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YOGURT ENRICHMENT WITH GRAPE POMACE: EFFECT OF GRAPE CULTIVAR ON PHYSICOCHEMICAL, MICROBIOLOGICAL AND SENSORY PROPERTIES

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ABSTRACT

Grape skin flours obtained from grape pomace of Chardonnay, Moscato and Pinot noir varieties were used as sources of polyphenolic compounds in yogurt formulation during 3 weeks of storage. Yogurt containing grape skin flour presented significantly higher total phenolic content (+55%), antioxidant activity (+80%) and acidity (+25%) whereas lower pH, syneresis (−10%) and fat (−20%) than control. Procyanidin B1 and vanillic acids were detected only in the yogurt added of Pinot noir flour while gallic acid, catechin and quercitrin were the major phenolic compounds found in the yogurts with Moscato or Chardonnay grape skins. Significant differences were highlighted for acidity and lactose content while total phenolic content, antioxidant activity and lactic acid bacteria trend were stable after production and storage. The liking test performed with consumers showed a loss of textural quality for yogurts fortified with grape skin flours.

PRACTICAL APPLICATIONS

Grape skin is a nutritious, but underused, by-product of winemaking containing fiber and antioxidants. Using a suitable production design, a new fortified yogurt formulation with grape by-product could be optimized for enhance antioxidant consumers' daily intake. The use of grape skin flour in the development of value-added food products will be a step toward making new functional foods, and partially solving waste management problem from wine production. The results of this study would provide an opportunity of dairy producer to develop a novel product in agreement with consumers' preferences. This research represents a new approach in the development of novel dairy foods with high nutritional quality and with great potential applications on food industry.

INTRODUCTION

Grape (*Vitis vinifera L.*) is one of the world's largest fruit crops. Winemaking process uses a considerable amount of fresh grape generating a huge mass of solid by-products that correspond to approximately 13% of the total grape weight. This by-product, usually referred to as grape pomace (GP), is generated after destemming and pressing grapes and is composed of grape seeds and skins. The disposal of GP is costly and complicated due to characteristics of its composition, such as its high sugar content and low pH. If not properly treated, these characteristics pose a crucial environmental problem (Cheng *et al.* 2010).

Currently, GP has different nonfood applications: cattle feed (Özvural and Vural 2011), solid fuel for gas production, compost fertilizer, effective adsorbent of pollutant heavy metals and even for the production of high-added value materials (e.g. pullulan and laccase) (Arvanitoyannis *et al.* 2006). Because it is well known that GP is an interesting source of fiber and antioxidants with significant nutritional activities, some research has been performed toward using GP for food applications. For example, grape skin

flour (GSF) obtained from GP has been used in baked goods (Walker *et al.* 2014), corn breakfast cereal (Camire *et al.* 2007) and tomato puree (Lavelli *et al.* 2014) whereas grape seed flour has been added to bread (Hoye and Ross 2011), meat (Özvural and Vural 2011), cereal bars, pancakes and noodles (Rosales Soto *et al.* 2012), and minced fish muscle (Sánchez-Alonso *et al.* 2007).

GP antioxidants can be considered completely safe in comparison with synthetic antioxidants and include polyphenol components such as anthocyanins, flavanols, catechins and proanthocyanidins (Rosales Soto *et al.* 2012). These compounds have a high antioxidant activity, which gives them potential health-promoting and disease-protective effects (Choi *et al.* 2010; Hogan *et al.* 2010). For this reason, these compounds have recently been considered as food additives or novel ingredients that can introduce extra health benefits to various food products (Peng *et al.* 2010) and, at the same time, could be a solution for the waste disposal problem.

Yogurt is already considered to be a healthy food because it contains viable probiotic bacteria; however, it does not contain fiber and phenolic antioxidant compounds (Karaaslan *et al.* 2011). Available data on the GP addition into yogurt (Tseng and Zhao 2013) are encouraging regarding the feasibility of using GP as novel ingredient. The objective of this study was to investigate the influence, over 3 weeks of storage at 4C, of GP addition from different unfermented grape varieties (Chardonnay, Moscato and Pinot noir) on gross composition, phenolic and volatile compounds, antioxidant activity, lactic acid bacteria and consumer preferences of yogurt.

MATERIALS AND METHODS

Chemicals

n-Hexane, sulfuric acid, sodium hydroxide, ethanol, methanol, trifluoroacetic acid, 2-octanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu's phenol reagent, sodium carbonate, pyruvic acid, lactic acid, citric acid, acetic acid, propionic acid, butyric acid, tartaric acid, malic acid, glucose, lactose, fructose, gallic acid, protocatechuic acid, procyanidin B1 (PB1), 2,3,4-trihydroxybenzoic acid (THA), catechin, vanillic acid, epicatechin, rutin and quercitrin were purchased from Sigma-Aldrich (Milan, Italy). All chemicals were of reagent or HPLC grade level. Ultrapure water was produced with a Milli-Q System (Millipore, Milan, Italy).

GSF Preparation

Nonfermented GP of three *Vitis vinifera* varieties – Chardonnay, Moscato and Pinot noir – were provided from a winemaking factory (Fontanafredda, Alba, Italy). Skins were mechanically separated, stored at −20C until drying, dried in an oven UFE 550 model (Memmert, Schwabach, Germany) at 54C for 48 h and then ground with a Retsch ZM200 grinder (Retsch Gmbh, Germany) to obtain GSF with a particle size of less than 250 μm. GPF was sterilized in an autoclave at 121C for 15 min before use in yogurt production.

Yogurt Production

Yogurt was prepared using UHT whole milk (fat 36.0 g/kg, proteins 31.0 g/kg and carbohydrates 48.0 g/kg) purchased at the local market. Milk was put in a vat and milk powder 3% (w/w) was added. When the temperature reached 42C, milk was inoculated with starter culture YO-MIX 401 (Santamaria, Burago di Molgora, Italy), containing a mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (2:1).

The inoculated milk was fermented at 42C until a final pH of 4.8 was obtained (approximately 6.5 h). At this point, the sterile GPF was mixed with yogurt to reach a concentration of 60 g/kg and separated into pots. Samples were stored at 4C and analyses were performed immediately after production and at 1, 7, 14 and 21 days of storage. Two different yogurt productions were realized. Within each production yogurt was divided in four batches in which one without GSF (control) and three fortified yogurts (FY) namely Chardonnay, Moscato and Pinot noir.

Physicochemical Characteristics of GPF

The moisture content of the GSF was determined using a Eurotherm EUR thermo-balance (Gibertini, Milano, Italy) at 105C. Protein, fat and ash contents were determined according to AOAC official methods of analysis (Tseng and Zhao 2013). The carbohydrate content was estimated by difference. Dietary fiber (TDF, SDF and IDF) was measured using the Megazyme total dietary analysis kit (Lee *et al.* 1992). All analyses were performed in triplicate.

Physicochemical Characteristics of Yogurt

pH was measured with a Crison microph 2002 pH-meter (Crison Strumenti SpA, Carpi, Italy). Titratable acidity was determined via a potentiometric method (IDF 1991) and expressed as lactic acid per 100 g of yogurt. Yogurt syneresis was determined according to Celik *et al.* (2006), with some modifications. Yogurt (20 g) was centrifuged at 16,800× g for 20 min at 4C using a Megafuge 11 R centrifuge (Thermo Fischer Scientific, Waltham, MA). Syneresis was expressed as the volume of separated whey per 100 mL of yogurt (Wacher-Rodarte *et al.* 1993). Samples were analyzed in triplicate.

Extraction of Bioactive Compounds

The extraction was carried out according to McCue and Shetty (2005), with slight modifications. Briefly, each yogurt sample (10 g) was diluted with distilled water (2.5 mL) and centrifuged (16,800× g, 40 min, 4C). The supernatant was harvested and filtered through a 0.45 μm polypropylene membrane filter (VWR, Milan, Italy). Extraction was carried out in triplicate on different pots and extracts were stored at 4C until analysis.

