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Addition of fruit purees to enhance quality characteristics of sheep yogurt with selected strains

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ABSTRACT

The aim of this research was to assess the effects of fruit purees of banana, kiwi, mango, red berry, and strawberry on the microbiological, physicochemical, antioxidant, and sensory properties of sheep yogurt. The fruit purees were characterized for their microbiological profile before yogurt production, and no spoilage or pathogenic microorganisms were detected in any of the purees analyzed. Yogurt productions were carried out under industrial conditions using pasteurized sheep's milk and selected starter cultures of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. Five experimental yogurt productions (EYP) were made by adding 10 % (w/w) of each fruit puree, while the control yogurt production (CYP) was puree-free. Plate counts revealed that levels of viable *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* exceeded 8.0 log cfu/g in all CYP and EYP samples after 3 d of refrigerated storage. The addition of fruit purees reduced fat percentage until to 7 % and increased of antioxidant activity, especially with red berry puree. Except for banana, the addition of fruit purees resulted in a statistically significant increase ($p < 0.0001$) in the total terpene VOC profiles of EYP. Notably, the terpene content in mango-flavored yogurt was eightfold greater than that observed in the control trial. Sensory evaluation revealed a reduction in unpleasant odor and off-flavor, and an increase of about 50 % in overall acceptance for all EYP in comparison to CYP. Therefore, adding fruit purees to sheep yogurt is a promising strategy for the valorization of Sicilian sheep's milk.

1. Introduction

Yogurt is a fermented milk product that has been consumed worldwide for centuries due to its nutritional and health benefits [1]. According to a report by Market Research [2] the global yogurt market was valued at USD 113.5 billion in 2022 and is expected to reach USD 163.8 billion by 2028. The consumption of yogurt has steadily increased over time [3]. The production of this dairy product relies on the beneficial symbiotic relationship (protocooperation) between *Lactobacillus delbruekii* subsp. *bulgaricus* and *Streptococcus thermophilus* [4,5]. Yogurt-fermenting bacteria and their metabolites can help modulate the gut microbiota and improve immune system function [6]. For this reason, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) recommend a daily intake of yogurt [7].

Yogurt is primarily made from cow's milk in the western world, but it can also be made from milk of other animals such as sheep [8]. Sheep's milk boasts several functional properties that position it as a compelling alternative to cow's milk. Remarkably, it offers elevated levels of protein, fat, vitamins, and minerals [9]. The smaller size of fat globules in sheep's milk, compared to cow's milk, contributes to its enhanced digestibility [10]. Additionally, the abundance of conjugated linoleic acids (CLA) and bioactive peptides in sheep's milk plays a significant role in cancer prevention, cardiovascular health, and diabetes management [11]. Italy is one of the main sheep's milk producer countries in the European Mediterranean area [12]. Sheep farming is mainly concentrated in the inner hilly areas of Sicily and Sardinia. These areas are known for their rugged terrain and are ideal for sheep farming [13]. The majority of sheep's milk produced in these regions is used to make

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"Pecorino" cheese, which requires a long ripening period and is known for its strong and persistent aroma [14]. As a result, dairy producers have encouraged academic research institutes to develop new products that can be brought to market more quickly and satisfy the tastes of modern consumers. Among dairy products, yogurt can be marketed soon after production and does not require any ripening period. Currently, yogurt producers offer consumers an impressive variety of products, spanning diverse qualities and price ranges [15]. Among these, fruit-flavored yogurts reign supreme as the most popular choice [16]. In traditional bovine yogurt, fruit components such as fruit cubes, fruit flavors, fruit purees, and flavor extracts are commonly incorporated. The most favored fruit flavors in yogurt production include banana, berry, cherry, kiwi, lemon, lime, mango, passion fruit, peach, and strawberry [17–19]. These fruits are added to yogurt not only to enhance its flavor, but also to boost its functionality and health properties [20–22]. Regarding Sicilian sheep yogurts, while Garofalo et al. [23] have provided data on their production using commercial and selected starter cultures, literature lacks studies on their fortification with fruit purees. In fact, adding fruit purees to sheep yogurt could mask the typical strong aromatic flavor attribute that some consumers may not appreciate [24], while also improving further its nutritional value [25]. It is widely recognized that daily consumption of fruits and vegetables can reduce or prevent the risk of non-communicable diseases [26]. The health benefits of fruit are mainly linked to their antioxidant components, particularly phenolic compounds and flavonoids [27].

The purpose of this study was to create new types of sheep yogurt by adding commercial fruit purees and selected *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* starter cultures for the valorization of Sicilian sheep's milk. The specific research objectives were: (i) to evaluate the microbiological quality of the sheep's milk and fruit purees; (ii) to monitor the presence of *Lb. delbruekii* subsp. *bulgaricus* and *S. thermophilus* during yogurt productions; (iii) to assess the physicochemical properties, antioxidant activity, volatile organic compounds and sensory characteristics of the final yogurts.

2. Materials and methods

2.1. Raw material and natural milk starter culture preparation

To produce new types of sheep yogurt, five single commercial fruit purees were purchased from Les Vergers Boiron (Châteauneuf-sur-Isère, France). These purees consisted of 100 % pulp from banana [with a total soluble solid (TSS) of 23 °Brix, protein content of 1.1 %, and sugar content of 17.8 %], kiwi (TSS 13 °Brix, protein 0.6 %, sugars 7.2 %), mango (TSS 19 °Brix, protein 0.8 %, sugars 13.9 %), red berry (TSS 10 °Brix, protein 0.5 %, sugars 8.8 %), and strawberry (TSS 8 °Brix, protein 0.5 %, sugars 5.1 %). These specific fruit flavors were chosen from those commonly used in commercial flavored yogurt production [17–19]. The raw milk from sheep of "Valle del Belice" breed was provided by several artisanal dairy farms located within Agrigento province (Sicily, southern Italy). The strains *Lb. delbrueckii* subsp. *bulgaricus* WT601 and *S. thermophilus* PON244, which were previously selected for their dairy aptitudes [28,29] and ability to improve the antioxidant properties of white sheep yogurt [23], were used to prepare a Natural Milk Starter Culture (NMSC). Briefly, *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* were grown in de Man-Rogosa-Sharpe (MRS) (Biotec, Grosseto, Italy) and Medium 17 (M17) (Oxoid, Hampshire, UK) broths, respectively, for 48 h at 44 °C. After growth, the cells of each strain were washed in Ringer's solution (Oxoid) and individually inoculated at 1 % (v/v) into UHT sheep's milk (Leeb Vital, Wartberg a der Krems, Austria). These milks, after incubation at 44 °C for 24 h, represented the two NMSCs to be used as fermenting agents in yogurt production.

