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Development and quality evaluation of a fermented dessert made from donkey milk fortified with oat flour, wheat germ oil, and pomegranate extract

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ABSTRACT

The dairy sector is constantly innovating in response to the critical issues connected to sustainability and healthiness and to the increasing consumer demand for products with nutritional benefits. This study aimed to develop an innovative fermented dessert made from donkey milk (FDMD) added with oat flour (OF) and wheat germ oil (WGO). Different concentrations of OF and WGO (8, 10, and 12, and 3, 5, and 7 % w/w) were tested to optimize texture and, for this purpose, D-optimal mixture design was used. The best structural formulation obtained (11.5% w/w of OF and 3.5% w/w of WGO) was fortified with two further ingredients including dark chocolate 3% w/w, and pomegranate extract rich in ellagic acid (0.5 w/w, FDMD-A, and 1% w/w, FDMD-B) for improving flavor and nutritional properties. The quality and safety characteristics of the product during 30 days of storage were evaluated. The application of the Design of Experiment (DoE) proved successful in achieving the desired structural characteristics using 11.5% w/w OF and 3.5% w/w WGO. The addition of pomegranate extract did not negatively influence the textural and rheological characteristics of the samples, instead acting positively on the stability of the product during storage. Moreover, it had an impact on total polyphenol content and antioxidant activity, showing better results at 1% level of fortification (FDMD-B). The shelf-life study demonstrated that both formulations were microbiologically safe and stable and were appreciated by the panel involved in the sensory analysis. Finally, poor proteolysis took place during the 30-day storage period, indicating that the two samples did not significantly change over the 30 days of storage.

1. Introduction

Consumption of fermented dairy products has shown an increase in recent years, and market trends suggest that this phenomenon is likely to further increase (García-Burgos et al., 2020). Consumer interest derives from the nutritional and health benefits provided by these products. Indeed, a diet that includes fermented milk products containing probiotics and prebiotics enriches the gut with beneficial microflora, improves the body's defense mechanisms, increases resistance to external stimuli stressors, and reduces the likelihood of disease (Xiang et al., 2019). Notably, fermented dairy desserts have emerged as a compelling option due to their widespread global consumption and the burgeoning market with diverse new products and concepts (Morais et al., 2014). In recent years, considerable interest has been focused on donkey milk (DM) and its use as a potential functional ingredient, also in connection with the increase in donkey farms particularly in European countries such as Italy, Greece, and Cyprus (Faccia et al., 2018). According to European regulations that support local breeds and agricultural diversification, donkeys can prosper in marginal locations that are not suitable for highly specialized dairy cows (Bragaglio et al., 2024). Interest in the use of DM resulted from its potential to produce innovative and nutraceutical foods. The chemical composition of donkey milk reveals a good similarity with human milk, except for the fat content, which is lower in donkey milk. From a nutritional standpoint, there is growing interest in producing fermented products from donkey milk owing to its potential health benefits, including immunomodulatory activity, antioxidant, and detoxifying effects, modulation of intestinal microbiota, and the reduction of blood sugar and triglycerides (Martini et al., 2021). In particular, the high lactose content in donkey milk makes it an ideal substrate for proper development of intestinal lactobacilli, evidencing a prebiotic effect (Bhardwaj et al., 2020). Moreover, the high level of lysozyme exerts a selective effect on the microbiota (Yvon et al., 2019).

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In addition, DM is recommended in the feeding of pathological subjects, the elderly and children with gastrointestinal diseases, and subjects allergic to cow's milk proteins, due to the low casein/protein ratio of DM (Cunsolo et al., 2017; Martini et al., 2021). Given its nutritional value and health properties, DM stands out as an ideal ingredient for the production of a new generation of fermented milk desserts. Besides, combining cereals and milk provides an opportunity to increase the nutritional value of this type of food product. Consumption of oats has been linked to numerous health benefits, such as anti-inflammatory and antioxidant activity, potential in reducing the risk of cardiovascular disease, type 2 diabetes, gastrointestinal disorders, and cancer. Its bioactive constituents include phenolic compounds, a high level of functional proteins with valuable amino acids, polyunsaturated fatty acids, tocopherols, avenanthramides, and β-glucans (Martínez-Villaluenga & Peñas, 2017). In particular, β-glucans, besides being immunostimulant, prebiotic and dietary fiber, can exert a technological action when used in fermented dairy products. The addition of β-glucans from OF in yogurt and fermented milk can increase viscosity, reduce acidity, and prevent texture defects (Buniowska-olejnik et al., 2022; Qu et al., 2021; Vasquez-Orejarena et al., 2018).