Total Phenolic Content and Radical Scavenging Activity of Yogurt

The total phenolic content (TPC) was determined in triplicate using an assay modified from Apostolidis *et al.* (2007). Briefly, 1 mL of extract was transferred into a test tube and mixed with 1 mL of 95% ethanol and 5 mL of distilled water. To each sample, 500 μL of 50% v/v Folin–Ciocalteu reagent was added and the resulting sample was mixed. After 5 min, 1 mL of 5% Na_2CO_3 was added and the reaction mixture was allowed to stand in the dark at room temperature for 60 min. Just before the end of the incubation time, samples were centrifuged (16,800× g, 10 min, 20C) and the supernatant absorbance was read at 725 nm with a UV-vis spectrophotometer (UV-1700 PharmaSpec, Shimadzu, Milan, Italy). The absorbance values were converted to the total phenolics and were expressed as μg gallic acid equivalents per gram sample (μg GAE/g). Standard curves were established using various concentrations of gallic acid in water ($R^2 = 0.997$).

The radical scavenging activity (RSA) was determined using the DPPH$^{\cdot}$ assay modified by Gadow *et al.* (1997). A sample extract (75 μL or distilled water for the blank) was placed in a test tube, and 3 mL of a 6×10^{-5} M methanolic solution of DPPH$^{\cdot}$ was added. The decrease in absorbance at 515 nm was determined at the steady state (60 min of incubation at room temperature in the dark) after a previous centrifugation step. All determinations were performed in triplicate on different pots. The inhibition percentage (IP) of the DPPH$^{\cdot}$ by yogurt extracts was calculated according to the following:

$$IP = [(A_{0\,min} - A_{60\,min})/A_{0\,min}] \times 100$$

where A_{0min} is the absorbance of the blank at t = 0 min, and A_{60min} is the absorbance of samples at 60 min.

HPLC-DAD Analysis

Phenolic Compound Profiles. HPLC-DAD analysis of yogurt extract was performed using a Thermo-Finnigan Spectra System HPLC system (Thermo-Finnigan, Waltham, MA) equipped with a P2000 binary gradient pump system, a SCM 1000 degasser, an AS 100 automatic injector, a UV6000LP DAD and the ChromQuest software for data processing. Separation was achieved on a C_{18} RP Lichrosphere 250 × 4.6 mm, 5 μm (Merck, Milan, Italy) column, equipped with a C_{18} RP Lichrosphere guard column 5 μm (Merck). The mobile phase was composed of trifluoroacetic acid/ultrapure water (0.1:99.9, v/v) (A) and methanol (B). The flow rate was 1 mL/min and the injection volume was 20 μL. The elution program was as follows: initial conditions of 95% A, held for 2 min, 80% A over 8 min, 25% A over 57 min, 0% A over 13 min, 95% A over 5 min. DAD spectra were recorded in full scan modality over the wavelength range of 200–600 nm and at a discrete wavelength of 525 nm. Identification was achieved by comparing the retention times and spectra with those of authentic standards. Phenolic compounds were quantified using the following external standards: gallic acid (λ_{max} = 270, $R^2 = 0.9998$, LOD = 0.01 mg/L), PB1 (λ_{max} = 277, $R^2 = 0.9997$, LOD = 0.50 mg/L), (+)–catechin (λ_{max} = 280, $R^2 = 0.9995$, LOD = 1.00 mg/L), (−)-epicatechin (λ_{max} = 280, $R^2 = 0.9998$, LOD = 0.50 mg/L), rutin (λ_{max} = 356, $R^2 = 0.9998$, LOD = 0.06 mg/L) and quercitrin (λ_{max} = 350, $R^2 = 0.9999$, LOD = 0.09 mg/L). Protocatechuic acid, THA and vanillic acid were quantified using the gallic acid calibration curve. The precision, evaluated by calculating the RSD% of the retention time and the peak area for each analyte collected over a period of 3 weeks, was 1.90–7.89% for gallic acid, 1.82–10.54% for protocatechuic acid, 1.18–6.04% for PB1, 1.59–9.57% for THA, 1.32–15.74% for (+)–catechin, 0.29–10.65% for vanillic acid, 2.13–9.17% for (−)-epicatechin, 1.22–11.36% for rutin and 1.39–9.34% for quercitrin.

Sugar and Acid Determination

Ion exchange high-performance liquid chromatography was used to determine the organic acid and sugar contents. The method of Adhikari *et al.* (2002) was used with slight modification.

Yogurt samples (5 g) were added to 20 mL of 0.013 N H_2SO_4 (mobile phase) and mixed for 30 min with a horizontal shaker (PBI, Milano, Italy) at 100 oscillation/min. The slurry was subsequently centrifuged for 30 min at 5,000× g and 10C and the supernatant was filtered through a 0.45 μm polypropylene membrane filter (VWR).

The HPLC system (Thermo Quest, San Jose, CA) was equipped with an isocratic pump (P1000), a multiple autosampler (AS3000) fitted with a 20 μL loop, a UV detector (UV100) set to 210 and 290 nm, and a refractive index detector (Spectra System RI-150, Thermo Electro Corporation). The detectors were connected in series. Data were collected using ChromQuest ver. 3.0 (Thermo Finningan).

The analyses were performed isocratically at 0.8 mL/min and 65C with a 300 × 7.8 mm i.d. cation exchange column (Aminex HPX-87H) equipped with a cation H^+ microguard cartridge (Bio-Rad Laboratories, Hercules, CA). The mobile phase was 0.013 N H_2SO_4, which was prepared by diluting reagent-grade sulfuric acid with ultrapure water and degassing under vacuum. Identification was achieved by comparison with retention times of authentic standards. A total of eight organic acids and three sugars were investigated, including pyruvic acid, lactic acid, citric acid, acetic acid, propionic acid, butyric acid, tartaric acid, malic acid, glucose, lactose and fructose.

Analysis of Volatile Compounds

The volatile compounds in the yogurt samples were extracted using headspace solid phase micro-extraction and analyzed by gas chromatography/mass spectrometry (GC/MS). The analysis was carried out as described by Coda *et al.* (2011) with slight modifications. All samples were analyzed in triplicate. The analysis was conducted using a 20 mL vial filled with 1.5 g of sample to which was added 5 μL of 2-octanol in ultrapure water (92.8 mg/L) as an internal standard. After an equilibration time of 30 min at 37C, the extraction was performed using the same temperature for 40 min with a 50/30 μm DVB/CAR/PDMS fiber (Supelco, Milan, Italy) with stirring (250 rpm) before injection. The fiber was desorbed at 260 for 4 min in splitless mode. GC/MS analysis was performed with a Shimadzu GC-2010 gas chromatograph equipped with a Shimadzu QP-2010 Plus quadruple mass spectrometer (Shimadzu Corporation, Kyoto, Japan) and a DB-WAXETR capillary column (30 m × 0.25 mm, 0.25 μm film thickness, J&W Scientific Inc., Folsom, CA).

The carrier gas (He) flow rate was 1 mL/min. The temperature program began at 40C for 5 min, and then the temperature was increased at a rate of 10C/min to 80C and 5C/min to 240C for 5 min. The injection port temperature was 250C, the ion source temperature was 240C and the interface temperature was 230C. The detection was carried out by electron impact mass spectrometry in total ion current mode, using an ionization energy of 70 eV. The acquisition range was m/z 30–330. The identification of volatile compounds was confirmed by injection of pure standards and the comparison of their retention indices (a mixture of a homologous series of C_5–C_{28} was used), MS data reported in the literature and in the database (http://webbook.nist.gov/chemistry/). Compounds for which pure standards were not available were identified on the basis of mass spectra and retention indices available in the literature. Semi-quantitative data (μg/kg) were obtained by measuring the relative peak area of each identified compound in relation to that of the added internal standard.

Microbiological Analysis

For each yogurt type, sampling points were analyzed using traditional microbiological methods (colony-forming unit [cfu]). Streptococci were counted on M-17 agar (Oxoid, Milan, Italy) and lactobacilli were counted on Man Rogosa Shape agar (Oxoid). Both medium were incubated under microaerophilic conditions at 37C for 48 h.

Liking Tests

Because a previous acceptance test that was carried out on a small scale with a restricted panel (data not shown) indicated that better liking was found for the Moscato and Chardonnay yogurts, we chose to use only the white varieties for liking test.

To assess the sensory acceptability of yogurt samples, a central location test was conducted in Turin (Italy). The consumer test was performed at a stand for the University of Gastronomic Sciences during a public event named "European Researchers' Night." A total of 256 regular consumers of yogurt (48% males, 52% females, 18–86 years, mean age 24) voluntarily participated in the sensory evaluation. Written informed consent was obtained from each subject after the experiment was described to them.