2.2. Yogurt production and sample collection

Yogurt production was carried out at a dairy pilot plant of the dairy factory "Cooperativa Agricola Tumarrano" located in Cammarata (Italy), during March 2022. The experimental design (Fig. 1) included a control yogurt production (CYP) and five experimental yogurt productions (EYP), one for each fruit puree. Briefly, 40 L of sheep's milk underwent conventional heat pasteurization at 72 °C for 15 s using a P75 50/2 pasteurizer (Tecnolat S. p.a., Nocera Inferiore, Italy), which did not include high-pressure milk homogenization. After pasteurization, the milk was transferred into the yogurt machine (Due Ci Inox s. r.l., Guastalla, Italy) previously sanitized with an alkaline chlorinated solution (Mersolat, Cercola, Italy). The milk was cooled at 42 °C and added with 400 mL of each NMSC under gentle agitation. The fermentation process took place for 6 h at 40 °C under static conditions, and it was stopped at pH around 4.5 [30]. For the CYP, the mixture was initially stirred and then packaged into 120 mL round plastic pots sealed with hermetic pressure caps (FD Store s. r.l., Vignola, Italy). In contrast, the EYP were enriched with 10 % (w/w) of each fruit puree, gently mixed for 2 min, and subsequently packaged.

All yogurts were kept under refrigerated condition (4 °C) for 3 d. Two independent yogurt productions were carried out at seven days of distance. Samples of fruit purees, pasteurized milk, inoculated milk with NMSC, and yogurts after three days of storage were collected for analysis.

2.3. Microbiological analyses

All samples collected along the production chain of CYP and EYP were serially diluted in Ringer's solution (Sigma-Aldrich, Milan, Italy). Cell suspensions of raw materials (fruit purees and pasteurized milk) were plated on: Skim Milk Agar (SMA) (Microbiol Diagnostici, Cagliari, Italy) incubated aerobically for 72 h at 30 °C for the enumeration of total mesophilic microorganisms (TMM); acidified MRS agar (Biotec) and M17 agar (Oxoid) incubated anaerobically for 48 h at 44 °C for total lactobacilli and streptococci, respectively; Hektoen Enteric Agar (HEA) (Microbiol Diagnostici) incubated aerobically at 37 °C for 24 h for *Escherichia coli* and *Salmonella* spp.; Baird Parker (BP) agar (Oxoid) incubated aerobically at 37 °C for 48 h for coagulase-positive staphylococci; *Listeria* Selective Agar Base (Oxoid) incubated aerobically at 37 °C for 24 h for *Listeria monocytogenes.* Inoculated milk and yogurt samples were analyzed only for TMM, *S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus.* The presence of the last two microorganisms was confirmed following the ISO 7889 [31] guidelines. Briefly, cell suspensions from the inoculated milk and yogurt samples were plated on: acidified MRS agar (Biotec) incubated anaerobically for 72 h at 37 °C for *Lb. delbrueckii* subsp. *bulgaricus* and M17 agar (Oxoid) incubated aerobically for 48 h at 37 °C for *S. thermophilus*. After growth, the colonies were collected, purified, tested for Gram reaction and catalase activity, and microscopically investigated [32]. Analyses were performed in duplicate.

2.4. Physicochemical and antioxidant analyses

The yogurt samples were subjected to the determination of colorimetric parameters by a Minolta Chroma Meter CR-300 (Minolta, Osaka, Japan), measuring lightness (L* = 0/100, from black to white), redness (a* = -a/+a, from green to red) and yellowness (b* = -b/+b, from blue to yellow), according to the CIE L*a*b* system [33]; these values (L*, a*, and b*) were used to calculate chroma $[(a \times {}^2 + b \times {}^2)^{0.5}]$, expressing the color intensity or saturation, hue angle $[\tan^{-1} (b^*/a^*)]$, as a measure of color tone, and the whiteness index $[100\text{-}((100\text{-}L^*)^2 + a \times {}^2 + b \times {}^2)^{0.5}]$, according to Vargas et al. [34].

All samples of yogurts were analyzed for pH by HI 9025 pH meter (Hanna Instruments, Ann Arbor, MI, USA), titratable acidity by Soxhlet-Henkel method (°SH/50 mL) and total soluble solids (TSS) with an optical Brix refractometer (Manual Refractometer MHRB-40 ATC, Mueller Optronic, Erfurt, Germany). Successively, the samples were first frozen at −20 °C and then lyophilized to be analyzed for dry matter (DM),

