0A00–0447122). Data privacy and protection were respected according to the legislation (GDPR 2016/679).

2.12.2.1. First consumer test. In the first test consumers got access to the questionnaire using a QR code they collected at the public event in different CNR locations. Basic information was collected about consumers' gender, age, chocolate types of preferences (milk chocolate, dark chocolate, white chocolate, chocolate with nuts, chocolate for food intolerances) and consumption habits (everyday, at least once a week, occasionally). Furthermore, participants were requested to indicate their knowledge of functional food and their inclination to try it. Then, they were invited to perform a co-creation task to elicit their preferences for a virtual pomace-enriched chocolate using a CATA test. Consumers were first request to choose among different options the preferred chocolate type (milk chocolate, dark 50 %, dark 70 %, dark 90 %, white chocolate), the pomace to add to the functional chocolate (red or white) and the sugar presence (sugar, sweeteners, both or sugar free). Fifteen attributes were chosen for CATA (Check All That Apply) questionnaire: salty, sweet, bitter, crunchy, melty, hard, floury, sticky, astringent, milk flavor, alcohol flavor, toasted flavor, nuts flavor, cocoa flavor, pomace flavor, and consumers were asked to check among the list the sensation they would like to find in their ideal pomace-added chocolate. The test recorded 190 participants correctly and completely filling out the questionnaire.

2.12.2.2. Second consumer test. In the second test, which took place only in Bologna, consumers answered to a hedonic acceptance test. Respondents were asked for both demographic information (gender, age) and their attitude towards functional food. Moreover, they were presented with nine different pomace-enriched chocolate prototypes. All participants signed informed consent prior to taking part in the study and, to the best of their knowledge, declared to be not allergic to any of the ingredients composing the chocolate.

Each consumer received a bag containing a QR code to answer the questionnaire and different chocolate prototypes (nine cubes, 5,5 g each) placed in a plastic bag coded with three-digit numbers. Samples were tested at room temperature in a randomized and balanced order among subjects. Consumers received instructions on how to taste the sample and were invited to select all the attributes on the list, chosen from the literature, that described their sensory perceptions the most (Ares et al., 2010): four related to taste (sweet, sour, bitter and salty), 7 related to flavour (vanilla, vegetable drink, winey, raisin, spicy, chocolate, nuts) and 9 to the texture (soft, crunchy, melting, sticky, hard, pungent, astringent, floury, grainy). Then they were asked to score the sample overall liking on a 9-point Likert scale from 1: “I don’t like it at all” to 9: “I extremely like it”. The total number of participants was 90.

2.13. Statistical analysis

The data of fatty acids are the mean values of replicate measurements ($n = 2$) with standard deviation. One-way ANOVA was applied to study the effects of different parameters on fatty acids profile of different samples. Three-way ANOVA was applied to fatty acids percentages, by considering an interaction model comprising main effects (cultivar, GPF granulometry and percentage added) and two-factor interaction effects. Differences due to single and interacting factors were considered significant for p-values of the null hypothesis less than, or equal, to 0.05. The calculations and visualisation of the ANOVA results were performed in the MATLAB environment (R2024a).

Sensory data were elaborated using IBM SPSS V. 27 and R Core Team 2023 (R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria), and SensoMineR: Sensory Data Analysis for R package version 1.2. A one-way ANOVA analysis on DA sensory and liking scores, and on volatile compounds data was performed. The Tukey post hoc test was applied to determine the differences among the samples. The significance level was fixed at $p < 0.05$. The three-way ANOVA was also performed to assess the interaction between the three factors. A Principal Component Analysis (PCA) was performed on the variance matrix of the average scores of each sensory attribute, to summarize through a map the main characteristics of each prototype sample and correlated them with liking score. Descriptive statistics were used for socio-demographic data. For Co-creation data analysis Attributes frequency of use in the CATA question was measured by counting the number of consumers selecting that term to characterize each sample. Frequency data, and the combination of gender and age class influence on sensory preferences were analysed, considering chi-square distances, through Multiple Correspondence Analysis (MCA). Hedonic data and consumers’ liking of prototypes were analyzed through Correspondence Analysis which was performed on attribute frequency selection data obtained by counting the number of assessors citing that term and considering chi-square distances. Correlation between DA data and Consumer liking was performed by PCA.

3. Results and discussion

3.1. Characterization of phenolic molecules and antioxidant activity of GPF

The total phenol content (TPC), antioxidant activity (AA) and HPLC polyphenols characterization of GPFs are presented in Table 1S. As shown, both flours were characterized by a high TPC values, but Fiano derived GPF was a significantly richer source of polyphenols than Negroamaro derived GPF in both particle sizes (200 µm and 500 µm). Fiano GPF with particle size of 200 and 500 µm showed TPC values of 60.13±8.13 and 49.61±5.27 mg of Gallic Acid Equivalent (GAE)/g of flour, respectively. While Negramaro derived GPF exhibited TPC values of 21.87±0.50 and 19.63±0.33 mgGAE/g of flour respectively. The same trend was observed for AA values. Fiano GPF showed AA values of 809.99±80.24 µmol of Trolox Equivalent (TE)/g of flour at a particle size of 200µm and 709.14±0.11 µmolTE/g of flour at 500µm, whereas for Negramaro GPF, values of 608.77±28.76 µmolTE/g of flour and 534.85±31.28 µmolTE/g of flour were measured at particle sizes of 200 and 500 µm, respectively. Both Fiano and Negroamaro GPF did not show significant differences in TPC and AA between flours with 200 µm and 500 µm granulometries (Table 1S). Higher TPC in Fiano derived GPF is due to fermentation without solid matter during white wine production process that determines a not complete extraction of polyphenols from pomace (De La Cerda-Carrasco et al., 2015). Higher AA in Fiano derived GPF is related to TPC values as has been shown in previous studies (Radulescu et al., 2024).

The predominant phenolic molecules in Fiano GPF belonged to the class of flavanols (catechin and epicatechin) regardless of the particle size with value between 655.30 and 1497.64 µg/g. In Negroamaro GPF catechin and epicatechin were abundant together with anthocyanins (Table 1S). HPLC-DAD analysis showed that the most abundant non acylated anthocyanin was malvidin-3-glucoside (oenin) and as reported for other phenolic molecules, no significant differences were observed between flours with different particle size. The results of TPC and AA in Table 1S are comparable to the same values reported for pomace flours obtained from other fruits (Nagarajaiah & Prakash, 2016; Struck et al., 2016). The results obtained from the analysis of the polyphenolic content and profile of GPF, along with their antioxidant activity, support the potential of this winery by-product as an alternative and sustainable source of polyphenols for application as a food ingredient aimed at improving nutritional quality. Both flours, derived from white grapes and black grapes, contain different classes of polyphenols, all of which exhibit preventive activity against degenerative diseases such as cardiovascular and metabolic disorders, neurodegenerative diseases as well as cancer (Jain et al., 2025).