The test consisted of a sensory evaluation of the FYs (Moscato and Chardonnay) and of the control sample. Yogurt samples (10 g) were served under blind conditions in opaque white plastic cups (38 mL) sealed with a clear plastic lid and coded with a random three-digit number. Samples were served in completely randomized order, with the control served as the last sample for all subjects to limit the contrast effect (Meilgaard *et al.* 2006). Consumers were asked to stir each sample with a plastic teaspoon, observe its appearance, smell and taste it and rate the yogurts for appearance, odor, taste, flavor, texture and overall acceptance. Liking was expressed on a 9-point hedonic scale ranging from "dislike extremely" (1) to "like extremely" (9) (Peryam and Pilgrim 1957). Purchase interest (*Would you buy this yogurt?*) was also rated on a 7-point scale (1 = absolutely no, 7 = absolutely yes). Participants were required to rinse their mouth with still water for about 1 min between samples. Consumers took between 15 and 20 min to complete the evaluation. Liking data (appearance, odor, taste, flavor, texture and overall acceptance) and declared purchase interest from consumers were independently submitted to a two-way analysis of variance (ANOVA) model, assuming sample and subject as main effects, by performing LSD ($P < 0.05$).

Data Analysis

A one-way ANOVA with Duncan's test for mean comparison was used to highlight significant differences among

TABLE 1. CHEMICAL COMPOSITION OF GRAPE SKIN FLOUR AND RESULTS OF ANALYSIS OF VARIANCE WITH DUNCAN'S TEST

Chemical parameters†	Moscato	Chardonnay	Pinot noir	Significance
Protein	93.5 ± 3.7^{b}	97.0 ± 0.3^{c}	88.3 ± 1.1^{a}	**
Fat	50.1 ± 1.6^{c}	41.0 ± 1.1^{b}	23.2 ± 1.1^{a}	***
Carbohydrates	271.4 ± 0.4^{a}	326.8 ± 1.6^{b}	501.2 ± 3.8^{c}	***
Moisture	57.9 ± 0.5^{c}	45.2 ± 1.1^{b}	20.8 ± 0.9^{a}	***
Ash	45.9 ± 0.6^{b}	63.9 ± 0.2^{c}	20.9 ± 0.7^{a}	***
IDF	390.9 ± 0.5^{c}	346.3 ± 3.9^{b}	285.0 ± 1.5^{a}	***
SDF	90.2 ± 1.7^{c}	81.5 ± 1.1^{b}	62.9 ± 0.5^{a}	***
TDF	481.0 ± 1.2^{c}	426.2 ± 0.12^{b}	345.5 ± 3.5^{a}	***

Notes: Different letters within a column are significantly different ($P < 0.05$).
** $P < 0.05$; *** $P < 0.01$.
† The results are reported as g/kg of dry weight and represented as means ± standard deviation.
IDF, insoluble dietary fiber; SDF, soluble dietary fiber; TDF, total dietary fiber.

samples. All calculations were performed using the STATISTICA for Windows statistical software (Release 7.0; StatSoft Inc., Tulsa, OK).

RESULTS AND DISCUSSION

Chemical Composition of GSF and Yogurts

Fat values were significantly different among varieties, with the lowest value for Pinot noir, probably due to more loss of grape seeds during preparation of the GSF (Table 1). Pinot noir showed also the lowest protein value (88.3 g/kg), whereas the highest was for Chardonnay at 97.0 g/kg. The highest values of soluble, insoluble and total dietary fiber were found in Moscato (90.2, 390.9 and 481.0 g/kg, respectively) followed by Chardonnay and Pinot noir.

Concerning FY, the lowest protein contents (Table 2) were observed in Pinot noir (208.4 g/kg) and Chardonnay (216.5 g/kg) yogurts, while the highest was found in Moscato yogurt (246.5 g/kg). Fat evaluation revealed that FY containing Pinot noir had a lower value than yogurt containing Moscato, with fat contents of 214.4 and 242.9 g/kg ($P < 0.05$), respectively.

Carbohydrate concentrations were significantly different between FY samples; they were higher in Pinot noir yogurt, followed by Chardonnay and Moscato yogurts. Moisture was significantly different between yogurts and the Moscato FY had the highest value, followed by yogurt containing Chardonnay and Pinot noir.

pH, Acidity and Syneresis of Yogurt

High significant differences ($P < 0.001$) were found for pH with respect to storage time and yogurt type, except on the 14th day (Table 3). The addition of GSF to yogurt instantly reduced the pH from 4.59 to 4.22–4.26, as previously reported by Tseng and Zhao (2013). The reduction in pH during storage corresponded to an increase in acidity (Tseng and Zhao 2013). The highest increase was found in Moscato yogurt (+17.9%), while the lowest observed was for Pinot noir yogurt (+11.4%).

FYs had higher values of syneresis compared with the control during storage due to the addition of GSF and statistically significant differences were found between yogurt types ($P < 0.001$, except on the first day) whereas no differences were found with respect to storage time ($P > 0.05$). The IDF present in GSF causes a rearrangement of the matrix gel, which was previously observed by García-Pérez *et al.* (2005) and Tseng and Zhao (2013). Chardonnay yogurt exhibited the highest value at each sampling time, while Pinot noir exhibited the lowest.

TPC and RSA of Yogurt

As expected, all FYs exhibited a high and statistically significant increase in the TPC compared with the control yogurt (about 38, 54 and 66% for Moscato, Chardonnay and Pinot noir, respectively) at each sampling time (Table 3).

TABLE 2. CHEMICAL COMPOSITION OF CONTROL AND FORTIFIED YOGURTS AND RESULTS OF ANALYSIS OF VARIANCE WITH DUNCAN'S TEST

Chemical parameters†	Moscato	Chardonnay	Pinot noir	Control	Significance
Protein	246.5 ± 9.4^{b}	216.5 ± 3.5^{a}	208.4 ± 4.8^{a}	260.4 ± 8.1^{c}	***
Fat	242.9 ± 3.8^{c}	236.5 ± 1.3^{b}	214.4 ± 2.8^{a}	311.3 ± 3.4^{d}	***
Carbohydrates	461.3 ± 1.6^{b}	488.2 ± 5.0^{c}	528.3 ± 5.5^{d}	365.9 ± 8.6^{a}	***
Moisture	839.1 ± 0.6^{c}	829.9 ± 0.2^{b}	827.1 ± 0.4^{a}	858.0 ± 1.2^{d}	***
Ash	57.0 ± 0.5^{ab}	58.0 ± 1.5^{b}	55.3 ± 1.1^{a}	61.8 ± 1.3^{c}	***

Notes: Different letters within a column are significantly different ($P < 0.05$).
*** $P < 0.01$.
†The results are reported as g/kg of dry weight and represented as means ± standard deviation.

TABLE 3. PHYSICOCHEMICAL PARAMETERS OF CONTROL AND FORTIFIED YOGURTS DURING STORAGE AND RESULTS OF ANALYSIS OF VARIANCE WITH DUNCAN'S TEST