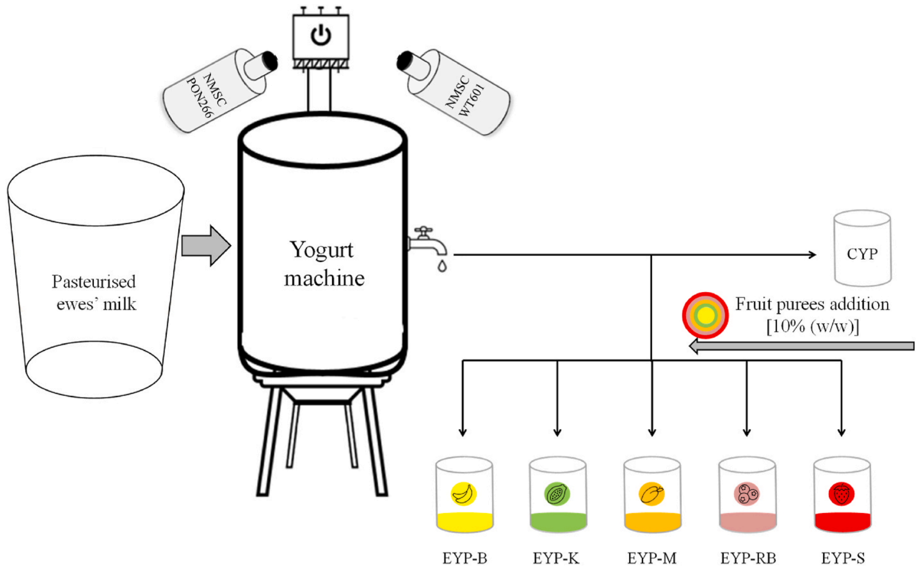

Fig. 1. Experimental design of yogurt productions. Abbreviations: NMSC PON244, natural milk starter culture produced with the starter *S. thermophilus* PON244; NMSC WT601, natural milk starter culture produced with the starter *Lb. delbrueckii* subsp. *bulgaricus* WT601; CYP, control yogurt production; EYP-B, experimental yogurt production enriched with banana puree; EYP-K, experimental yogurt production enriched with kiwi puree; EYP-M, experimental yogurt production enriched with mango puree; EYP-RB, experimental yogurt production enriched with red berry puree; EYP-S, experimental yogurt production enriched with strawberry puree. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

protein, fat and ash content, according to standard of the International Dairy Federation [35–38].

From the lyophilized samples, extracts were prepared according to the method of Rashidinejad et al. [39], with minor modifications, for the subsequent analysis to determine the antioxidant capacity. Briefly, 0.5 g of each sample was suspended in 25 mL of methanol aqueous solution (95 % v/v) containing 1 % HCl. The mixture was shaken by vortex for 30 s. Then the suspension remained in an ultrasonic bath (LBS1 Sonicator; Falc Instruments, Treviglio, Italy) at 40 °C for 30 min, during which it was shaken by vortex every 10 min for a few seconds. As soon as cold, the suspension was filtered with linen cloth and centrifuged at 7000 rpm/min for 10 min at 9 °C, then stored at −18 °C until analysis.

The yogurt extracts were analyzed in duplicate to determine the total polyphenols by the Folin–Ciocalteau colorimetric method, with gallic acid as standard, and the antioxidant capacity by TEAC assay using Trolox as standard, as described by Ponte et al. [40]; the respective results were expressed in gallic acid equivalent (GAE)/kg DM and mmol Trolox/kg DM. Regarding TEAC assay, the ABTS radical cation was obtained by reacting 14 mM ABTS aqueous solution with an equal volume of 4.9 mM persulfate of potassium and incubating the mixture in the dark for 16 h at 22 °C (room temperature). For the assay, the ABTS radical cation solution was diluted with 5 mM phosphate-buffered saline (PBS) at pH 7.4 to an absorbance of 0.795 (±0.02) at 734 nm. The absorbance of a mixture of 75 μL of PBS with 1425 μL of a diluted ABTS solution was read at 734 nm after incubation for 6 min at 30 °C. In the same way, 75 μL of each extracted sample were mixed with 1425 μL diluted ABTS radical cation solution, and after incubation at 30 °C for 6 min the absorbance read at 734 nm was used to calculate the percentage decrease of the absorbance due to decolorization in comparison with the absorbance read with PBS. Trolox solutions in PBS, between 0 and 2.5 mM, were used to develop a calibration curve ($R^2 = 0.99$).

The oxidative stability of yogurt fat was evaluated by determining the peroxide values (POV, meq O_2 kg/fat), as a primary lipid oxidation index [41], and the thiobarbituric acid reactive substances [TBARS, mg malondialdehyde (MDA)/kg DM] as secondary lipid oxidation index, performed according to the method proposed by Tarladgis et al. [42] and modified by Mele et al. [43], as described by Ponte et al. [40].

2.5. Volatile organic compounds determination

The volatile organic compounds (VOCs) analysis was conducted using headspace solid-phase microextraction (SPME) coupled with the Gas Chromatography-Mass Spectrometry (GC-MS) technique. Five grams of CYP and EYP samples were exposed to an SPME fiber (DVB/CAR/PDMS 50 mm, Supelco) for 15 min at 60 °C with continuous stirring. Following adsorption, the SPME fiber was thermally desorbed through a splitless GC injector at 250 °C for 1 min. Chromatographic separation was achieved using a DB-624 capillary column (Agilent Technologies, 60 m, 0.25 mm, 1.40 μm) with a carrier gas (helium) at 1 mL/min. The oven temperature program was initiated with a 5 min isotherm at 40 °C, followed by a linear increase of 5 °C per min up to 200 °C, and the final temperature was held at 200 °C for 2 min. The acquisition was performed under scanning (SCAN) conditions with the interface temperature set at 230 °C, and the acquisition mass range spanning from 40 to 400. Identification of VOC compounds was carried out by comparing the MS spectra with the NIST05 commercial library. The relative proportions of identified compounds were expressed as percentages, obtained by normalizing GC-MS peak areas with the total area of selected peaks. Three replicates of each sample were carried out.

2.6. Sensory evaluation

A descriptive panel of 15 judges (8 females and 7 males, aged between 27 and 63 years) evaluated the sensory traits of all yogurts. The evaluation was carried out in individual chambers using an iPad connected to the Smart Sensory Box software (Smart Sensory Solutions S. r. l., Sassari, Italy). The judges underwent training for evaluating yogurt attribute, following the guidelines outlined in ISO 8586 [44] indications. Approximately 35 mL of each yogurt were placed in white plastic glasses and served in a random order. The panelists were asked to score the following sensory attributes: odor intensity, unpleasant odor, color, homogeneity, viscosity, sweet, acid, bitter, taste persistency and overall acceptance. The intensity each attribute was quantified on a line scale ranging from 0 (low quality) to 9 (high quality) cm.