3.2. Total phenolic content and antioxidant activity of Pba_WCh fortified samples

TPC of PbA_WCh and GPF fortified samples (Fig. 1a) were evaluated using Folin-Ciocalteu (Fig. 1b). Fortified samples showed a significantly higher TPC compared to PbA_WCh. In particular, PbA_WCh had a TP content of 140.98±25.99 µgGAE/g, while supplemented samples exhibited a higher concentration of these compounds. More favorable effects were observed in samples after the addition of Fiano GPF with a granulometry ≤200 µm, which resulted in a TPC of 1481.95 ±69.72µgGAE/g for 5 % addition and 3538.80±286.96 µgGAE/g for 10 % addition. Samples enriched with Fiano GPF of ≤500 µm granulometry showed TPC values of 900.27±73.26 µg GAE/g and 2479.75±281.24 µg GAE/g for 5 % and 10 % addition, respectively. On the other hand, the addition of Negroamaro GPF led to more modest increases in TPC, reaching 313.27±10.92 µg GAE/g and 545.70± 14,92 µg GAE/g for 5 % and 10 % addition of Negramaro GPF of ≤200 µm granulometry respectively. Samples fortified with Negramaro GPF of ≤500 µm granulometry showed TPC values of 328.69±18.24 µg GAE/g and 631,99 ±80.20 µg GAE/g for 5 % and 10 % addition, respectively. Assuming a serving size of 30 g, corresponding to the weight of the mini chocolate bar showed in Fig. 1a, the intake of total polyphenols ranged from approximately 4.23 mg GAE/serving in PbA_WCh to about 106.14 mg GAE/serving in samples fortified with 10 % Fiano GPF (≤200 µm), highlighting the potential of grape pomace fortification to markedly enhance the polyphenolic intake per portion. It is well documented in the literature that the particle size of ingredients can influence several food properties, such as shelf life, functionality, texture, nutritional value, and bioavailability (Dacanal, 2024). Our results confirm that, in terms of functionality, the release of bioactive molecules becomes more efficient as particle size decreases. Future studies will further investigate the influence of GPF particle size on qualities of fortified PbA_Wch samples.

Antioxidant activity (Fig. 1b) was assayed by two chemical assays, TEAC and ORAC. Both assay results showed the same trend described for the TPC. PbA_WCh had an AA value of 1.91±0.36 µmolTE/g and 2.74 ±0.82 µmolTE/g when assayed by TEAC and ORAC assay respectively. Fiano 200_5 % (TEAC value: 25.95±1.26 µmolTE/g; ORAC value: 25.33 ±2.42) and Fiano 200_10 % (TEAC value: 64.36±5.71 µmolTE/g; ORAC value: 52.71±8.45) samples showed higher antioxidant activity than

Fiano 500_5 % (TEAC value: 17.74±1.08 μmolTE/g; ORAC value: 20.32 ±0.64) and Fiano 500_10 % (TEAC value: 37.06±5.37 μmolTE/g; ORAC value: 32.37±3.63). As showed in Fig. 1b, Negroamaro GPF addition determined an increase of AA when compared to PbA_WCh, but smaller when compared to Fiano GPF addition. Moreover, the granulometry of Negroamaro GPF does not affect the increase in antioxidant activity observed in fortified samples compared with control samples. Our data confirm the results of previous works indicating that fortification of baked foods (Tolve et al., 2021; Walker et al., 2014), pasta (Balli et al., 2021; Gerardi et al., 2023), beverages (Gerardi et al., 2020b) and dairy products (Aliakbarian et al., 2015), with 2 to 15 % grape pomace improves their total polyphenol content and antioxidant properties. To our knowledge the addition of whole grape pomace (seeds and skins) in white chocolate formulations has been investigated only by Altınok et al. (2022) that reported a preliminary study assessing the polyphenol content of fortified samples after *in vitro* digestion; however, due to methodological differences, the reported data are not directly comparable with the results of the present study. Bolenz and Glöde (2021) reported 3 times increase of TPC content in milk chocolate product added with 10 % of grape pomace. The same study confirmed our data about the correlation between the antioxidative capacity increase and pomace incorporation. The level of TPC in our samples is lower if compared to Bursa et al. (2022) The result may be related to the utilization of separated skins of Cabernet sauvignon pomace as bulking agent in chocolate formulations. Therefore, compared to Negroamaro grape pomace, Fiano grape pomace appears to be a more suitable source for enhancing the TPC content and antioxidant activity of PbA_WCh. Fiano grape pomace can be considered more efficient than other polyphenol plant sources such as *Moringa oleifera*, black carrots, muscadine grape pomace and apple pomace exploited in previous studies to enhance the nutritional value of chocolate compounds and spreads (Baycar et al., 2021; Büker et al., 2021; Darwish et al., 2023; Poliński et al., 2022). These encouraging results should be confirmed by stability studies evaluating the biomolecules from GPF added to PbA_WCh during storage. Previous studies conducted on both Fiano and Negroamaro GPF have shown that the polyphenol and fatty acid contents of flours are largely preserved when stored at 4 and 25 °C in the dark (Angilè et al., 2025; Gerardi et al., 2022); however, these findings need to be confirmed in fortified PbA_WCh samples. Moreover, only the assessment of the bioaccessibility of the added polyphenolic compounds through *in vitro* digestion studies will be able to demonstrate the real fortification of the PbA_WCh.

3.3. Polyphenols profile of Pba_WCh fortified samples

The polyphenols profile of PbA_WCh control and fortified samples obtained by HPLC analysis are reported in Table 1. Epicatechin and catechin were identified in PbA_WCh sample. Indeed, the most abundant phenol compounds in cocoa powder are procyanidin dimers, catechin and epicatechin (Ortega et al., 2008). Flavanols such as catechin and epicatechin showed a significant increase in PbA_WCh added with both Fiano and Negroamaro GPF. Higher GPF added percentage corresponds to higher epicatechin content while GPF granulometry doesn't affect epicatechin content. In GPF added samples were identified polyphenol compounds not detected in PbA_WCh. Fiano GPF added samples contained high amounts of rutin that were not detected in Negroamaro GPF added PbA_WCh. In both Fiano and Negroamaro GPF added samples, quercetin, quercetin 3-glucoside and kaempferol 3-glucoside were measured. Also, the class of soluble acids was represented in PbA_WCh fortified samples characterized by gallic acid and cutaric acid that was not detected in control samples. As expected, polyphenolic compounds identified in PbA_WCh fortified samples were the same compounds predominant in GPF (Tab.1S). Negroamaro GPF added samples were characterized by high content of total anthocyanins, but no significant difference was observed among samples with different GPF granulometry or addition level. Oenin content was highest in PbA_WCh added with 10 % of Negroamaro GPF, whereas the lowest content was detected in the sample added with 5 % of Negroamaro GPF with a granulometry ≤200 μm. This result could be explained by degradation of anthocyanins in the presence of oxygen (Calabuig-Jiménez et al., 2022; Jackman & Smith, 1996; Kim et al., 2021) since fine granulometry allows greater exposure to oxygen during GPF production. The extraction and characterization of polyphenolic compounds in the PbA_WCh samples are strongly influenced by the interactions between polyphenols and the food matrix. Such interactions are well documented in the literature, as they also affect the bioaccessibility of functional molecules (Cianciosi et al., 2022). In the case of polyphenols, these interactions are particularly complex because they depend on the nature of the interactions and on the affinity between the different classes of compounds and the components of the food matrix. The use of grape pomace as a food ingredient is often associated with the perception of astringent and bitter tastes (Beres et al., 2019). This characteristic is correlated with the content of polyphenolic compounds, and in particular with the presence of flavonols, both in monomeric and polymeric forms (Lesschaeve & Noble, 2005). The characterization of the

Table 1
Phenolic compounds in plant-based alternative of white chocolate characterized by HPLC. Data represented the mean values ± SD, different letters within the same row indicate significant differences among the samples according to ANOVA with Tukey's honestly significant differences (HSD) post hoc test; p value < 0.05.