Parameter†	Days	Control	Moscato	Chardonnay	Pinot noir	Significance
pH	0	4.59 ± 0.02^{cB}	4.22 ± 0.02^{eA}	4.26 ± 0.01^{eA}	4.24 ± 0.01^{cA}	***
	1	4.52 ± 0.13^{cB}	4.12 ± 0.01^{dA}	4.15 ± 0.01^{dA}	4.13 ± 0.01^{bA}	***
	7	4.30 ± 0.02^{bC}	4.07 ± 0.01^{cA}	4.09 ± 0.10^{cA}	4.12 ± 0.10^{bB}	***
	14	4.00 ± 0.04^{aB}	3.90 ± 0.02^{bA}	3.92 ± 0.01^{bA}	3.96 ± 0.02^{aAB}	**
	21	4.00 ± 0.01^{aD}	3.86 ± – ^{aA}	3.88 ± 0.01^{aB}	3.93 ± – ^{aC}	***
	Significance	***	***	***	***	
Acidity (lactic acid %)	0	0.72 ± 0.03^{aA}	0.90 ± – ^{aB}	0.89 ± 0.01^{aB}	0.92 ± 0.01^{aB}	***
	1	0.79 ± – ^{bA}	0.96 ± – ^{bC}	0.94 ± 0.01^{aB}	0.97 ± – ^{bC}	***
	7	0.89 ± – ^{cA}	1.00 ± 0.02^{cB}	1.01 ± 0.03^{bB}	1.00 ± 0.01^{cdB}	**
	14	0.99 ± 0.01^{d}	1.04 ± 0.01^{d}	1.06 ± 0.04^{b}	0.99 ± 0.01^{bc}	ns
	21	0.99 ± –^{dA}	1.07 ± 0.01^{eC}	1.04 ± 0.02^{bBC}	1.02 ± 0.01^{dB}	**
	Significance	***	***	**	**	
Syneresis (%)	0	32.73 ± 0.31^{A}	45.49 ± 0.39^{C}	49.60 ± 0.35^{D}	43.05 ± 0.28^{B}	***
	1	32.34 ± 0.91^{A}	46.92 ± 1.99^{C}	50.86 ± 2.21^{D}	42.87 ± 1.11^{B}	**
	7	33.38 ± 0.25^{A}	46.39 ± 0.58^{C}	48.33 ± 0.32^{D}	43.21 ± 0.63^{B}	***
	14	34.03 ± 0.35^{A}	46.03 ± 0.57^{C}	48.43 ± 1.27^{D}	43.34 ± 0.19^{B}	***
	21	32.82 ± 0.18^{A}	45.82 ± 0.33^{C}	48.15 ± 0.67^{D}	43.13 ± 0.42^{B}	***
	Significance	ns	ns	ns	ns	
TPC (μg GAE/g)	0	9.38 ± 0.04^{A}	12.88 ± 0.60^{Bab}	13.96 ± 0.66^{B}	15.83 ± 1.13^{C}	***
	1	9.17 ± 0.05^{A}	13.30 ± 0.42^{Bb}	14.43 ± 0.11^{C}	15.14 ± 0.10^{D}	***
	7	9.30 ± 0.13^{A}	12.23 ± 0.20^{Ba}	13.60 ± 0.73^{C}	15.09 ± 0.58^{D}	***
	14	9.40 ± 0.01^{A}	12.21 ± 0.07^{Ba}	13.94 ± 0.01^{C}	14.61 ± 0.08^{D}	***
	21	9.35 ± 0.17^{A}	12.94 ± 0.46^{Bab}	14.37 ± 0.46^{C}	15.25 ± 0.51^{D}	***
	Significance	ns	*	ns	ns	
RSA (–i%)	0	20.21 ± 3.31^{Ac}	23.35 ± 1.12^{A}	23.98 ± 1.64^{A}	30.79 ± 2.80^{B}	*
	1	13.37 ± 0.50^{Abc}	22.60 ± 1.99^{B}	25.29 ± 3.11^{BC}	29.04 ± 0.76^{C}	**
	7	12.31 ± 0.42^{Abc}	18.88 ± 4.29^{AB}	18.95 ± 4.68^{AB}	28.24 ± 1.76^{B}	*
	14	12.18 ± 0.55^{Aab}	17.62 ± 5.28^{A}	18.61 ± 1.80^{AB}	28.86 ± 1.18^{B}	*
	21	11.53 ± 0.61^{Aa}	18.97 ± 1.54^{B}	20.67 ± 1.11^{B}	25.31 ± 0.68^{C}	***
	Significance	*	ns	ns	ns	
Glucose (g/L)	0	1.53 ± 0.08^{cA}	4.94 ± 0.11^{B}	7.13 ± 0.01^{C}	10.13 ± 0.05^{aD}	***
	1	1.33 ± 0.12^{bcA}	5.20 ± 0.09^{B}	7.46 ± 0.27^{C}	10.62 ± 0.06^{cD}	***
	7	1.27 ± 0.11^{abA}	4.95 ± 0.09^{B}	7.21 ± 0.03^{C}	10.57 ± 0.01^{cD}	***
	14	1.07 ± 0.13^{aA}	4.78 ± 0.31^{B}	7.16 ± 0.20^{C}	10.57 ± 0.10^{cD}	***
	21	1.11 ± 0.13^{aA}	4.90 ± 0.23^{B}	7.25 ± – ^{C}	10.29 ± 0.13^{bD}	***
	Significance	**	ns	ns	***	
Lactose (g/L)	0	41.37 ± 0.47^{dB}	36.40 ± 0.14^{dA}	36.02 ± 0.10^{cA}	36.24 ± 0.13^{cA}	***
	1	41.15 ± 0.40^{dB}	35.42 ± 0.26^{cA}	35.30 ± 0.09^{cA}	36.18 ± 0.35^{cA}	***
	7	39.71 ± 0.10^{cD}	34.53 ± 0.14^{bC}	33.86 ± 0.09^{bA}	34.19 ± 0.24^{bB}	***
	14	37.52 ± 0.21^{bB}	33.66 ± 0.35^{aA}	32.41 ± 0.97^{aA}	33.47 ± 0.39^{aA}	**
	21	35.85 ± 0.66^{aB}	33.32 ± 0.19^{aA}	33.15 ± 0.04^{abA}	33.62 ± 0.18^{aB}	***
	Significance	***	***	***	***	
Fructose (g/L)	0	nd	7.32 ± 0.04^{aA}	8.70 ± 0.16^{aB}	12.36 ± 0.22^{aC}	***
	1	nd	7.75 ± 0.09^{bA}	9.32 ± 0.03^{bB}	13.13 ± 0.17^{bC}	***
	7	nd	7.91 ± 0.02^{bA}	9.51 ± 0.13^{bB}	13.26 ± 0.11^{bC}	***
	14	nd	7.92 ± 0.18^{bA}	9.47 ± 0.11^{bB}	13.20 ± 0.30^{bC}	***
	21	nd	8.24 ± 0.03^{cA}	9.85 ± 0.14^{cB}	13.23 ± 0.02^{bC}	***
	Significance	ns	***	***	***	
Pyruvic acid (g/L)	0	0.05 ± 0.01^{c}	0.04 ±	0.05 ±	0.04 ± 0.01	ns
	1	0.05 ± ^{c}	0.05 ±	0.04 ± 0.01	0.04 ± 0.01	ns
	7	0.04 ± ^{b}	0.05 ±	0.04 ± 0.01	0.05 ± 0.01	ns
	14	0.02 ± ^{aA}	0.04 ± ^{B}	0.04 ± 0.01^{B}	0.04 ± 0.01^{B}	**
	21	0.02 ± ^{aB}	0.04 ± ^{B}	0.04 ± 0.01^{B}	0.04 ± 0.01^{B}	*
	Significance	***	ns	ns	ns	
Lactic acid (g/L)	0	11.48 ± 0.10^{aD}	8.67 ± 0.02^{aC}	8.46 ± 0.06^{aB}	8.22 ± 0.02^{aA}	***
	1	11.70 ± 0.10^{aC}	9.29 ± 0.04^{bA}	9.49 ± 0.11^{bB}	9.18 ± 0.13^{bA}	***
	7	13.63 ± – ^{bD}	10.22 ± 0.08^{cB}	10.51 ± 0.01^{cC}	9.86 ± 0.08^{cA}	***
	14	14.50 ± 0.37^{cC}	10.55 ± 0.21^{dB}	10.55 ± 0.34^{cB}	9.80 ± 0.16^{cA}	***
	21	15.63 ± 0.24^{dD}	11.11 ± 0.01^{eB}	11.38 ± 0.02^{dC}	10.65 ± 0.09^{dA}	
	Significance	***	***	***	***	
Citric acid (g/L)	0	1.99 ± 0.10^{B}	1.76 ± 0.08^{A}	1.75 ± 0.09^{A}	1.74 ± 0.07^{A}	*
	1	1.97 ± 0.10^{B}	1.75 ± 0.07^{A}	1.78 ± 0.08^{A}	1.78 ± 0.09^{A}	*
	7	2.00 ± 0.09^{B}	1.76 ± 0.08^{A}	1.77 ± 0.08^{A}	1.74 ± 0.06^{A}	*
	14	1.89 ± 0.04^{B}	1.74 ± 0.05^{AB}	1.72 ± 0.12^{A}	1.71 ± 0.09^{A}	ns
	21	2.01 ± 0.06^{B}	1.75 ± 0.07^{A}	1.77 ± 0.08^{A}	1.76 ± 0.06^{A}	**
	Significance	ns	ns	ns	ns	