2.7. Statistical analyses

One-Way Variance Analysis (ANOVA) was used to analyze data for plate counts and total terpene compounds. The generalized linear model (GLM) procedure with the yogurt productions (6 levels) as unique factor was used to analyze physicochemical traits. Tukey's test was applied for pairwise comparisons at a significance level of $p < 0.05$. Heat map cluster analysis was used to identify the distribution of VOCs emitted from yogurts. Two-factor ANOVA, with panelists ($p = 1\ldots 15$) and yogurt ($y = 1\ldots 6$) as fixed factors, was applied to test the data on sensory evaluations. Statistical processing of plate counts, VOCs and sensory data, were carried out using XLStat software version 2020.3.1 for Excel (Addinsoft, New York, NY, USA), while the physicochemical data were analyzed using the SAS 9.2 software (SAS Institute Inc., Campus Drive Cary, NC, USA).

3. Results and discussion

3.1. Evolution of microbiological parameters during yogurt production

The microbiological evaluation of commercial fruit purees used in this study was performed to detect the presence of the main pathogenic and spoilage microorganisms. This assessment is mandatory before their use in food applications because, despite undergoing heat treatment, they are known to be vehicles for undesirable microorganisms [45]. The specific search for spoilage and pathogenic microorganisms did not reveal any colonies in any of the analyzed fruit purees (data not shown), indicating the hygienic suitability of these products for use in yogurt production. Fig. 2 reports a graphic representation of the microbiological plate counts performed on raw and pasteurized sheep's milk. Raw milk hosted consistent levels of TMM and streptococci (6.0 log cfu/mL), while lactobacilli were two log cycles lower. Raw milk used for traditional Sicilian cheese production commonly hosts consistent levels of these microorganisms [46]. The presence of high levels of TMM and LAB is mainly due to the milking practices and conservation conditions [47].

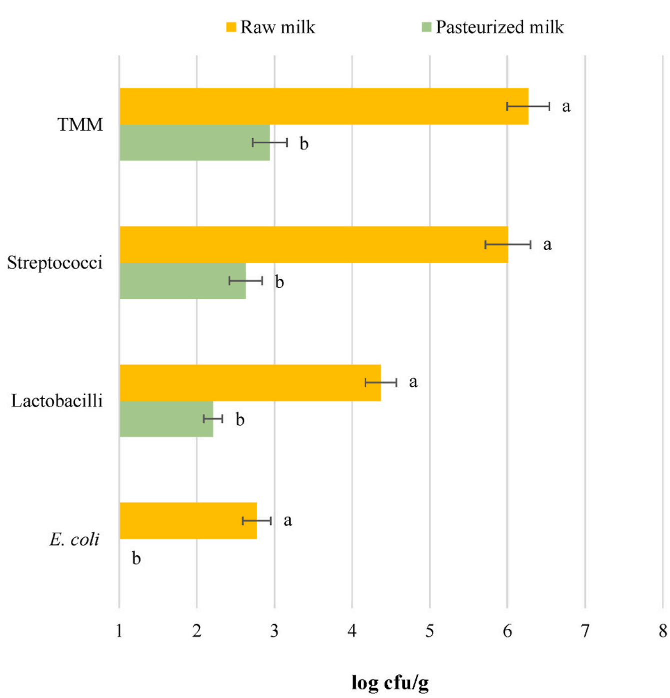

Fig. 2. Microbiological loads of sheep's milk samples before and after pasteurization. Units are log cfu/mL. Results indicate mean values ± S.D. of four plate counts (carried out in duplicate for two independent productions). Abbreviations: TMM, total mesophilic microorganisms; *E.*, *Escherichia*. a, b = $p < 0.05$.

Within the main dairy pathogenic bacteria [48], *L. monocytogenes*, CPS, and *Salmonella* spp., were undetectable (<1 log cfu/mL), while *E. coli* was around 3.0 log cfu/mL. *E. coli* is often detected in raw milk, and its presence is usually attributed to fecal contamination [49]. Following pasteurization, milk only contained TMM, streptococci, and lactobacilli at levels of 2.94, 2.63, and 2.21 log cfu/mL, respectively. Similar results were previously reported by Busetta et al. [50] and Barbaccia et al. [51] in pasteurized sheep's milk to be transformed into pressed and stretched cheeses, respectively. These results are not surprising since the pasteurization process does not have the ability to inhibit the growth of the thermoduric indigenous milk microbiota [52]. The growth of the selected starter cultures during yogurt productions is reported in Table 1. The analysis of milk after NMSCs addition showed levels of TMM, *Lb. delbrueckii* subsp. *bulgaricus*, and *S. thermophilus* above 7.0 log cfu/mL. It is widely recognized that starter cultures should be added at levels of 10^7 CFU/g of viable bacteria to promptly initiate the milk fermentation process [53]. No significant differences ($p > 0.05$) were found for the levels of TMM, *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* among control and experimental yogurt samples. In particular, TMM and *S. thermophilus* reached values of about 10^9 cfu/mL, while *Lb. delbrueckii* subsp. *bulgaricus* reached levels one order of magnitude lower. Other authors have reported this trend in yogurt produced with ovine and bovine milk [54,55]. These results indicated that the addition of 10 % (w/w) of each fruit puree did not have a negative impact on the survival of *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* used as fermenting agents in yogurt production.

3.2. Physicochemical and Antioxidant characterization of yogurts

The physicochemical properties of control yogurt and the five experimental yogurt productions with fruit puree are indicated in Table 2. As expected, the addition of fruit puree had a visible effect on the color traits of yogurt, such as lightness, red index and yellow index (respectively L*, a*, b*) shown in Table 2. In the research of Ścibisz et al. [56], yogurt with blueberry puree compared to that with strawberry puree showed lower lightness and yellow index values. By assimilating the blueberry puree to the red berry puree, this trial revealed similar results regarding the values of L* and b*, that are lower in yogurt with the addition of red berry puree in comparison with each other. Nevertheless, it is evident that the addition of fruit puree reduced the lightness values (L*) of all yogurts [57], especially those with the addition of red berry puree. Understandably, the same yogurt showed the significantly

Table 1
Levels of selected starter culture during yogurt production.