	PbA_WCh	FIANO				NEGROAMARO			
Compound (μg/g)		500_5 %	500_10 %	200_5 %	200_10 %	500_5 %	500_10 %	200_5 %	200_10 %
Total Anthocyanins (μgOE/g)	nd	nd	nd	nd	nd	149.5 ± 33.8^{a}	163.8 ± 21.9^{a}	139.24 ±7.69^{a}	183.3 ± 26.9^{a}
	μg/g								
Oenin	nd	nd	nd	nd	nd	21.68±0.55^{a}	26.24±0.79^{b}	16.93±0.11 ^{c}	28.10±0.61^{b}
Catechin	64.42 ±0.93^{b,c}	85.33 ±3.39^{b,c}	90.48 ±17.2^{b,c}	93.65 ±11.1^{a,b}	131.73 ±7.76^{a}	89.90 ±15.29^{b,c}	69.69±0.33 ^{b,c}	53.98±7.61 ^{c}	72.49±3.90 ^{b,c}
Epicatechin	82.98±15.6^{e}	471.25 ±3.02^{b,c}	573.74±50 ^{a}	468.39 ±22.3^{b,c}	531.33 ±8.25^{a,b}	386.35 ±18.18^{c}	434.49 ±4.86^{b,c}	218.77 ±35.96^{d}	375.78 ±25.56^{c}
Gallic ac	nd	7.37±0.03^{b,c,d}	13.71±4.5^{a,b}	9.72±0.92^{b,c}	19.70±0.63^{a}	2.91±0.43 ^{c,d}	4.73±0.97 ^{c,d}	2.02±1.14^{d}	6.58±0.43^{b,c,d}
Cutaric ac	nd	2.26±0.03 ^{b,c}	2.69±1.34^{a,b,c}	4.16±0.53^{a,b}	4.77±0.08^{a}	2.48±0.10 ^{a,b,c}	1.29±0.57 ^{c}	2.28±0.57 ^{b,c}	3.62±0.35^{a,b,c}
Rutin	nd	31.92±1.15^{b}	31.34 ±2.67^{a}	21.70 ±1.42^{a,b}	14.75±0.30^{a}	nd	nd	nd	nd
Quercetin	nd	11.46±0.02^{c}	13.33±1.0^{a,b}	11.73±0.18^{c}	13.47±0.22^{a,b}	12.55±0.11^{b,c}	14.19±0.11^{a}	11.39±0.33^{c}	14.47±0.21^{a}
Quercetin 3-glucoside	nd	8.39±0.0^{b,c}	14.54 ±4.04^{a,b}	9.59±0.75^{a,b,c}	15.73±0.70^{a}	6.40±0.54^{c}	9.37±0.13^{a,b,c}	5.09±0.2^{c}	10.36±0.41^{a,b,c}
Kaemperol 3-glucoside	nd	8.85±0.14^{c}	12.55 ±0.23^{a}	8.95±0.77^{c}	10.76±0.58^{b}	0.80±0.07^{e}	0.86±0.20^{e}	nd	2.76±0.22^{d}

polyphenolic profile and content of the PbA_Wch samples supplemented with GPF suggests a greater impact on the sensory perception of astringency in the samples enriched with Fiano GPF when compared to samples added with Negramaro GPF, as confirmed by the sensory analysis described in Section 3.9.2.

3.4. Fatty acid profile

Fatty acids from the different chocolate samples were identified by GC–MS analysis, and the percentages values were defined as reported in Table 2. Twelve saturated and unsaturated fatty acids were identified in all samples. Quantitatively, C16:0 (>20 %), C18:0 (>30 %), C18:1 (>15 %), C18:2 (>7 %) were the most important fatty acids detected. Our findings agree with previous research by Liendo et al. (1997), Lipp et al. (2001) and Jahurul et al. (2013) which also highlighted the significance of C16:0, C18:0, and C18:1 as the primary fatty acids in cocoa butter. To evaluate the significant differences among samples, one-factor ANOVA was carried out (Table 2S). Results obtained showed significant differences among saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids. In details, among SFA acids, differences ($p < 0.05$) were detected for myristic, stearic and eicosanoic acids, while differences for $p < 0.01$ were found in margaric and docosanoic acids. Among MUFA acids, significant differences were observed for palmitoleic ($p < 0.01$) and oleic, and 11-eicosenoic acids ($p < 0.05$). Finally, regarding PUFA group, linoleic acid showed significant differences ($p < 0.01$) among samples.

A three factor ANOVA (cultivar, GPF dimension and percentage added) with interactions, was performed to study the impact of these parameters on fatty acids profile of samples.