Table 3. *Continued*

Parameter†	Days	Control	Moscato	Chardonnay	Pinot noir	Significance
Tartaric acid (g/L)	0	nd	2.59 ± 0.01^{bC}	2.51 ± 0.04^{bB}	2.01 ± 0.02^{cdA}	***
	1	nd	2.25 ± 0.01^{a}	2.09 ± 0.24^{a}	2.05 ± 0.09^{d}	ns
	7	nd	2.68 ± 0.02^{bB}	2.79 ± 0.20^{bB}	1.89 ± 0.06^{bcA}	***
	14	nd	2.55 ± 0.20^{bB}	2.85 ± 0.03^{bC}	1.72 ± 0.10^{aA}	ns
	21	nd	2.74 ± 0.11^{bB}	2.67 ± 0.23^{bB}	1.77 ± 0.08^{abA}	***
	Significance		**	**	**	
Malic acid (g/L)	0	nd	$0.19 \pm {}^{bA}$	0.32 ± 0.01^{cB}	0.50 ± 0.01^{C}	***
	1	nd	$0.19 \pm {}^{bA}$	0.31 ± 0.01^{cB}	0.51 ± 0.02^{C}	***
	7	nd	0.16 ± 0.01^{aA}	0.28 ± 0.01^{bB}	0.49 ± 0.01^{C}	***
	14	nd	$0.15 \pm {}^{aA}$	0.28 ± 0.01^{bB}	0.48 ± 0.02^{C}	***
	21	nd	0.17 ± 0.02^{aA}	0.27 ± 0.01^{aB}	0.51 ± 0.03^{C}	***
	Significance	ns	**	***	ns	

Notes: Values in each column having different lowercase letters are significantly different at $P < 0.05$ within storage time. Values in each row having different capitals letters are significantly different at $P < 0.05$ within yogurt type.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns not significant.

† The results are represented as means ± standard deviation.

GAE, gallic acid equivalent; nd, not detected; where not specified, standard deviation are less than 0.01; ns, not significant; RSA, radical scavenging activity; TPC, total phenolic content.

The TPC was generally stable during storage for all samples and only Moscato yogurt showed statistically significant differences during the storage time ($P < 0.05$). The $DPPH^{\cdot}$ values indicated that all FYs had higher antiradical activity compared with the control. The RSA did not decrease significantly during storage for FYs, whereas it changed significantly in the control yogurt ($P < 0.05$). The RSA control value was lower on the 21st day of sampling than for day 0, with a reduction of 75%. Similar studies (Karaaslan *et al*. 2011; Tseng and Zhao 2013) stated that the RSA dropped during storage in yogurt containing 10% of red grape extract and yogurt containing 3, 2 and 1% of red wine GP. As expected, in our work, yogurt containing Pinot noir GSF exhibited the highest RSA during all storage times, whereas there was no statistically significant difference between yogurt containing Moscato and Chardonnay.

Sugar and Organic Acid Contents

The glucose values were higher in FYs compared with the control due to the addition of GSF (Table 3) and were very different at each time of sampling ($P < 0.001$). The control and FY containing Pinot noir were also significantly different during storage ($P < 0.01$ and $P < 0.001$, respectively). The glucose content dropped during storage in the control, with a reduction of 38% between 0 and 21 days of storage ($P < 0.01$). The glucose content of FY containing Pinot noir increased on the first day (10.62 g/L) and remained approximately the same until the 14th day (10.67 g/L), followed by a decrease at the last sampling time (10.29 g/L). This trend could be explained by the dissolution of glucose from GSF into yogurt. Changes in the glucose contents of Moscato and Chardonnay yogurts were not significant during the storage time ($P > 0.05$). As expected, the lactose content decreased during storage in all yogurts. Lactose content at the beginning of storage was approximately 36 g/L in FY, while at the end it was approximately 33 g/L. Fructose was observed in all FYs, and the highest content was found in Pinot noir yogurt, followed by Chardonnay and Moscato yogurts. As expected, the content of lactic acid increased during storage in all yogurts, and by a higher percentage in the control yogurt than in FY. As a consequence, large statistically significant differences were found at each sampling time among yogurt type ($P < 0.001$). Citric acid content was similar among FYs but slightly different from control yogurt ($P < 0.05$) and storage did not affect its content in the yogurts ($P > 0.05$). Malic and tartaric acids are the most important organic acids of grape and they were found in all FYs. FY containing Pinot noir exhibited the lowest content of tartaric acid during storage (1.72–2.05 g/L), while FY containing Moscato and Chardonnay showed similar values, except at 0 and 14th day of storage. During storage, highly significant differences were observed in the malic acid contents of Moscato and Chardonnay yogurts ($P < 0.001$), which exhibited a decreasing trend, while that of Pinot noir did not change during storage and had the highest values at each sampling time (0.48–0.51 g/L). The lowest values were found in Moscato yogurt (0.15–0.19 g/L). Butyric, propionic and acetic acids were not found in any yogurt.

Profiles of Phenolic Compounds

A total of nine compounds were identified and quantified: gallic acid, protocatechuic acid, PB1, THA, catechin, vanillic acid, epicatechin, rutin and quercitrin (Table 4). None of these phenolic compounds were detected in control yogurt.

TABLE 4. PHENOLIC COMPOUNDS OF CONTROL AND FORTIFIED YOGURTS DURING STORAGE AND RESULTS OF ANALYSIS OF VARIANCE WITH DUNCAN'S TEST

	Days	Moscato	Chardonnay	Pinot noir	Significance
Gallic acid†	0	3.6 ± 0.5^{B}	2.7 ± 0.2^{AB}	1.5 ± 0.1^{A}	*
	1	4.2 ± –^{C}	2.6 ± 0.2^{B}	1.7 ± 0.1^{A}	**
	7	3.9 ± 0.3^{C}	2.7 ± ^{B}	1.6 ± 0.2^{A}	**
	14	3.8 ± ^{C}	2.6 ± – ^{B}	1.6 ± 0.1^{A}	***
	21	4 ± 0.3^{C}	2.7 ± 0.2^{B}	1.7 ± 0.1^{A}	**
	Significance	ns	ns	ns	
Protocatechuic acid	0	1.1 ± 0.3	1.2 ±	0.7 ± 0.1	ns
	1	1.5 ± 0.1	1.2 ± 0.1	1.1 ± 0.4	ns
	7	1.2 ± 0.9	1.2 ± 0.1	0.8 ± 0.1	ns
	14	1.4 ± 0.2^{B}	1.2 ± 0.1^{AB}	0.8 ± ^{A}	*
	21	1.1 ± 0.2	1.1 ± 0.3	1.2 ± 0.1	ns
	Significance	ns	ns	ns	
Procyanidin B1	0	nd	nd	2.6 ± 0.1	–
	1	nd	nd	2.7 ± 0.1	–
	7	nd	nd	2.9 ± 0.4	–
	14	nd	nd	2.9 ±	–
	21	nd	nd	3.0 ± 0.2	–
	Significance	–	–	ns	
2,3,4-Trihydroxybenzoic acid	0	nd	nd	1.7 ± 0.1	–
	1	nd	nd	2.2 ± 0.1	–
	7	nd	nd	2.3 ± 0.3	–
	14	nd	nd	2.4 ± 0.1	–
	21	nd	nd	1.7 ± 0.6	–
	Significance	–	–	ns	
Catechin	0	17.9 ± 1.5	22.9 ± 3.4	5.1 ± 0.2	ns
	1	19.3 ± 0.1^{B}	18.8 ± 0.6^{B}	5.3 ± 0.6^{A}	**
	7	18.8 ± 0.1^{B}	18.1 ± 1.2^{B}	6.6 ± 3.1^{A}	***
	14	18.0 ± 0.1^{B}	19.0 ± 0.7^{B}	7.0 ± 0.3^{A}	***
	21	16.1 ± 1.7	17.2 ± 0.1	6.7 ± 0.3	ns
	Significance	ns	ns	ns	
Vanillic acid	0	nd	nd	3.5 ± 0.3	–
	1	nd	nd	3.4 ± 0.1	–
	7	nd	nd	3.1 ± 0.5	–
	14	nd	nd	2.9 ± 0.2	–
	21	nd	nd	3.3 ± 0.2	–
	Significance	–	–	ns	
Epicatechin	0	0.3 ±	0.4 ±	nd	–
	1	0.4 ±	0.3 ±	nd	–
	7	0.3 ±	0.3 ±	nd	–
	14	0.3 ±	0.3 ±	nd	–
	21	0.3 ±	0.3 ±	nd	–
	Significance	ns	ns	–	
Rutin	0	3.1 ± 0.1	3.7 ± 0.4	5.3 ± 1.0	ns
	1	3.9 ± ^{A}	3.4 ± 0.1^{B}	5.6 ± 0.1^{C}	***
	7	3.7 ± 0.7	3.4 ± 0.1	5.1 ± 1.0	ns
	14	4.0 ± 0.1^{B}	3.3 ± ^{A}	5.2 ± 0.1^{C}	***
	21	4.3 ± 0.3	4.1 ± 0.1	5.0 ± 0.4	ns
	Significance	ns	ns	ns	
Quercitrin	0	6.3 ± 0.6^{AB}	9.9 ± 1.2^{abB}	4.6 ± 1.0^{A}	*
	1	8.4 ± 0.6^{B}	8.9 ± 0.5^{abB}	4.9 ± 0.2^{A}	**
	7	7.7 ± 1.4	8.6 ± 0.5^{a}	4.5 ± 0.9	ns
	14	8.9 ± ^{B}	8.8 ± 0.1^{aB}	4.7 ± 0.1^{A}	***
	21	9.3 ± 2.3^{AB}	11.4 ± 0.4^{bB}	4.6 ± 0.5^{A}	*
	Significance	ns	*	ns	