Similar to DM, oats have been demonstrated to promote the growth of lactic acid bacteria (Wu et al., 2018). In addition, oats' supply of phenolic compounds and antioxidant capacity can improve the fermentation of a substrate when yeast or bacteria are used (Bei et al., 2020). Therefore, oats are becoming a popular matrix for innovative and functional foods (Angelov et al., 2018).

In the formulation of commercial desserts, a vegetable fat component is often part of the ingredient list. Vegetable oils include wheat germ oil (WGO), a vegetable fat obtained from germ of the wheat seed, a by-product generated by the milling industry. Compared to more commercially popular oils, such as olive oil and sunflower oil, WGO boasts high antioxidant power, due to the presence of high concentrations of α- and β-tocotrienol, and a higher presence of unsaturated fatty acids, such as linoleic, and polyunsaturated fatty acids (PUFAs) (Squeo et al., 2022; Vurro et al., 2022).

Pomegranate (Punica granatum) emerges as a significant source of bioactive compounds with historical use in folk medicine. Particularly, ellagic acid, followed by ellagitannins, are the main polyphenols identified in pomegranate. These substances offer a multitude of health benefits, including anticancer, antimicrobial, anti-inflammatory, antimutagenic, and antioxidant properties (Pirzadeh et al., 2021). The technological application results in antioxidant activity, contributing to reduced oxidation and limiting microbial growth, thereby increasing shelf-life (Difonzo et al., 2023). In the dairy industry, pomegranate extract has been applied in numerous products such as cheeses, creams, fermented milk, and yogurt, thus providing bioactive antioxidant and antimicrobial molecules that improved nutritional and technological features, as well as red fruit flavors to the products (Al-Hindi & Abd El Ghani, 2020; Alsubhi et al., 2022; Difonzo et al., 2023; Khademi et al., 2024).

Considering the expansion of donkey milk production, and its known functional properties, the scientific community is increasingly investigating on its application in the production of alternative dairy products. So far, several innovative dairy products have been developed, such as cheese, ice cream, kefir, yogurt, and fermented drinks (Faccia et al., 2018; Perna et al., 2019; Salgado et al., 2021; Tidona, 2017; Tidona et al., 2015). In this scenario, our study regarding the use of donkey milk to produce fermented milk dessert, combined with oat flour and wheat germ oil as further ingredients. The work had the following specific aims: i) optimizing the structural parameters by applying a D-optimal mixture design approach; ii) fortifying the optimized product by adding dark chocolate (flavoring ingredient) and pomegranate extract (ingredient for providing antioxidant activity); studying the chemical, sensory and safety characteristics of the product throughout a 30 days storage period.

Table 1
Formulations of fermented donkey milk dessert (FDMD) according to the Design of Experiment (DoE) (expressed as %).

Sample	Oat flour (A)	Wheat germ oil (B)	Other ingredients[1]
FDMD-1	8	7	85
FDMD-2	10	5	85
FDMD-3	12	3	85

The sum of the two variables in all samples was 15 %. The remaining 85 % consisted of other ingredients.
[1] Other ingredients (85 %): donkey milk 82.5 %; guar gum 0.5 %; sugar 2 %.

2. Materials and methods

2.1. Structural optimization of fermented dessert made from donkey milk (FDMD)

2.1.1. Ingredients, experimental domain, and design settings

The ingredients for the FDMD formulation were food grade and were chosen considering the possible benefits in the final product in terms of nutritional and structural characteristics. To formulate FDMD, a commercial product (oat dessert) with fitting structural characteristics, was chosen as the comparison sample. The commercial oat dessert (CD) had the following ingredients: water, oat (11.6%), cane sugar, tapioca starch, low-fat cocoa powder (3%), chocolate (1%), sunflower oil, safflower oil, sea salt, locust bean gum, gellan gum.