The incorporation of grape pomace flour (GPF) had a significant influence on the fatty acid profile of the chocolate samples, with the greatest impact arising from the cultivar and the interaction between the cultivar and particle size (Cv × dimensions). Samples formulated with 10 % Fiano GPF at a particle size of 200 µm exhibited the most pronounced modifications in fatty acid composition. For most fatty acids, a significant increase in concentration was observed, primarily due to the interaction between cultivar and particle size, as revealed by the three-way ANOVA. An exception to this general trend was oleic acid (C18:1), which decreased in samples containing Fiano GPF, suggesting cultivar-specific modulation of this fatty acid. Also, the addition of Negramaro GPF was associated with a slight decrease in oleic acid content; however, this reduction was not statistically significant and was observed in formulations with a larger particle size (500 µm) at a 5 % addition level, and with a smaller particle size (200 µm) at both a 5 % and a 10 % addition level. Negramaro GPF also led to a decrease, particularly at 500 µm and 10 % inclusion (25.13 ± 1.81 %), whereas at 500 µm at 5 % and then smaller particle size (200 µm) mitigated this effect, maintaining oleic acid levels closer to the control (34.67 ± 1.67 %). These results suggest that oleic acid behaves differently in response to the addition of GPF, depending on the grape cultivar and processing conditions. Overall, Fiano GPF with a particle size of 200 µm appeared to be the most effective in enhancing the fatty acid content of the samples, whereas Negramaro GPF had a stronger effect at a particle size of 500 µm. These cultivar- and size-dependent responses highlight the importance of carefully selecting the source of grape pomace and its granulometry to modulate the lipid composition of plant-based white chocolate alternatives. The findings reported in Table 2S, highlighted the important role of the cv used. Indeed, the influence of cv is statistically significant for all acids identified, except for pentadecanoic, palmitic and linolenic acids. Much more weakly appears to be the impact of the percentage of GPF added, which affects the content of eicosanoic, docosanoic ($p < 0.05$) and linoleic ($p < 0.01$) acids. The "dimension" factor seems to have no noticeable effect. However, in relationship with the cv factor it notably impacts most of the acids under consideration ($p < 0.05$, $p < 0.01$), despite its insignificant individual influence. In Table 2, is reported the SFA/UFA ratio useful to evaluate the relationship between main groups of fatty acids identified. According to literature (Torres-Moreno et al., 2015), its ratio was higher than 1 and ranged from 1.28 to 2.32, which indicates a high percentage of SFA in chocolate sample. From a nutritional standpoint, the predominance of saturated fatty acids compared with unsaturated fatty acids is generally regarded as unfavourable. For decades, saturated fatty acids with chain lengths ranging from C12:0 to C16:0 have been considered to contribute to atherosclerosis and have been associated with an increased risk of cardiovascular disease (CVD). Consequently, due to its relatively high saturated fat content, chocolate has often been assumed to have a hypercholesterolemic effect. However, more recent clinical studies have suggested that stearic acid (C18:0), a saturated fatty acid that is neither cholesterolemic nor atherogenic, has a neutral metabolic effect. These studies indicate that chocolate intake does not adversely affect total cholesterol or LDL-cholesterol levels in the blood, nor does it reduce HDL-cholesterol concentrations (Kris-Etherton et al., 1999; Thijssen & Mensink, 2005). Wan et al. (2001) reported that consuming 22 g of cocoa powder and 16 g of dark chocolate daily for four weeks resulted in

Table 2
Fatty acid composition of PbA_WCh and GPF added samples. Means ± standard deviations are reported.

	PbA_WCh	FIANO				NEGROAMARO			
Fatty Acid (Relative percentage)		F-500_5 %	F_500_10 %	F_200 5 %	F_200_ 10 %	N_500_5 %	N_500_10 %	N_200_5 %	N_200 10 %
SFA									
Myristic acid (14:0)	0.17±0.01	0.15±0.01	0.18±0.05	0.23±0.08	0.30±0.02	0.15±0.01	0.18±0.02	0.17±0.03	0.15±0.00
Pentadecanoic acid (15:0)	0.06±0.01	0.05±0.001	0.06±0.02	0.070±0.01	0.10±0.004	0.04±0.01	0.06±0.01	0.06±0.02	0.06±0.01
Palmitic acid (16:0)	22.30±0.99	21.14±0.36	20.13±1.52	22.56±1.40	20.85±0.13	21.07±0.06	21.56±0.22	21.07±0.67	21.28±0.39
Margaric acid (17:0)	0.32±0.02	0.38±0.04	0.39±0.07	0.44±0.05	0.70±0.01	0.33±0.06	0.40±0.01	0.36±0.002	0.29±0.01
Stearic acid (18:0)	31.90±0.81	38.29±5.48	36.78±1.32	40.87±1.44	44.11±2.22	35.60±3.02	40.47±0.55	35.13±1.14	33.39±1.19
Eicosanoic acid (20:0)	1.25±0.09	1.76±0.53	1.89±0.04	1.77±0.26	3.03±0.30	1.54±0.09	1.90±0.11	1.44±0.14	1.47±0.09
Docosanoic acid (22:0)	0.20±0.02	0.36±0.14	0.34±0.04	0.38±0.02	0.67±0.13	0.35±0.02	0.40±0.01	0.24±0.04	0.31±0.03
MUFA									
Palmitoleic acid (16:1)	0.51±0.02	0.55±0.022	0.65±0.14	0.74±0.13	1.08±0.02	0.57±0.08	0.64±0.07	0.57±0.02	0.46±0.01
Oleic acid (18:1)	35.98±1.14	28.88±5.77	29.78±4.31	23.81±0.80	15.31±2.10	32.17±4.07	25.13±1.81	33.30±0.1	34.67±1.67
11-Eicosenoic acid (20:1)	0.13±0.09	0.25±0.03	0.19±0.04	0.20±0.01	0.32±0.02	0.16±0.05	0.20±0.02	0.15±0.02	0.17±0.04
PUFA									
Linoleic acid (18:2)	6.85±1.10	7.79±0.15	9.20±1.17	8.47±0.72	12.70±0.30	7.61±0.04	8.60±0.84	7.10±0.28	7.39±0.06
Linolenic acid (18:3)	0.29±0.01	0.35±0.04	0.34±0.09	0.40±0.03	0.66±0.03	0.32±0.03	0.41±0.14	0.34±0.06	0.32±0.02
∑ SFA	56.19	62.12	59.77	66.32	69.77	59.09	64.96	58.46	56.94
∑ MUFA	36.62	29.67	30.63	24.74	16.71	32.9	25.96	34.01	35.3
∑ PUFA	7.15	8.14	9.54	8.87	13.36	7.93	9.01	7.44	7.72
∑ UFA	43.77	37.81	40.17	33.61	30.07	40.83	34.97	41.45	43.02
Ratio SFA/UFA	1.28	1.64	1.49	1.97	2.32	1.45	1.86	1.41	1.32

a 4 % increase in HDL-cholesterol levels. Similarly, Mursu et al. (2004) observed that daily intake of 75 g of dark chocolate over three weeks led to a 11–14 % rise in HDL-cholesterol concentration. Kurlandsky and Stote (2006) also demonstrated that a daily intake of 41 g of dark chocolate alongside a self-selected diet for six weeks was associated with improved serum triacylglycerol levels in healthy women. Furthermore, the available evidence indicates that stearic acid has a similar impact on lipid profiles to oleic and linoleic acids, which are recognised for their protective effect against cardiovascular disease (Hooper et al., 2004; Hu et al., 2001).

3.5. VOC profiles

The volatile profile was carried out to characterize the aroma of the plant-based chocolate samples enriched with two different GPFs. The HS-SPME extraction method using a fiber coated with DVB/CAR/PDMS was chosen based on literature as the appropriate extraction method for its generic adaptability to a wide range of compounds and specifically to the food matrix focus of this work: cocoa butter and grape pomace (Giosuè et al., 2024; Wardencki et al., 2004). All the volatile organic compounds detected by HS-SPME/GC–MS analysis in plant-based white chocolate unenriched and enriched with GPF are reported in Table 3S. A total of 71 volatile compounds across 16 chemical classes were extracted and recognised by mass spectra (MS) and retention time (RT). The most abundant chemical classes were aldehydes, followed by acids and alcohols. Samples enriched with 10 % of Negroamaro GPF, in particular with a granulometry of 500 µm, recorded the highest amount of total volatile compounds, while chocolates enriched with 10 % of Fiano grape pomace flour were characterized by the lowest total amounts. The PbA_WCh sample was characterized by the lowest volatile concentration of all chemical classes except aldehydes, represented almost entirely by benzaldehyde (851.18 ng/g). This high benzaldehyde level can be explained by a higher amount of pure almond paste since this compound is well-known to be the key odorant of almond (Xiao et al., 2014). As grape pomace flour increases, pure almond paste decreases. However, benzaldehyde was also abundant in chocolate with Fiano grape pomace flour and especially in the sample with 500 µm of granulometry. Genovese et al. (2007) explained its high presence derives from the degradation of odorant compounds occurring in Fiano wine.