Notes: Values in each column having different lowercase letters are significantly different at $P < 0.05$ within storage time. Values in each row having different capitals letters are significantly different at $P < 0.05$ within yogurt type.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns not significant.

† The results are reported as μg/g and represented as means ± standard deviation.

nd, not detected; where not specified, standard deviation are less than 0.1.

In yogurt containing Moscato and Chardonnay GSF, gallic acid, protocatechuic acid, catechin, epicatechin, rutin and quercitrin were detected, while all phenolic compounds except for epicatechin were detected in yogurt containing Pinot noir GSF.

Statistically significant differences were found between yogurt types with respect to gallic acid, while there were no statistically significant differences within each yogurt type during storage.

Moscato FY exhibited the highest gallic acid content (3.6–4.2 μg/g), followed by FY containing Chardonnay and Pinot noir. Protocatechuic acid was detected in all types of FYs, and its content did not change significantly during storage ($P > 0.05$). The only significant difference for protocatechuic acid content was found on the 14th day, in which reporting levels of protocatechuic acid decreased in the following order: Moscato > Chardonnay > Pinot noir. PB1 and THA were only detected in Pinot noir yogurt and their contents did not change during storage ($P > 0.05$). The PB1 content ranged from 26 to 30 mg/g. Catechin was the predominant polyphenol in all FYs, with the highest levels in Moscato yogurt on the first day (19.3 μg/g) and Chardonnay yogurt on day 0 (22.9 μg/g). Its content did not change significantly during storage ($P > 0.05$). On the first, seventh and 14th day of storage, statistically significant differences in catechin content were found between yogurt types. Yogurts containing Moscato and Chardonnay exhibited higher levels of catechin compared with yogurt containing Pinot noir. Epicatechin was present at similar levels in Moscato and Chardonnay yogurts. During the storage of these yogurts, the epicatechin content did not change significantly ($P > 0.05$). According to Karaaslan *et al.* (2011), the catechin concentration was higher than epicatechin in yogurt to which grape callus extract had been added (*Vitis vinifera* cv. Merlot).

Vanillic acid was exclusively detected in Pinot noir yogurt, in which its content did not change significantly during storage ($P > 0.05$).

Rutin was detected in all three FYs, with higher values in Pinot noir (first and 14th day) than in Chardonnay and Moscato yogurts ($P < 0.001$) and its content did not change significantly during the storage of the three yogurts ($P > 0.05$).

A higher content of quercitrin was found at day 21 in Chardonnay yogurt with respect to Moscato and Pinot noir yogurts ($P < 0.05$). At days 14 and 21, the Pinot noir yogurt was characterized by the lowest amount of quercitrin (4.7 and 4.6 μg/g, respectively). Quercitrin content did not change significantly during the storage, except for the Chardonnay yogurt for which a slight increase in the quercitrin level was observed at day 21. This could be due to an increase in compound solubilization into the yogurt, due to its ability to be extracted into water.

Analysis of Volatile Compounds

A total of 48 compounds were found in control and FYs, which corresponded to 10 ketones (2-pentanone, 2,3-pentanedione (diacetyl), 2-heptanone, acetoin, 6-methyl-5-hepten-2-one, 3-hydroxy-2-pentanone, 2-nonanone, 6-methyl-3,5-heptadien-2-one, 2-undecanone, 2-tridecanone), four aldehydes (nonanal, benzaldehyde, 4-methylbenzaldehyde, dodecanal), 12 alcohols (isobutanol, 1-pentanol, 3-methyl-1-butanol, 1-hexanol, 2-hexen-1-ol, 1-octen-3-ol, 1-octanol, 1-nonanol, benzyl alcohol, phenylethyl alcohol, 1,4-butanediol, 1-dodecanol), 11 acids (acetic acid, isobutyric acid, butanoic acid, methacrylic acid, pentanoic acid, hexanoic acid, 2-ethylhexanoic acid, heptanoic acid, octanoic acid, nonanoic acid, benzenecarboxylic acid), one ester (β-phenylethyl acetate), two lactones (γ-caprolactone, δ-decalactone), three furan derivatives (2-pentyl-furan, furfural, 2-furanmethanol), four terpenoids (limonene, *cis*-linalool oxide, linalool, α-terpineol) and one phenol (phenol) (Supporting Information Table S1). Table 5 displays the sums of all of the volatile compounds in each of these chemical classes. Carbonyl compounds, such as aldehydes and ketones, are the major volatile compounds responsible for the desirable flavor of yogurt (Cheng 2010). Their content is affected by the symbiotic relationship that occurs between *Streptococcus thermophilus* and *Lactobacillus bulgaricus* that are added as starter cultures (Routray and Mishra 2011). As reported in Table 5, ketones were the most abundant compounds observed, and their values increased significantly during storage in all three FYs ($P < 0.001$). Highly statistically significant differences ($P < 0.001$) were found between yogurt type at sampling days 0 and 21. On the 21st day of storage, the contents of ketones found in control and yogurt containing Pinot noir, 1,153.28 and 1,092.65 μg/kg, respectively, were lower compared with those found in white grape varieties. The ketone contents of yogurts containing Moscato and Chardonnay were not significantly different. The ketone content increased at a rate of 11% (control), 23% (Pinot), 47% (Moscato) and 55% in Chardonnay. Of the ketones, 2,3-pentanedione, 2-heptanone and acetoin were the most abundant (Supporting Information Table S1), and they play an important role in yogurt flavor, as reported by Routray and Mishra (2011). The most abundant aldehyde was benzaldehyde. Its content ranged (Supporting Information Table S1) from 2.63 (control at 14th day) to 15.89 μg/kg (Moscato at 14th day). Moreover, all FYs demonstrated higher amounts of these volatile compounds compared with the control. Sánchez-Palomo *et al.* (2005) studied the volatile compound contents of the pulp and skin of Muscat grapes, and reported that benzaldehyde was found in its skin. The same was found in Chardonnay

TABLE 5. VOLATILE COMPOUNDS OF CONTROL AND FORTIFIED YOGURTS DURING STORAGE AND RESULTS OF ANALYSIS OF VARIANCE WITH DUNCAN'S TEST