The technological scheme developed for FDMD was as follows: DM (azienda agricola Lamacarvotta, Laterza, Italy) was mixed with OF (Molino Rossetti S.P.A., Codevigo, Italy) and table sugar at room temperature. The mixture was stirred at room temperature at 800 rpm for a few minutes to promote solubilization by AREX heating magnetic stirrer (Velp Scientifica SRL). Successively, the mixture was stirred at 1300 rpm at 80 °C for 10 minutes with a heating magnetic stirrer and then cooled to 50 °C. Guar gum was added and the mixture was homogenized by using an Ultra-Turrax (model T-25, IKA-Werke GmbH & Co. KG, Staufen, Germany) at 24,000 rpm for 3 minutes. Once the temperature of 40 °C had been reached, two microbial starter cultures (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) were inoculated, and the resulting mixture was incubated at 40 °C until pH of 4.5–4.6, resulting in acid coagulation. Once the desired pH was obtained, WGO was added, emulsified by Ultra-Turrax at 24,000 rpm for 5 minutes and the final product was stored in hermetically sealed glass containers at 4 °C.

Once defined the basic production technology for FDMD, experimental trials were carried out for optimizing the process using a mixture design approach. Preliminary, different amounts of each ingredient were tested to establish the experimental domain in which to perform the experimentation. It was done by comparing the physical behavior of the mixtures with the characteristics of commercial control, in terms of physical stability and consistency. With this approach, it is possible to assess the influence of ingredients and rationally identify their optimum combinations (Squeo et al., 2021).

Based on the preliminary test results, the experimental domain was defined according to the following component constraints (%): OF ($8 \leq X_1 \leq 12$); WGO ($3 \leq X_2 \leq 7$). The sum of the components was 15%, while the remaining 85% consisted of the other ingredients, held constant. Subsequently, 3 experimental points (FDMD-1, FDMD-2, FDMD-3) within the domain were chosen, to these, three production replicates were added, one for each point, useful for estimating the pure error. The experimental design is shown in *Table 1*.

Two mathematical models were used to represent the relationship between factors and the different responses:

- *Linear model*: $y = b_1X_1 + b_2X_2$
- *Quadratic model*: $y = b_1X_1 + b_2X_2 + b_{12}X_1X_2$

Where y, X and b are respectively the response variables, the investigated components, and the coefficients of the model. The quality of the models was evaluated by means of the coefficient of determination (R^2), the predicted coefficient of determination (R^2_p), and by the analysis of variance (ANOVA) at a significant level of 0.05. The DoE was built and evaluated by Design-Expert 12 (Stat-Ease, Inc., Minneapolis, MN, USA).

2.1.2. Textural and rheological analysis

The back extrusion of the FDMD samples was carried out by a Z1.0 TN texture analyzer (Zwick Roell, Ulm, Germany) equipped with a compression disk of 40 mm diameter and a standard back extrusion container of 50 mm diameter, filled with 90 gs of sample. The analysis consisted in a double compression cycle at 1 mm/s until 50% of compression was achieved with 50 N load cell. Analyses were performed in triplicate and the samples had constant temperature at 5 °C. The rheological properties of the samples were measured using a HAAKE MARS iQ Air rheometer (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA), equipped with a flat plate geometry of 35 mm diameter. The system was maintained at 5 °C and the samples were applied to the bottom plate. After adjusting the headspace to 1 mm, measurements were conducted within the linear viscoelastic region, which was determined with the amplitude sweep test (with a constant frequency at 1 Hz and a variable strain from 0.05 to 100 per cent). The frequency sweep test was then performed to assess the microstructure and complex viscosity of the stressed samples. The frequency was changed logarithmically from 0.01 to 10 Hz, at a constant amplitude of 0.5 percent and a temperature of 5 °C. The modulus of elasticity (G'), modulus of viscosity (G'') and tan δ (G''/G') were determined.