The concentration of other chemical classes increased by adding grape pomace flour to the control sample. Within the acids chemical class, the Negroamaro samples showed the highest concentration, of which the most abundant was hexanoic acid. In the same way alcohols, mainly represented by phenylehtyl alcohol, increased their concentration in particular with the addition of Negroamaro GPF. The acids and alcohol chemical classes deriving from grape pomace addition contribute to fermented odor and wine odor intensity (Capone et al., 2013). According to Giosuè et al. (2024) the ester class was almost absent in the control sample demonstrating to be strongly linked to the fruit-based component added to chocolate. Esters are known for imparting fruity notes, and their abundance in Negroamaro grape pomace can be explained the contribution of these samples in wine odor and flavour. Furthermore, Negroamaro enriched chocolate presents an ester compound, butanedioic acid, diethyl ester, as peculiar of these samples and absent in the other chocolate samples.

The only furan found, 2-pentyl furan, increased in the enriched chocolate samples, and was particularly abundant in that samples with 10 % of Negroamaro GPF. Giosuè et al. (2024) and Gerardi et al. (2023) also reported the presence of this compound in pomace-enriched products, and its increase related to thermal processes. Likewise, ketones compounds, mostly represented by acetophenone, were higher in the Fiano and Negroamaro samples and associated with honey aroma (Genovese et al., 2021). Some monoterpenes and terpenoids were found in all samples with lower concentration in PbA_WCh and chocolate enriched with 5 % of Negroamaro GPF. Finally, three pyrazines were identified, two of them belonging to the chocolate aroma and associated with nutty and grassy flavour (Barišić et al., 2019). Pyrazines were found in all samples, with higher concentration in 5 % added Fiano GPF samples.

To evaluate the significant differences and the effect of the interdependencies between the three factors (cultivar, dimensions, and percentages) on the sample's VOC profiles, one-way and three-way ANOVA were performed (Table 4S). The one-way ANOVA showed statistical differences in all chemical classes except for monoterpenes. The main results derived from factors and their interaction effect on the most abundant chemical classes linked to the final aroma were that cultivar, dimensions, and their combination indicated a strong effect on acids and alcohols. Interaction between cultivar and dimension also impacted the concentration of aldehydes, together with the percentage of GPF added. Cultivar and its interaction with percentages also affected this class but to a lesser extent. For chemical classes, represented both by a lower number of compounds and lower concentrations, results showed that cultivars had a strong influence on the presence of the esters, less influenced by percentages; ketone concentration highly depended only on the interaction between dimension and percentages; dimension, percentages, and the interaction cultivar/dimension conditioned the presence of pyrazines and terpenoids. The chemical classes most explored in this work were those represented by high concentrations, but especially those commonly linked to the final aroma profile of chocolate and grape pomace based on literature (Abreu et al., 2023; Barišić et al., 2019). To our knowledge, the literature on VOC components found in Fiano and Negroamaro grape pomace is scarce, while more information is available on Fiano and Negroamaro wines.

3.6. Color profile

Among quality parameters, the color influences appearance and consumer perception. The results of colorimetric analysis are reported in Table 5S. The plant-based alternative of white chocolate GPF added samples showed a net decrease of L^* value depending on GPF added percentage either for Fiano or Negroamaro flours. The decrease of brightness is a negative effect for chocolate, but in the present study, this result attests changes in the visual properties of the used fortifying agent. Moreover, b^* value decreased and a^* value increased in GPF added samples when compared to control samples indicating that GPF supplement led the samples redder and less yellow than control. These changes in the color parameters are more evident for Negroamaro GPF addition in comparison to Fiano GPF, due to anthocyanins found in Negroamaro GPF. In addition, for both cultivar, Fiano and Negroamaro, the samples prepared with GPF at $\leq$200 µm granulometry showed higher a^* values in comparison with sample prepared with GPF at $\leq$500 µm granulometry. This finding suggested showing particle size plays a role in the coloring effect of GPF. Similar results were reported by Altınok et al. (2022) in white chocolate samples added with grape pomace powder. Moreover, the role of GPF particle size on color parameters is confirmed by Troilo et al. (2021) on muffins added with 15 % of grape pomace powders at different particle size.

3.7. Descriptive Analysis (DA) profiles

Sensory evaluation data expressed by the expert panel showed a significant impact of Cv on cocoa odor, bitter and acid tastes, and on flavors such as nuts, spicy, raisin, vanilla and winy (Table 6S). Pomace percentage showed an impact on the perception of grape odor, bitter and acid tastes, spicy, raisin, vanilla and vegetable drink flavors, and on texture attributes such as flouriness and granulosity, and also on pungency. The dimension GPF did not affect perception. Were recorded interactions Cv-GPF percentage and GPF percentage-GPF Dimension on crunchiness (Table 6S).

The panel evaluated also PbA_WCh in comparison with the pomace-enriched samples (Table 3). Differences were registered for most of the attributes analysed. In relation to the odors, the PbA_WCh sample was

Table 3
Mean intensity scores for the sensory attributes of the different pomace-enriched chocolate samples. Different letters (a,b,c) correspond to different mean values according to one-way Anova followed by Tuckey post hoc test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