	Days	Control	Moscato	Chardonnay	Pinot noir	Significance
Σ Ketones†	0	1,030.51 ± 36.98^{bB}	927.48 ± 20.49^{aA}	914.32 ± 11.02^{bA}	887.81 ± 28.12^{aA}	***
	1	995.20 ± 38.40^{abC}	969.05 ± 14.49^{bBC}	899.04 ± 50.88^{bAB}	881.72 ± 54.39^{aA}	*
	7	1,017.23 ± 43.42^{ab}	857.83 ± 100.24^{a}	912.81 ± 61.76^{b}	978.09 ± 17.87^{b}	ns
	14	893.43 ± 75.08^{aB}	898.02 ± 12.06^{abB}	761.96 ± 56.57^{aA}	959.24 ± 5.74^{bB}	**
	21	1,153.28 ± 109.31^{cA}	1,367.59 ± 49.81^{cB}	1,425.31 ± 25.26^{cB}	1,092.65 ± 14.77^{cA}	***
	Significance	*	***	***	***	
Σ Aldehydes	0	9.87 ± 0.66^{cA}	18.43 ± 2.15^{abC}	23.69 ± 2.61^{cD}	14.92 ± 0.53^{B}	***
	1	5.60 ± 0.27^{aA}	17.51 ± 0.51^{aB}	23.28 ± 1.34^{cD}	19.94 ± 0.31^{C}	***
	7	5.91 ± 0.31^{aA}	20.75 ± 2.02^{bcB}	23.30 ± 0.57^{cB}	18.31 ± 6.74^{B}	**
	14	7.70 ± 1.50^{bA}	22.51 ± 0.47^{cD}	19.84 ± 1.91^{bC}	15.34 ± 0.79^{B}	***
	21	8.28 ± 1.02^{bcA}	16.95 ± 1.05^{aC}	15.59 ± 0.54^{aC}	13.23 ± 1.83^{B}	***
	Significance	***	*	***	ns	
Σ Alcohols	0	55.52 ± 1.00^{cA}	263.87 ± 34.02^{bC}	332.55 ± 22.25^{bD}	115.79 ± 3.42^{bcB}	***
	1	27.04 ± 6.05^{aA}	224.23 ± 5.97^{aB}	310.12 ± 9.11^{abD}	267.21 ± 3.68^{dC}	***
	7	38.51 ± 9.66^{bA}	336.39 ± 21.52^{cC}	311.27 ± 23.85^{abC}	98.54 ± 19.98^{abB}	***
	14	21.78 ± 5.56^{aA}	365.00 ± 8.29^{cD}	287.43 ± 25.08^{aC}	87.73 ± 6.64^{aB}	***
	21	49.05 ± 4.35^{bcA}	346.83 ± 9.32^{cC}	413.31 ± 18.42^{cD}	127.19 ± 15.58^{cB}	***
	Significance	***	***	***	***	
Σ Acids	0	71.01 ± 7.53^{aA}	231.68 ± 18.40^{aB}	284.10 ± 29.23^{bC}	210.70 ± 0.78^{aB}	***
	1	72.24 ± 13.01^{aA}	219.25 ± 20.83^{aC}	173.78 ± 14.20^{aB}	177.86 ± 8.45^{aB}	***
	7	115.16 ± 3.05^{bA}	288.38 ± 15.67^{bC}	210.47 ± 14.20^{aB}	276.20 ± 41.93^{bC}	***
	14	144.20 ± 8.09^{cA}	298.44 ± 42.52^{bB}	179.12 ± 9.39^{aA}	351.33 ± 9.24^{cC}	***
	21	134.60 ± 26.78^{bcA}	287.25 ± 2.29^{bC}	201.87 ± 31.62^{aB}	277.07 ± 8.54^{bC}	***
	Significance	***	**	***	***	
Esters	0	0.75 ± 0.12^{cB}	13.58 ± 0.82^{bD}	10.09 ± 0.20^{C}	0.56 ± 0.03^{bA}	***
	1	0.22 ± 0.01^{abA}	11.36 ± 0.15^{aC}	10.41 ± 0.26^{B}	0.48 ± ^{abA}	***
	7	0.18 ± 0.01^{abA}	17.62 ± 0.92^{dC}	11.06 ± 0.08^{B}	0.45 ± 0.09^{aA}	***
	14	0.13 ± 0.01^{aA}	21.52 ± 1.03^{eC}	10.33 ± 0.98^{B}	0.48 ± 0.03^{abA}	***
	21	0.29 ± 0.02^{bA}	15.62 ± 0.72^{cC}	12.32 ± 2.53^{B}	0.56 ± 0.02^{bA}	***
	Significance	***	***	ns	*	
Σ Lactones	0	1.17 ± 0.03^{A}	2.93 ± 0.17^{bcB}	4.09 ± 0.67^{C}	1.24 ± 0.08^{aA}	***
	1	1.18 ± 0.10^{A}	2.54 ± 0.35^{abB}	3.52 ± 0.19^{C}	1.09 ± 0.07^{aA}	***
	7	1.09 ± 0.15^{A}	3.34 ± 0.27^{cBC}	3.86 ± 0.13^{C}	2.42 ± 0.93^{bB}	***
	14	1.21 ± 0.29^{A}	3.81 ± 0.27^{dD}	3.16 ± 0.13^{C}	2.23 ± 0.10^{bB}	***
	21	1.13 ± 0.20^{A}	2.35 ± 0.05^{aB}	4.00 ± 1.06^{C}	1.02 ± 0.03^{aA}	***
	Significance	ns	***	ns	**	
Σ Furan derivatives	0	12.08 ± 0.76^{cA}	98.88 ± 10.06^{abC}	85.82 ± 3.84^{bB}	112.45 ± 5.18^{D}	***
	1	4.34 ± 0.08^{aA}	89.77 ± 6.05^{aBC}	97.77 ± 7.25^{cC}	84.25 ± 5.36^{B}	***
	7	4.22 ± 0.07^{aA}	110.08 ± 9.68^{bC}	85.91 ± 6.21^{bB}	96.61 ± 13.26^{BC}	***
	14	3.78 ± 0.19^{aA}	124.11 ± 2.28^{cD}	79.16 ± 4.10^{bB}	94.80 ± 0.28^{C}	***
	21	5.07 ± 0.33^{bA}	87.65 ± 5.55^{aC}	62.67 ± 4.80^{aB}	100.05 ± 15.02^{C}	***
	Significance	***	***	***	ns	
Σ Terpenoids	0	32.02 ± 2.55^{A}	49.79 ± 2.22^{aB}	66.85 ± 6.71^{cC}	31.03 ± 3.06^{A}	***
	1	32.87 ± 3.33^{A}	46.46 ± 3.46^{aB}	33.75 ± 2.32^{bA}	33.24 ± 2.09^{A}	***
	7	30.69 ± 2.82^{A}	51.73 ± 3.06^{aB}	27.35 ± 1.42^{abA}	44.89 ± 13.31^{B}	**
	14	25.42 ± 4.06^{A}	58.48 ± 2.48^{bC}	24.40 ± 2.21^{aA}	31.43 ± 1.61^{B}	***
	21	28.98 ± 0.64^{A}	64.91 ± 2.98^{cB}	31.10 ± 0.62^{bA}	28.97 ± 1.68^{A}	***
	Significance	ns	***	***	ns	

Note: Values in each column having different lowercase letters are significantly different at $P < 0.05$ within storage time. Values in each row having different capitals letters are significantly different at $P < 0.05$ within yogurt type.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

† The results are reported as μg/kg and represented as means ± standard deviation.

nd, not detected; where not specified, ns, not significant; standard deviation are less than 0.01.

grape skin and juice by Rosillo *et al.* (1999). We could confirm a major portion of the benzaldehyde content is due to the addition of GSF.

On the 21st day of storage, FYs containing Moscato and Chardonnay exhibited higher amounts of aldehydes compared with the Pinot noir and control yogurts.

The amount of alcohols increased during yogurt shelf life in FY, and their levels were higher in FYs compared with the control. Moscato and Chardonnay showed an average of ~300 μg/kg of alcohols during storage, which was higher compared with the alcohol content in Pinot noir yogurt (~140 μg/kg). In FYs containing Moscato, phenylethyl alcohol was the most abundant alcohol observed, and it ranged from 92.19 μg/kg (21st day) to 157.69 μg/kg (14th day). This alcohol was also the most abundant compound found in Moscato skin flour according to Sánchez-Palomo *et al.* (2005). The acid content within yogurt types and sampling time was always highly significantly different ($P < 0.001$), except for FY containing Moscato ($P < 0.01$). The total acids increased during storage (21st day > 0 day) in all yogurts except for Chardonnay. The percentage increase was 90% (control), 24% (Moscato) and 31% for Pinot noir. FY exhibited higher acid values compared with control yogurt during storage, which is due to the typical acidity of GSF and the microbial activity of starter microorganisms. On the 21st day of storage, FYs containing Moscato and Pinot noir exhibited the highest acid levels compared with yogurt containing Chardonnay.