Samples	Bacterial counts		
	TMM	*Lb. delbrueckii* subsp. *bulgaricus*	*S. thermophilus*
Inoculated milk	7.37	7.09	7.23
CYP	9.21	8.15	8.93
EYP-B	9.33	8.02	8.97
EYP-K	9.01	7.93	8.85
EYP-M	9.25	8.09	8.94
EYP-RB	9.02	7.95	8.76
EYP-S	9.13	8.05	9.01
SEM	0.04	0.04	0.05
p value	0.310	0.777	0.652

Units are log cfu/mL for inoculated milk samples and log cfu/g for yogurt samples. Results indicate the mean values of four plate counts (carried out in duplicate for two independent productions). Abbreviations: TMM, total mesophilic microorganisms; *Lb.*, *Lactobacillus*; *S.*, *Streptococcus*; CYP, control yogurt production; EYP-B, experimental yogurt production enriched with banana puree; EYP-K, experimental yogurt production enriched with kiwi puree; EYP-M, experimental yogurt production enriched with mango puree; EYP-RB, experimental yogurt production enriched with red berry puree; EYP-S, experimental yogurt production enriched with strawberry puree; SEM, standard error of the mean.

Table 2
Physicochemical traits, antioxidant capacity and oxidation products of sheep yogurts.

	Samples						SEM	p *value*
	CYP	EYP-B	EYP-K	EYP-M	EYP-RB	EYP-S		
Color								
Lightness L*	94.85 a	93.20 a	93.26 a	89.49 b	57.923 d	82.97 c	0.552	<0.0001
Redness a*	−3.59 cd	−2.76 c	−3.98 d	−3.24 cd	24.77 a	4.52 b	0.186	<0.0001
Yellowness b*	8.49 b	7.48 b	9.11 b	35.61 a	−3.005 d	3.79 c	0.723	<0.0001
Chroma	95.07 a	93.35 a	93.48 a	92.99 a	57.964 c	83.02 b	1.10	<0.0001
Hue angle (°)	−66.98 c	−69.67 d	−66.43 c	−84.74 e	−6.94 b	39.88 a	0.566	<0.0001
Whiteness index	89.37 a	89.44 a	87.98 a	62.72 c	51.08 d	81.97 b	0.774	<0.0001
pH	4.38 a	4.25 c	4.10 d	4.31 b	3.96 e	4.11 d	0.009	<0.0001
Titratable acidity, °SH	1.20 d	1.25 c	1.32 b	1.18 e	1.40 a	1.30 b	0.006	<0.0001
TSS, °Brix	8.01 f	11.60 a	10.30 d	11.20 b	10.80 c	9.00 e	0.008	<0.0001
Dry matter (DM), %	17.09 d	21.51 b	22.01 a	17.16 d	16.26 e	21.03 c	0.058	<0.0001
Ash, % DM	4.58 a	4.31 b	3.69 c	4.63 a	4.35 b	3.57 d	0.021	<0.0001
Protein, % DM	33.04 a	30.30 c	30.29 c	31.68 b	30.48 c	30.71 c	0.160	<0.0001
Fat, % DM	34.62 a	27.68 d	28.65 c	27.75 d	31.22 b	28.83 c	0.116	<0.0001
Polyphenols, g GAE/kg DM	2.41 BCE	2.51 b	2.31 c	2.05 d	4.58 a	2.38 c	0.028	<0.0001
TEAC, mmol/kg DM	16.46 d	36.88 c	45.43 BCE	45.60 BCE	132.29 a	47.91 b	2.34	<0.0001
POV, mEq O_2/kg fat	0.432 d	1.19 b	0.964 c	0.952 c	1.32 a	0.269 e	0.008	<0.0001
TBARs, mg MDA/kg DM	0.074 e	0.140 c	0.118 d	0.106 d	1.75 a	0.186 b	0.003	<0.0001

Abbreviations: CYP, control yogurt production; EYP-B, experimental yogurt production enriched with banana puree; EYP-K, experimental yogurt production enriched with kiwi puree; EYP-M, experimental yogurt production enriched with mango puree; EYP-RB, experimental yogurt production enriched with red berry puree; EYP-S, experimental yogurt production enriched with strawberry puree; SEM, standard error of the mean; TSS, total soluble solid; GAE, gallic acid equivalent; TEAC, trolox equivalent antioxidant capacity; POV, peroxide value; TBARs, thiobarbituric acid–reactive substances; MDA, malonylaldehyde. On the row: a, b, c, d = $p < 0.05$.

highest and positive red index (a*), which was followed by that of strawberry yogurt, while less marked but statistically significant differences were recorded among the other yogurts. The yellow index (b*) was much higher for yogurt with the addition of mango puree, which was visibly yellow. With regard to whiteness index, yogurt with banana and kiwi purees were not different than control yogurt. Instead, the yogurt with mango, red berry and strawberry purees showed lower levels of whiteness index due to the more intense color of puree. The trend of chroma and hue angle, being influenced by a* and b* values, reflects those of the original red and yellow indices.

As expected, the addition of fruit purees was responsible of a reduction of both pH and titratable acidity, more markedly with red berry puree. The Brix values of the six yogurt samples were significantly different from each other; this diversity is certainly due to the variable water content of the fruits used in the productions, as emerged by their respective TSS level. In each experimental production, the addition of fruits significantly decreased the protein and fat content of yogurt in comparison with the control yogurt. A similar result also emerged in Amal et al. [58] in the comparison between yogurts without and with the addition of fruit puree. Among yogurts with fruit puree, that with mango puree had a significantly higher protein content (31.68 %), whereas, as emerged also in Concha-Meyer et al. [59], yogurts with strawberry puree and kiwi puree had almost similar protein levels, along with that with banana puree and berries. Fat content of yogurts, on the other hand, ranged from 27.68 % to 34.62 %. The consistent decrease in the fat content of the yogurts containing fruits puree, is evidently due to the low fat content in fruit puree [58]. As regards the white yogurt without the addition of fruit puree (CYP), its chemical composition was almost similar to that of sheep yogurt produced in the Garofalo et al. [23].