	PbA_WCh	FIANO				NEGROAMARO			
Product Name		200_10 %	500_10 %	200_5 %	500_5 %	200_10 %	500_10 %	200_5 %	500_5 %
Grape odor***	1.4 b	3.2 a	3.3 a	2.6 a	2.8 a	3.5 a	3.3 a	2.8 a	3.2 a
Nuts odor***	1.9 b	3.1 a	3.3 a	3.0 a	3.0 a	3.2 a	3.0 a	2.9 a	3.1 a
Vanilla odor***	2.6 a	1.8 b	1.8 b	2.0 ab	2.0 ab	2.0 b	1.7 b	1.8 b	1.8 b
Cocoa odor	3.0	2.3	2.3	2.3	2.4	2.8	2.7	2.8	2.6
Sweet**	6.3 a	5.2 b	5.5 ab	5.7 ab	5.4 ab	5.2 b	4.9 b	5.5 ab	5.3 b
Salty	1.8	2.5	2.4	2.2	2.5	2.6	2.6	2.5	2.5
Bitter***	1.5 b	2.9 a	2.7 a	2.3 ab	2.4 a	3.0 a	3.0 a	2.6 a	2.7 a
Acid***	1.7 c	2.6 ab	2.8 ab	2.5 b	2.2 bc	3.3 a	3.3 a	2.8 ab	2.6 ab
Spicy flavor***	1.7 b	3.2 a	3.2 a	2.5 ab	2.7 a	3.2 a	3.2 a	3.0 a	3.2 a
Nuts flavor***	1.9 b	3.5 a	3.7 a	3.1 a	3.7 a	3.1 a	3.0 a	3.2 a	3.1 a
Chocolate flavor	3.9	3.0	3.0	3.0	2.9	3.0	3.1	3.7	3.2
Raisin flavor***	1.4 c	4.0 ab	3.1 b	3.1 b	3.0 b	4.7 a	4.2 ab	3.8 ab	3.8 ab
Vanilla flavor***	3.4 a	1.9 bc	2.2 bc	2.4 bc	2.5 b	1.8 c	1.8 c	2.0 bc	2.0 bc
Vegetable drink*	3.0 ab	2.5 ab	2.6 ab	2.7 ab	3.4 a	2.5 ab	2.3 b	2.8 ab	2.6 ab
Winy flavor***	1.3 f	2.7 cde	2.8 cde	2.2 def	2.0 ef	4.4 a	4.0 ab	3.3 bc	3.1 bcd
Crunchiness	4.9	5.9	5.8	5.1	5.7	5.4	5.0	5.2	6.1
Firmness	4.6	5.3	5.1	4.9	5.1	5.3	4.8	5.1	5.6
Flouriness***	2.3 c	4.9 ab	5.3 a	3.8 b	4.4 ab	4.5 ab	4.5 ab	4.1 ab	4.1 ab
Granulosity***	2.1 b	3.5 a	3.6 a	2.7 ab	3.5 a	3.7 a	3.4 a	2.8 ab	3.3 a
Meltiness**	5.4 a	4.2 b	4.2 b	5.0 ab	4.3 b	4.6 ab	4.5 ab	4.5 ab	4.2 b
Adhesiveness	3.8	3.5	3.3	4.0	3.5	3.3	3.8	3.5	3.5
Pungent**	2.3 b	3.3 ab	3.4 ab	3.2 ab	2.9 ab	3.8 a	3.5 a	3.4 ab	3.0 ab
Astringent	3.0	3.5	4.1	3.7	3.5	3.7	3.8	3.4	3.6

characterized by a vanilla odor, while for all the pomace-added samples, the main odor notes were nuts and grapes (Table 3). Regarding basic tastes, the PbA_WCh sample was the sweetest one, with the lowest intensity of bitterness and acidity. In accordance with a recent study conducted by Jaeger et al. (2024) which examines the sensory characteristics of plant-based milk alternatives, rice milk has been described as having a sweet and delicate flavor. Among pomace-enriched samples, F 200_5 % recorded the highest score for sweetness, while N 500_10 % was the least sweet. All of the pomace-added samples were evaluated as more bitter than PbA_WCh, with the highest bitterness registered for N 200_10 % and N 500_10 %, which also recorded the highest scores for acidity.

In relation to the aroma, pomace added chocolate, unlike the control sample, was characterized by the spicy and nuts flavor. This is in agreement with (Capone et al., 2013) that describes the taste profile of Negroamaro with good acidity and notes of ripe red fruits and spices. Instead, the nuts flavor (*e.g.*hazelnut and almond) that characterized Fiano pomace sample is frequently used in the oenological literature to describe Fiano aroma (Genovese et al., 2007, 2021). Raisin flavor characterized the sample N 200_10 % unlike the PbA_WCh sample, that was characterized by the highest vanilla flavor. The vegetable drink flavor was clearly perceived in F 500_5 % (3.4) followed by the PbA_WCh sample (3.0), while the lowest value was registered for N 500_10 %. In line with VOCs profiles data, winy flavor was higher in N 200_10 % (4.4) and N 500_10 % (4.0) than in the PbA_WCh sample (1.3) but was also slightly perceived in N 200_5 % (3.3) and N 500_5 % (3.1), while other samples had intermediate values.

All the pomace-added samples were crunchy and firm, texture attributes showed F 500_10 % characterized by the highest flouriness, followed by F 200_10 % and the PbA_WCh sample was the least floury. N 200_10 % characterized by the highest granulosity followed by F 500_10 % and F 200_10 % (Giosuè et al., 2024). Most of the pomace-enriched samples showed low meltiness with the lowest values for F 200_10 %, F 500_10 % and N 500_5 %. The PbA_WCh sample was the meltiest one followed by F 200_5 %. The highest pungency was found in N 200_10 % while the lowest value was found in PbA_WCh sample.

3.8. *Consumer test*

3.8.1. *First consumer test: ideal product*

One hundred and ninety consumers took part to the co-creation test.

MCA analysis of the co-creation data shows consumers' preference for the ideal pomace-added chocolate (Figure 1S). Consumers check among the list of CATA questionnaire the sensation they would like to find in their ideal pomace-added chocolate (Bruzzone et al., 2015; Ruark et al., 2016). Some differences were recorded among consumers' segments. Consumers under 30 preferred a sweet product, which reminds milk chocolate, thus their ideal product was a sweet, melty chocolate added with white pomace.

Consumers over 30 preferred red pomaces added to a sugar free product. In the "over 30″ consumer segment, clear differences were observed between genders, with males more appealed by pomace alcoholic flavor added to a strong 90 % dark chocolate. Females "over 30″ would appreciate more a toasted, cocoa, nuts flavor added to a crunchy 50–70 % dark chocolate. A considerable age-related difference was recorded also about the addition of sugar: desirable for the younger segment, while "over 30" show more interest for the sugar-free choice. The preferences toward dark and extra dark chocolate are better explained in (Merlino et al., 2021), which demonstrated a male preference for chocolate with a strong and bitter taste, in comparison to female preferences. A recent study has identified different chocolate consumer preferences based on age (Massaglia et al., 2023), and illustrate how chocolate preferences vary significantly with age, influencing marketing strategies and product offerings for chocolate companies.

3.8.2. *Second consumer test: evaluation of prototypes*

Ninety consumers evaluated the chocolate protoypes answering the survey in Bologna.