Esters were represented by β-phenylethyl acetate, which was found in all FYs. The amount of this ester was higher in Moscato and Chardonnay (15.62 and 12.32 μg/kg, respectively), whereas less than 1 μg/kg was found in Pinot and control yogurts.

Lactones originate from lipolysis that occurs during yogurt fermentation, in which unsaturated fatty acids lead to the formation of 4- or 5-hydroxy acids that readily cyclise to γ- or δ-lactones (Cheng 2010). The trend of the total lactones in control and FY containing Chardonnay was not statistically significant during the storage time ($P > 0.05$). On the 21st day of storage, the highest total lactone content was found in yogurt containing Chardonnay (4.00 μg/kg), followed by yogurt containing Moscato (2.35 μg/kg).

The amount of furan derivatives in samples was significantly higher in FY ($P < 0.001$) compared with the control, probably due to the drying and sterilization process used to prepare GSF before yogurt production.

During all sampling times, the highest levels of terpenes were found in Moscato yogurt, which was expected because Moscato grape is an aromatic variety characterized by linalool, geraniol and nerol (Sánchez-Palomo *et al.* 2005). Varietal terpenoids such as limonene, *cis*-linalool oxide and α-terpineol increased in FY containing Moscato skin flour during storage ($P < 0.001$), probably due to release from aromatic grape skin, whereas they decreased in FY containing Chardonnay.

Microbiological Analyses

The addition of GSF to yogurt did not affect the survival of starter strains during storage conditions and both *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* survived the addition of flours in all FY. After 21 days, *S. thermophilus* reached a concentration very similar to the control in all three FYs (data not reported). The final concentration of *S. thermophilus* in control yogurt was 9.33 log cfu/mL, whereas for FY the average concentration was 9.20 log cfu/mL.

The same trend was recorded for *L. bulgaricus*, which, at the end, reached a lower concentration of approximately 7.8 log cfu/mL for all yogurt tested compared with *S. thermophilus*. This result was expected, as different amounts of starter were added to the product (*S. thermophilus : L. bulgaricus* ratio of 2:1).

Liking Test

The effect of fortification on overall consumer liking and purchase interest for yogurts is shown in Fig. 1. A significant difference was found in liking among samples based on appearance ($F = 22.74$; $P < 0.0001$), odor ($F = 42.80$; $P < 0.0001$), taste ($F = 125.46$; $P < 0.0001$), flavor ($F = 72.84$; $P < 0.0001$), texture ($F = 40.50$; $P < 0.0001$), overall liking ($F = 102.04$; $P < 0.0001$) and purchase interest ($F = 54.98$; $P < 0.0001$). The control sample was acceptable and exhibited the highest scores for its appearance, odor, taste, flavor and texture. In general, the results for the FYs distinguished them from each other. Both of them had a low liking score that never reached the central value of the scale (5 = neither like nor dislike). The Moscato yogurt was disliked more, with a very low mean liking score, especially for taste and flavor. In contrast, Chardonnay was the sample with the highest mean scores for appearance, flavor and overall liking. Considering the overall liking, Chardonnay yogurt was significantly better liked than Moscato yogurt. Thus, samples prepared with Chardonnay reported a generally higher hedonic performance than samples fortified with Moscato, suggesting a more suitable use in combination with yogurt. The results for purchase interest were highly correlated to overall liking ($r^2 = 0.9996$), which demonstrated the key role of liking on declared buying behavior. Sensory evaluation results suggested the need of further optimization of prototypes, indicating as Chardonnay GSF as most suitable for use in this application. In general, the observed low acceptability for FYs was not surprising because a decrease in liking due to fortification was expected. Indeed, the addition of bioactive compounds or plant-based phytonutrients can result in a change in the

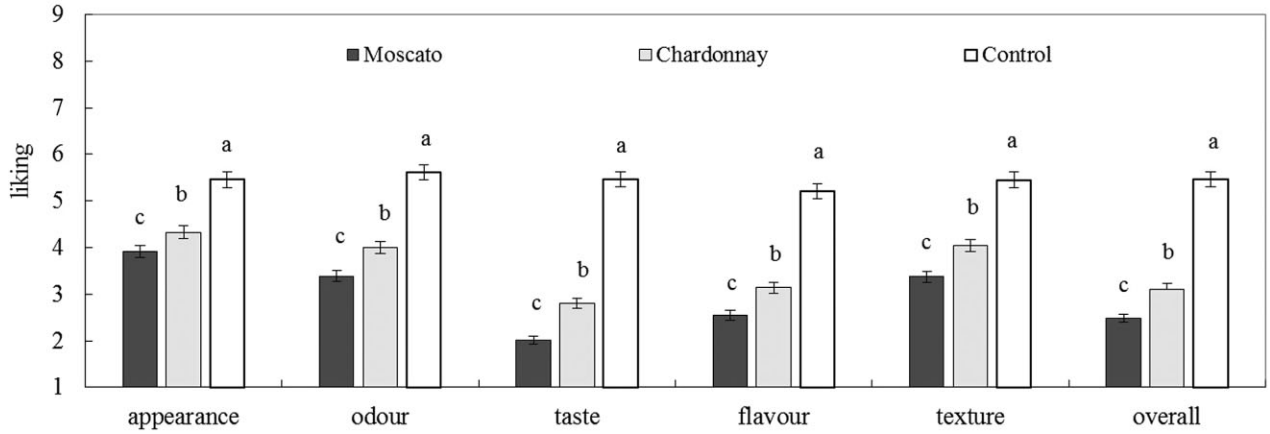

FIG. 1. LIKING OF APPEARANCE, ODOR, TASTE, FLAVOR, TEXTURE AND OVERALL LIKING EXPRESSED BY 256 CONSUMERS FOR THE CONTROL AND FORTIFIED YOGURTS

Means within a sensory modality with different letters are significantly different; Fisher's test, $P \leq 0.05$; error bars are standard deviations of means.

sensory quality of enriched foods, which can strongly affect the consumers' acceptance of such foods (Verbeke 2006). Verbal comments informally collected by participants after the end of the test indicated that the FYs were perceived as "too sour," "not enough sweet," with "unpleasant flavors," "not homogeneous" and "grainy/sandy." It is probable that the unpleasant texture was due to the perception of the GSF particles.

It should be taken into account that the mean overall liking score obtained for the control sample was just above the acceptability limit. Therefore, it can be hypothesized that the fortification of a more pleasant control yogurt could induce a similar decrease in the liking score, resulting in an overall liking above the acceptability limit (e.g. starting from an overall liking of eight, a decrease in two points of the liking score would result in a final overall liking equal to six, which would be higher than the acceptability limit).

In the future, it would be interesting to investigate the consumers' acceptance of the FY under informed conditions instead of in a blind test. Indeed, it has been demonstrated that information regarding the health benefits of GSF fortification can increase the consumers' acceptance of fortified products (Cheng *et al.* 2010).

CONCLUSIONS

The feasibility of using grape skin pomace as an ingredient in yogurt production was evaluated. The addition of GSF to yogurt resulted in a significant increase in the TPC and RSA with respect to control yogurt. The TPC and RSA values of FYs were retained during yogurt storage and no significant changes were observed. Regarding the differences found between grape cultivars, yogurt containing Pinot noir, a red cultivar, showed the highest TPC and RSA values. At the same time, phenolic compounds, which were only found in FY, were not influenced by storage. It is noteworthy that the addition of GSF did not affect the survival of starter strains during storage. The results obtained based on acceptance testing suggested that Pinot noir cannot be used for addition to yogurt due to the production of an undesirable aroma.

Results of the liking tests suggested that obtaining a higher preference by consumers will require decreasing the sour taste perception (using sweeteners or a different yogurt with a lower acidity) and improving the texture using GSF with a lower particle size.

The results obtained in this study demonstrated that GSF could be an alternative and safe source of antioxidants in the daily diet. Grape skin might be used in dairy applications, in particular for yogurt production, which could be a new way to use grape by-products.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Volatile compounds (mean ± standard deviation; μg/kg) of control and fortified yogurts during storage.