The total polyphenols content did not increase with the addition of fruit purees in the yogurt, with the only exception when the red berry puree was used. On the contrary, the values of antioxidant capacity (TEAC) of sheep yogurt greatly improved when it was enriched with each of fruit purees, and more markedly with the red berry puree by which the highest level was reached. Accordingly, Razola-Díaz et al. [60] observed that fruits such as blueberries and blackberries (commonly called berries) and strawberries have higher antioxidant capacity than other fruits; indeed, in their work, determining the antioxidant capacity of several smoothies, the authors found the best antioxidant capacity values for samples that contained red fruits in their ingredients. The same trend was observed by Müller et al. [61] and Nowicka et al. [62] who in smoothies with higher amounts of red fruits obtained a higher antioxidant capacity than in those composed of mango, banana, apple, and pear. This greater antioxidant activity can be explained by the major presence of tannins and anthocyanins in the red fruits, in line with the highest polyphenols content found in the red berry yogurt. Instead, the increase of TEAC value recorded in the other yogurts was not supported by a concomitant increase of polyphenols in comparison with the CYP, suggesting that other components have exerted antioxidant power, such as terpenes and vitamins [27], or that the phenolic compounds from fruits could have formed complexes with yogurt proteins, responsible for a reduction of the measured polyphenols amount [56].

Moreover, it can be noticed that the high antioxidant action of red berry was not able to protect the yogurts fat from oxidation, as emerged by the higher POV and TBARS values in comparison with the other yogurts. These results imply the possibility that the more intense red color could have interfered with the analytical determination. Nevertheless, in general both primary and secondary lipid oxidation recorded after 3 days from production were higher in yogurts with fruits than in CYP, although the increase was less consistent with mango, presumably due to its greatest richness in terpenes. In literature, there is no study that reports POV and TBARS values of sheep yogurt, therefore the higher values of lipid oxidation induced by the addition of fruit purees could be explained by a greater microbial activity, as hypothesized by Garofalo et al. [23]. However, on the basis of sensory evaluation (Table 3), the lipid oxidation apparently induced by the addition of fruits puree does not seem to have altered the yogurt taste or appearance.

3.3. *Volatile organic compounds emitted from yogurts*

The analysis of the volatile composition was conducted on both control and experimental yogurt productions to evaluate how the addition of fruit puree affects the volatile profile of yogurts in terms of sensory-active compounds. Fig. 3 reports the volatile profiles generated by the yogurt samples analyzed. The control yogurt emitted a total of 17 VOCs, which consisted of 7 ketones, 3 aldehydes, 3 acids, 2 alcohols, 1 ester, and 1 terpene. Ketones are prevalent components of yogurt and their formation occurs through both the β-oxidation of saturated fatty acids, followed by the decarboxylation of β-ketoacids in the metabolic pathway [14,63,64]. Diacetyl, acetone, and acetoin were the main

Table 3
Evaluation of the sensory traits of yogurts.

Attributes	Trial						SEM	*p* value	
	CYP	EYP-B	EYP-K	EYP-M	EYP-RB	EYP-S		Panelists	Yogurt
Odor intensity	5.33 c	5.89 b	5.96 b	6.22 ab	6.43 a	6.67 a	0.07	0.824	<0.0001
Unpleasant odor	2.22 a	1.09 BCE	1.41 b	1.15 BCE	1.04 BCE	0.77 c	0.07	0.995	<0.0001
Color	4.04 d	4.21 d	4.33 d	5.62 c	7.47 a	6.34 b	0.16	1.000	<0.0001
Homogeneity	6.13 a	5.83 ab	5.61 b	5.80 b	5.65 b	5.72 b	0.04	0.911	0.0001
Viscosity	4.90 b	6.34 a	6.41 a	6.37 a	6.19 a	6.23 a	0.07	1.000	<0.0001
Sweet	2.13 e	4.22 a	3.17 cd	2.80 d	3.25 c	3.73 b	0.09	0.997	<0.0001
Acid	5.20	5.12	5.36	5.24	5.49	5.32	0.05	0.437	0.223
Bitter	1.99 a	1.55 ab	1.47 b	1.33 b	1.61 ab	1.37 b	0.05	0.745	0.001
Taste persistency	6.16 a	4.92 b	5.21 b	5.04 b	5.31 b	5.12 b	0.07	0.996	<0.0001
Off-flavor	2.59 a	1.42 BCE	1.34 BCE	1.26 c	1.55 BCE	1.66 b	0.06	0.993	<0.0001
Overall acceptance	4.25 b	6.22 a	6.01 a	6.09 a	6.13 a	6.11 a	0.09	0.996	<0.0001

Results indicate mean value. Abbreviations: CYP, control yogurt production; EYP-B, experimental yogurt production enriched with banana puree; EYP-K, experimental yogurt production enriched with kiwi puree; EYP-M, experimental yogurt production enriched with mango puree; EYP-RB, experimental yogurt production enriched with red berry puree; EYP-S, experimental yogurt production enriched with strawberry puree; SEM, standard error of the mean. On the row: a, b, c, d = p < 0.05.