2.2. Preparation and validation of the final product

2.2.1. Manufacturing process of FDMD

For the final product, the best formulation obtained from the application of DoE (11.5% w/w of OF and 3.5% w/w of WGO) was chosen as the starting point for the subsequent work. Therefore, the production same process described above was followed and two further ingredients including dark chocolate (3% w/w) and pomegranate extract with 40% ellagic acid (PE) (Farmalabor, Canosa di Puglia, Italy), were added during the last homogenization step of the WGO. The level of addition of these ingredients was rather low in order to not affect texture of the product. The two ingredients were added in FDMD formulation and homogenized for about 10 minutes. Specifically, dark chocolate was added to the formulation to enhance the flavor of the product, instead PE was added to improve the antioxidant properties (two different doses were tested). As to this latter point, the doses were 0.5% and 1% (w/w) of PE in FDMD-A and FDMD-B, respectively. The samples were stored under refrigerated conditions and analyzed at 0, 10, 20, and 30 days after production. The storage and analysis time was determined according to the shelf-life of commercially available products with similar characteristics.

2.2.2. Physicochemical characterization

Protein (total nitrogen × 6.38), ash, lipids, and total fiber content were determined using the AOAC methods 979.09, 923.03, 945.38, and 991.43 respectively. The carbohydrate content was determined as difference subtracting the total dietary fiber, protein, ash, moisture, and lipid contents from 100. Moisture was determined by a moisture analyzer (Mod. MAC 110/NP, Radwag Wagi Elektroniczne, Poland) at 105 °C, until a constant weight. The pH value was measured using a pH meter (HANNA instruments, Woonsocket, RI, USA). Water activity (a_w) was determined using Water Activity Meter Aqua Lab 4TE (Meter Group Inc., Pullman, WA, USA) following the manufacturers' instructions. The evolution of the protein fraction of the samples during storage was investigated by SDS-PAGE as reported by Harper et al. (1989). The identification of the bands was done by considering their molecular weight and by comparison with the literature (Faccia et al., 2018; D'Alessandro et al., 2019; Miao et al., 2020). For the Total Phenolic Content (TPC) and antioxidant activity assay (ABTS-TEAC and DPPH), the phenolic extraction from FDMD-A and FDMD-B was carried out following the procedure reported by Caponio et al. (2019) with some modifications. To remove lipid, an amount of 4 mL of *n*-hexane was added to 5 g of sample and 20 mL of methanol:water (80:20 v/v). After stirring with vortex at 11,000 rpm for 15 minutes, samples were sonicated (Ultrasonic cleaner CP104, EIA) for 15 minutes. Finally, the samples were centrifuged at 6000 rpm for 10 minutes at 5 °C (Thermo Fisher Scientific, Osterodeam Harz, Germany). The hydroalcoholic phase was collected, subjected to another centrifugation at the same speed and time. Then, the hydroalcoholic phases were filtered through a nylon filter (pore diameter 0.45 μm, Sigma, Ireland) within an amber vial. The TPC was determined according to the Folin-Ciocalteu method (Difonzo et al., 2021). The DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS-TEAC assay were performed following Tarantino et al. (2020) methods.

The extraction of *α*- and *β*-tocopherol and tocotrienols by FDMD samples was obtained according to Rotondo et al. (2021) method with some modification. Briefly, 2 g of lyophilized samples were mixed with 4 mL of *n*-hexane, and stirred for 3 minutes at 2000 rpm (Vortex Multi Reax, Heidolph, Sigma). Then 2 mL of potassium hydroxide methanolic solution (2 M) were added, the oxygen removed with nitrogen flow, the vessel capped, and stirred again with in same condition. After that, the final mixture was sonicated at 20 °C for 10 min and subsequently centrifuged for 10 minutes at 4000 rpm, in refrigerate condition (4 °C). The supernatant was isolated and the nitrogen flow was used to remove *n*-hexane. For the determination of tocopherols and tocotrienols HPLC-FD method was used, according to Vurro et al. (2022).

The CM-600d colorimeter (Konica Minolta, Tokyo, Japan) and SpectraMagic NX software were used for the color analysis. The sample was placed in a specific container for analysis. Brightness (L^*), red index (a^*), and yellow index (b^*) are considered in accordance with the International Commission on Illumination (CIE).

Back extrusion and rheological analysis were performed as described in paragraph 2.1.2.