The moon plot (Fig. 2) shows consumers' perception of the pomace-enriched chocolate prototypes obtained through CATA questionnaire (Ares et al., 2010, 2014). The plot highlights the sensory attributes characterizing each prototype (indicated by the largest character and the distance from each attribute). The prototypes with Negroamaro (N) are perceived as more "winy", "acid", "raisin" and "spicy", while those based on Fiano (F) were more "grainy", "floury", "astringent" and with a nuts flavor with the exception of F 500_5 % which is reminiscent of a

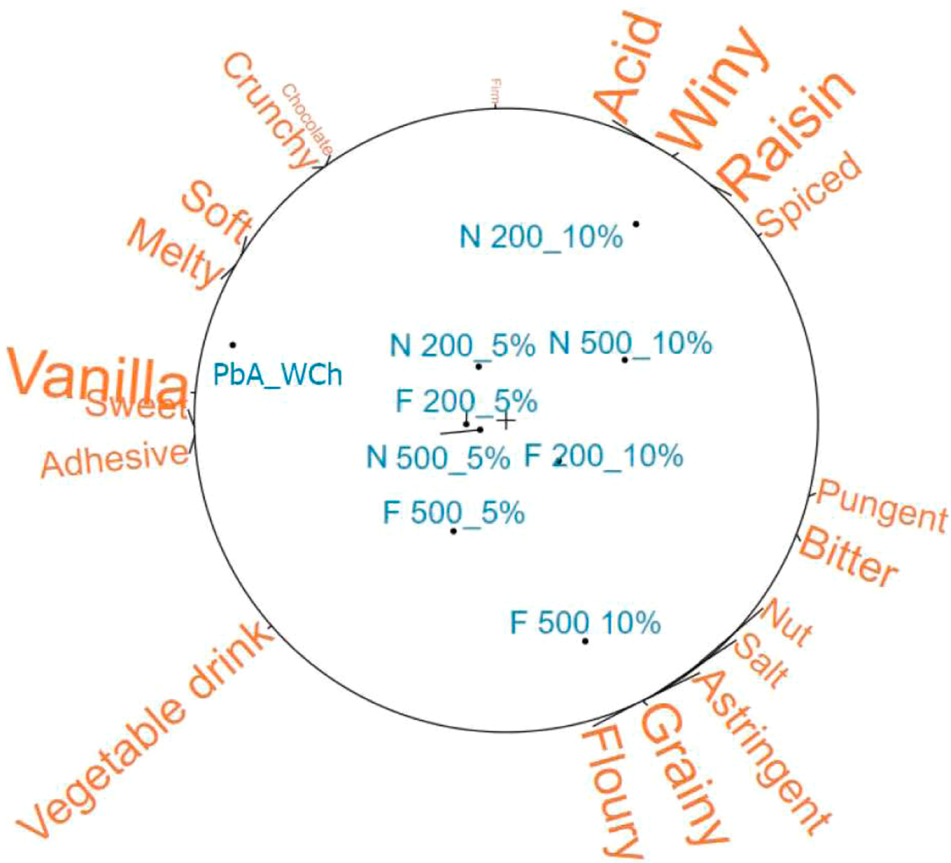

Fig. 2. Moon plot: evaluation of real chocolate samples through CATA questionnaire.

vegetable drink. The PbA_WCh sample is positioned far from other samples because it is sweeter and characterized by a vanilla flavor, meltiness and softness. The samples N 200_10 % was mainly characterized by attributes reminding raisin, while the sample F 500_10 % was characterized by texture attributes "grainy", "astringent", and "floury", also present in F 500_5 %. Moreover, samples enriched with 5 % pomace, were recognized as different from the control, but did not show relevant differences among them. The findings are consistent with the Descriptive Analysis (DA) profiles assessed by a panel of experts.

3.8.3. Overall liking

Consumer test indicated how liking was affected by Cv and GPF percentage, not by GPF dimension (Table 7S). A significant influence of gender and age was also observed. Male provided higher scores for all samples (average 4.7), as compared to female (average 3.6), younger consumers showed a higher appreciation of the products (average 4.4) than older consumers (average 4.0) (Table 4).

Consumers expressed the highest appreciation for PbA_WCh, although in line with the lowest threshold of acceptability (5) (Table 4). However, Negramaro pomace-enriched chocolates, received close evaluations, averaging a 4.4 score, and an average reduction of 11 % acceptance score when compared with PbA_WCh. Fiano pomace-enriched samples had lower acceptance scores, averaging 3.9, about 20 % lower than control. PbA_WCh received a positive acceptance score from 60 % of participants. Negramaro enriched samples enriched with 5 % and 10 % with 200 granulometry, were found acceptable by nearly half of the consumers (47–48 %). Consumer gender affected appreciation, with male expressing higher scores for all samples and also for PbA_WCh. Male evaluated N 500_5 % in line with acceptance threshold. Also, consumer age affected acceptance score, with younger, under 31, providing higher scores (average 4.4), than older ones (average 4.0). The younger segment evaluated N 500_5 % and N 200_5 % adequate to acceptance threshold.

Table 4
Overall liking score (1 not liking at all; 9: liking extremely; 5: acceptance threshold) of pomace-enriched chocolates compared with control. Different letters, in rows, correspond to different mean values according to one-way Anova followed by Tuckey post hoc test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Product Name	PbA_WCh	Fiano				Negroamaro			
		200_10 %	500_10 %	200_5 %	500_5 %	200_10 %	500_10 %	200_5 %	500_5 %
Overall Liking	5.0 a	3.7c	3.7c	3.9 bc	4.2 abc	4.4 abc	4.2 abc	4.4 abc	4.6 ab
Acceptance *vs.* PbA_WCh (%)	-	−27	−26	−21	−16	−11	−16	−11	−7
Percent consumer acceptance	60	29	29	32	38	47	37	48	47
Overall Liking Male	5.5 a	4.1 bc	4.0 c	4.5 abc	4.6 abc	4.8 abc	4.6 abc	4.9 abc	5.1 ab
Overall Liking Female	4.4 a	3.1 b	3.3 ab	3.2 ab	3.6 ab	4.0 ab	3.6 ab	3.9 ab	3.8 ab
Overall Liking Under 30	5.2 a	3.9 bc	3.8 c	4.3 abc	4.4 abc	4.6 abc	4.5 abc	4.9 ab	5.0 ab
Overall Liking Over 31	4.9 a	3.4 c	3.7 bc	4.0 ab	4.1 ab	4.3 ab	3.8 bc	4.1 ab	4.4 ab

3.8.4. Expert panel sensory evaluation and consumers'acceptance of chocolate prototypes

Descriptive analysis (DA) results, obtained through panel test, and liking scores, obtained through consumer test, were analysed together in a multidimensional way through PCA (Fig. 3), which showed that the first and the second principal components accounted for 69.3 % and 13.4 %, respectively, of the experimental variability of the data, representing a total of 82.7 %. The first component was positively correlated with aromatic flavor, reminding chocolate and wine/raisin, salty and acid, and texture characteristics as pungent and adhesiveness, and negatively correlated with texture attributes (astringent, firmness, flouriness, granulosity, and crunchiness), sweet, vanilla and vegetable drink flavor, nuts odor and flavour. The incorporation of grape pomace resulted in notable textural differences compared to PbA_WCh sample. The enriched samples exhibited a crisp texture and a more granular mouthfeel, in accordance with (Giosuè et al., 2024) who showed similar results when adding red grape pomace to biscuits. The second component was positively correlated with attributes reminding wine/raisin and most of the texture attributes, and negatively correlated with attributes reminding chocolate, adhesiveness and vegetable drink. Consumers' liking pointed mainly towards the PbA_WCh sample. Samples N 200_10 % and N 500_10 % were located at the positive area of the principal components indicating they were characterized by attributes reminiscent of wine and raisin that still earned consumer appreciation. The sample N 200_5 % was closer to the attributes reminding chocolate and the closest to consumers' liking among the chocolate enriched samples. The samples F 200_5 % and F 500_5 % were on the negative side of both components, indicating they were not in line with consumer liking and mainly characterized by the attribute vegetable drink flavor, vanilla odor and flavor, nuts flavor and sweetness. Finally, the samples N 500_5 %, F 200_10 % and F 500_10 % were mainly characterized by texture attributes, which didn't earn consumers' liking. Several studies indicate that consumers generally accept products enriched with grape pomace, especially when these products offer improved nutritional value and sensory qualities (Almanza-Oliveros et al., 2024; Antonić et al., 2020; Baldissera et al., 2022).