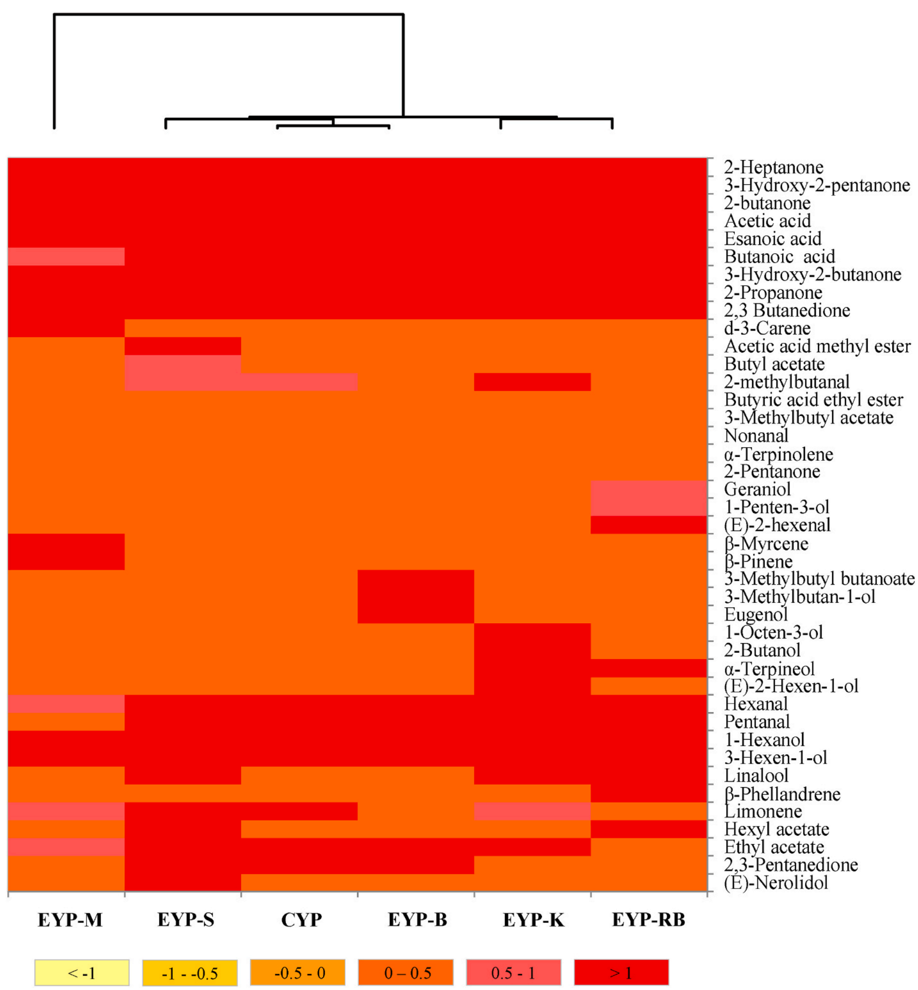

Fig. 3. Distribution of volatile organic compounds among yogurts. The heat map plot depicts the relative concentration of each VOC. Abbreviations: CYP, control yogurt production; EYP-B, experimental yogurt production enriched with banana puree; EYP-K, experimental yogurt production enriched with kiwi puree; EYP-M, experimental yogurt production enriched with mango puree; EYP-RB, experimental yogurt production enriched with red berry puree; EYP-S, experimental yogurt production enriched with strawberry puree. Specific sensory notes of volatile compounds identified in yogurt productions are reported in Table S1. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

ketones emitted by the control yogurt. Diacetyl, a direct product of glucose and citrate catabolism [65,66], is the predominant significant flavor compound among these C4 chemicals. Both *S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* are capable of producing diacetyl. Acetoin, the reduced form of diacetyl, is generated by the enzyme diacetyl reductase acting on diacetyl. Acetoine plays a crucial role in reducing the sharpness of diacetyl and contributes to the pleasant, creamy flavor of yogurt [67–70]. Additionally, acetone, originating from bacteria in milk and starter cultures, imparts a sweet fruity aroma and positively influences the flavor of yogurt [71]. Other ketones like 2-butanone, 2, 3-pentanedione, 3-hydroxy-butanone, and 2-heptanone were also detected. A similar ketone composition was also reported in goat yogurt [72]. The concentration of carboxylic acids was significantly higher than those of aldehydes, esters, and alcohols. Acetic acid was identified as the most abundant acid in CYP, followed by hexanoic acid and butanoic acid. Acetic acid results from the metabolism of lactose, citric and lactic acid [14] or derives from the catabolism of amino acids [73]. Hexanoic acid was generally found in goat's yogurt, reflecting the high amounts of short-chain fatty acids in goat's milk [74]. Overall, the carboxylic acids identified in this study, including hexanoic, butanoic, and acetic acids, contribute to the flavor of yogurt. Specifically, hexanoic acid is responsible for a sour note, butanoic acid imparts a cheesy flavor, and acetic acid is responsible for vinegar and acidic notes [14].

Compounds belonging to the classes of aldehydes (4-pentanal, hexanal, 2-methylbutanal), esters (ethyl acetate), alcohols (1-hexenol, 3-hexen-1-ol), and terpenes (limonene) have been identified in yogurt, but they constitute only a minor fraction of the total composition. Similar profiles have been reported in yogurt made from ovine milk [68, 72]. Compared to the control yogurt, the addition of fruits resulted in a decrease in the content of ketones and acids in experimental yogurt production. On the other hand, the addition of fruit purees in experimental yogurt productions particularly affected sensory-active compounds, increasing the overall content of the terpene class (Fig. 4) as observed in other studies on similar productions [25,75,76]. Specifically, the highest levels of terpene class were found in yogurt enriched with mango puree, followed in descending order by strawberry, red berry and kiwi. The consistent terpenes content registered in the yogurt with mango puree may contribute to the limited increase observed in its secondary lipid oxidation (Table 2). The absence of predominant VOCs in banana fruits [77–79] resulted in minimal aromatic influence on the final product, leading to no significant differences ($p > 0.05$) between banana-flavored yogurt and control production. Mango yogurt contained high levels of d-3-carene, which is characteristic of mango fruit and is the most abundant terpene found in the yogurt. Many studies reported values between 60 and 80 % of the total VOCs [80,81]. The increase in terpenes in yogurt enriched with strawberry puree is mainly due to the detection of nerolidol and linalool. According to literature findings, compounds such linalool, β-phellandrene, and α-terpineol are responsible for the increase in terpenes in red berry flavored yogurt [82]. These compounds are reported as the predominant chemical class in red berries [83]. Similarly, the increase in terpene compounds in kiwi-enriched yogurt is attributed to the detection of linalool, geraniol, and α-terpineol, which are present in kiwi fruit [84–86].

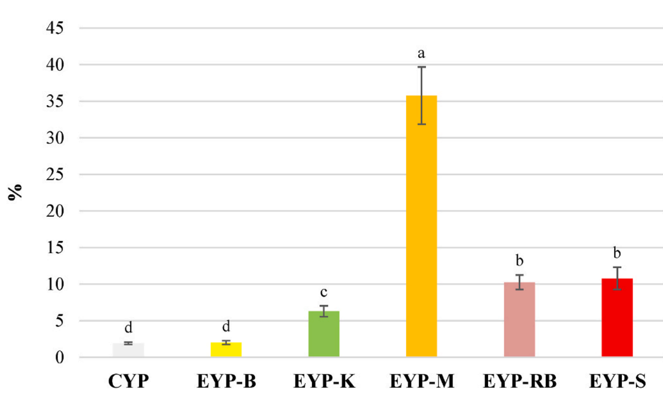

Fig. 4. Distribution of total terpene compounds among yogurts. Results indicate mean percentage values ± S.D. of four measurements (carried out in duplicate for two independent productions) and are expressed as relative peak areas x 100. Abbreviations: CYP, control yogurt production; EYP-B, experimental yogurt production enriched with banana puree; EYP-K, experimental yogurt production enriched with kiwi puree; EYP-M, experimental yogurt production enriched with mango puree; EYP-RB, experimental yogurt production enriched with red berry puree; EYP-S, experimental yogurt production enriched with strawberry puree. a, b, c, d = $p < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

The findings suggest that the aromatic profile is primarily influenced by the addition of fruit, as evidenced by the increase in terpenes and the detection of compounds absent in the control yogurt. However, despite the differences in physicochemical parameters observed between the control and experimental productions, such as the reduction in fat content due to the addition of fruits, it is not possible to determine if these variations influenced the final aromatic profile because the same production method was used for all samples. Therefore, further investigation is needed to evaluate the influence of production type (milk and starter cultures) on the final VOC profile.

In general, the presence of terpenes can positively impact consumer acceptability [87]. Terpenes significantly enhance aromatic perception [88], and numerous studies on fruits and vegetables have highlighted their role in enhancing flavor perception and consumer preference [89–93]. Furthermore, our results suggest that while there is an overall increase in terpenes, the specific terpene sensory-active compounds differ between the various production methods, which may influence sensory perceptions.

3.4. Sensory traits of yogurts

Both the control and experimental yogurts underwent sensory analysis to assess the impact of fruit puree addition on appearance, aroma, flavor, and texture attributes. This analysis is essential for evaluating consumer acceptance of new yogurts before their market launch [94]. Table 3 reports the results of the quantitative descriptive analysis of yogurts. Although it is well known that the aspect, aroma, taste, and texture of yogurt depend on the type and composition of milk, as well as the starter cultures used as fermenting agents [95], the addition of fruits and vegetables significantly impacts the sensory properties of this dairy product [96]. In this study, all sensory parameters except for acid perception attribute were scored differently among yogurts. Specifically, the addition of fruit purees, regardless of their taste, increased odor intensity, color, viscosity and sweetness, while reducing unpleasant odors, bitter, taste persistence and off-flavors. Sobti et al. [20] tested different commercial fruit purees to enrich camel yogurt and observed similar trends. In terms of color, the higher score given to the yogurt with mango puree is consistent with its intensely yellow index, whereas the scores for the red berry and strawberry puree correspond to their respective red color indices. The overall satisfaction, defined as the overall sensory acceptability of the food product evaluated [51], was higher for all yogurt productions enriched with fruit purees, regardless of their taste, compared to the control. Despite significant lipid oxidation in the experimental yogurts, there seems to be no negative impact on sensory properties.

4. Conclusion

This study delved into an extensive analysis of Sicilian sheep yogurts enriched with fruit purees at a concentration of 10 % (w/w). These novel yogurts were manufactured at the pilot plant scale using selected starters of *Lb. delbrueckii* subsp. *bulgaricus*. The addition of fruit purees did not affect hygiene and safety aspects of the final product. The two starter cultures (*Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*) drove the acidification process. The addition fruit purees decreased led to a 7 % reduction of fat content across all experimental yogurts. Antioxidant properties were enhanced, especially with red berry puree. The terpene class significantly impacted the VOC profiles of all yogurts, except for banana yogurt. The addition of fruit purees resulted in a reduction of the typical strong aromatic flavor associated with sheep yogurt.

These results suggest that these new types of sheep yogurt may offer health benefits and provide dairy producers with products that cater to the tastes of the post-modern consumer. Further studies will validate this manufacturing method at an industrial level and explore the beneficial effects of these novel yogurts on human health through gastrointestinal digestion and radical scavenging activities over time.

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CRediT authorship contribution statement

Giuliana Garofalo: Software, Investigation, Formal analysis, Data curation. **Raimondo Gaglio:** Writing – review & editing, Writing – original draft, Validation, Methodology, Conceptualization. **Gabriele Busetta:** Investigation, Formal analysis. **Marialetizia Ponte:** Software, Investigation, Formal analysis, Data curation. **Marcella Barbera:** Writing – original draft, Investigation, Formal analysis, Data curation. **Silvia Riggio:** Investigation, Formal analysis. **Daniela Piazzese:** Methodology. **Adriana Bonanno:** Writing – original draft, Methodology. **Hüseyin Erten:** Methodology. **Maria Teresa Sardina:** Resources, Project administration, Funding acquisition. **Luca Settanni:** Writing – review & editing.

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.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jafr.2024.101153.

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