3.9. Conclusion

The here reported polyphenol characterization of GPF suggest that the content of total phenols is higher in Fiano pomace than in Negroamaro as well as specific classes of compounds (flavanols, stilbenes and soluble acids) are more abundant in Fiano pomace. The higher content of specific compounds and the synergy between them resulted in higher antioxidant activity in PbA_WCh enriched with Fiano pomace when compared to samples added with Negramaro pomace. On the other side, the addition of GPF to plant-based chocolate provides a large amount of aromatic compounds. Negroamaro GPF was richer than Fiano GPF in the most aromatic chemical classes. Future studies could include gas-chromatography-olfactometry (GCO) analysis to identify key aroma compounds and better understand their sensory relevance, as well as extend this approach to other by-products within a circular economy framework. The sensory analysis of PbA_WCh showed a clear difference between samples and the consumer's preference. The control PbA_WCh sample, characterized by sweetness, vanilla and cocoa odor, chocolate flavor, and smoothness was preferred by consumers. However, the Negroamaro-enriched samples, while more acidic and pungent, presented a notable aromatic profile with notes of grapes, raisin, and wine. Whereas the Fiano-enriched samples were noted for their nutty odor and flavor. The sensory analysis has proven fundamental in outlining the profile of the newly developed foods, highlighting the peculiar characteristics that drive consumer preferences. The results will also be useful

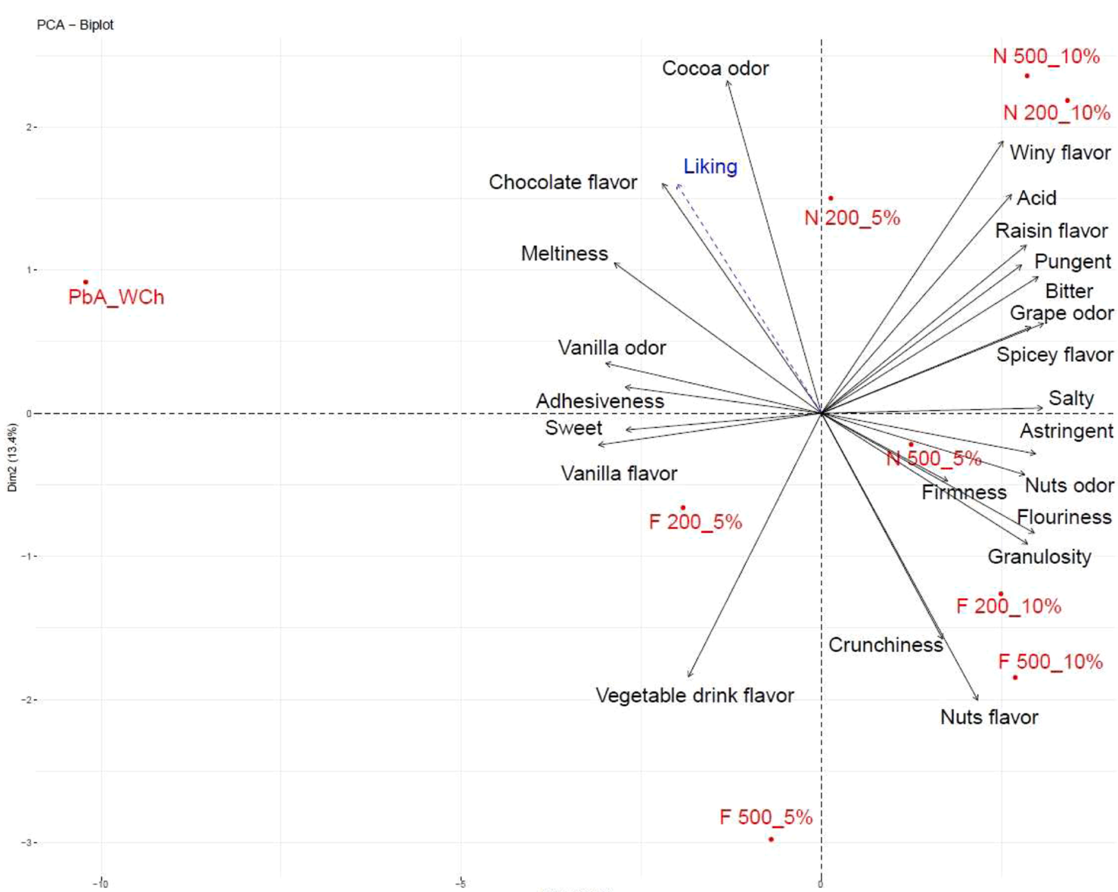

Fig. 3. PCA analysis of DA data and consumer acceptance.

to improving the formulation of fortified PbA_WCh. Future studies will focus on evaluating the bioaccessibility of the polyphenolic fraction of PbA_WCh samples fortified with grape pomace through *in vitro* digestion studies, in order to determine the release of phenolic compounds and thus assess the actual extent of fortification of the new products. Based on the results of this study, it is possible to conclude that grape pomace can be utilized as low-cost raw material for functional and plant-based confectionary products.

Ethical statement

All procedures for sensory evaluation were carried out in accordance with relevant laws, institutional guidelines, and the European Code of Conduct for Research Integrity and were approved by the Italian National Research Council Ethical Commission, 18 November 2024, as NUTR-AGE Project activity. Participants gave informed consent.

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Different percentages and granulometries of GPF in PbA_WCh formulation were defined

GPF as plant alternative ingredient in WCh resulted in higher fiber content and antioxidant activity

71 VOCs were identified using HS-SPME coupled with GC–MS, the addition of GPF to PbA_WCh provided cultivar-related aroma compounds.

The sensory attributes of enriched WCh were analyzed, showing a relevant impact of GPF of texture, odor and flavor.

Consumers showed a relatively higher appreciation for samples enriched with Negroamaro GPF.

CRediT authorship contribution statement

Federica Angilè: Methodology, Data curation, Conceptualization. **Maria Tufariello:** Methodology, Formal analysis, Conceptualization. **Marta Cianciabella:** Writing – review & editing, Writing – original draft, Conceptualization. **Chiara Medoro:** Methodology, Data curation. **Giulia Maria Daniele:** Writing – review & editing, Writing – original draft, Data curation. **Cinzia De Benedictis:** Data curation. **Stefano Predieri:** Writing – review & editing, Writing – original draft, Investigation. **Giovanna Giovinazzo:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources. **Carmela Gerardi:** Writing – review & editing, Writing – original draft, Resources, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing 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.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2026.101817.

Data availability

Data will be made available on request.

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