According to Difonzo et al. (2018), solid-phase headspace microextraction (HS-SPME) was used in combination with gas chromatography/mass spectrometry (GC–MS) to assess the volatile chemicals in the samples. 100 μL of 1-propanol internal standard (Sigma-Aldrich, Milan, Italy), 0.5 g of sample, and 4 mL of NaCl aqueous solution (200 g/L) were weighed in 200 ml vials. The aluminum caps on the vials were sealed with a septum made of butyl rubber. An Agilent 6850 gas chromatograph equipped with an Agilent 5975 mass spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA) and an HP-Innowax polar capillary column (60 m length x 0.25 mm i.d. x 0.25 μm film thickness) were used to separate the volatile chemical. A 75 μm SPME fiber of carboxen/polydimethylsiloxane (CAR/PDMS) (Supelco, Bellefonte, PA, USA) was exposed to the volatile substances in the vial headspace for 50 minutes at 40 °C. After that, the fibers were desorbed for 6 minutes at 40 °C and then injected into the gas chromatograph's injection port, which was running in split-less mode for 3.5 minutes at 230 °C. Helium, the carrier gas, was used to separate the volatile chemicals at a flow rate of 1.5 mL/min at the injector temperature of 250 °C. After being maintained at 35 °C for five minutes, the oven's temperature was raised by 5 °C per minute until it reached 50 °C. After remaining at this temperature for five minutes, the temperature was increased to 210 °C at a pace of 5.5 °C per minute, and it was then held there for an additional five minutes. The mass detector was set up with the following parameters: source temperature of 230 °C, interface temperature of 230 °C, ionization energy of 70 eV, and scanning range of 33–260 Amu. By standardizing the peak regions of the compounds of interest with the internal standard, 1-propanol, volatile chemicals were measured.

Table 2
Textural and rheological parameter (modulus of elasticity (*G'*), modulus of viscosity (*G''*) and of tan δ at frequency 1 Hz), and analysis of predicted model equation of samples.

Response	CD	FDMD-1	FDMD-2	FDMD-3	Model	R^2	R^2_p	F-value	P-value
Firmness (N)	0.98 ± 0.01^{ab}	0.55 ± 0.02^{c}	0.72 ± 0.07^{bc}	1.24 ± 0.21^{a}	Quadratic	0.92	0.66	16.32	<0.05
Consistency (mJ)	12.13 ± 0.66^{a}	3.19 ± 0.09^{b}	4.85 ± 0.11^{b}	12.89 ± 1.28^{a}	Quadratic	0.99	0.97	236.80	<0.05
Cohesiveness (N)	-0.64 ± 0.03^{c}	-0.32 ± 0.04^{a}	-0.45 ± 0.04^{b}	-0.72 ± 0.05^{c}	Linear	0.94	0.87	68.16	<0.05
Viscosity (mJ)	7.06 ± 0.71^{a}	2.34 ± 0.30^{c}	5.39 ± 0.46^{b}	7.74 ± 0.47^{a}	Linear	0.99	0.97	339.59	<0.05
G'	152.40 ± 7.55^{ab}	92.89 ± 10.79^{c}	124.71 ± 12.61^{b}	166.61 ± 21.42^{a}	Linear	0.88	0.68	28.29	<0.05
G''	57.69 ± 2.87^{b}	45.98 ± 3.84^{bc}	58.60 ± 6.31A^{b}	79.19 ± 10.18^{a}	Linear	0.86	0.65	24.57	<0.05
tan δ	0.47 ± 0.03^{a}	0.49 ± 0.10^{a}	0.46 ± 0.01^{a}	0.46 ± 0.01^{a}	-	-	-	-	-

^{a,c}Values in the same row bearing different letters are different at $P < 0.05$. Values shown are mean ± SD.
Abbreviation: CD: commercial dessert; FDMD-1: fermented donkey milk dessert with 8 % oat flour and 7 % wheat germ oil; FDMD-2: fermented donkey milk dessert with 10 % oat flour and 5 % wheat germ oil; FDMD-3: fermented donkey milk dessert with 12 % oat flour and 3 % wheat germ oil.

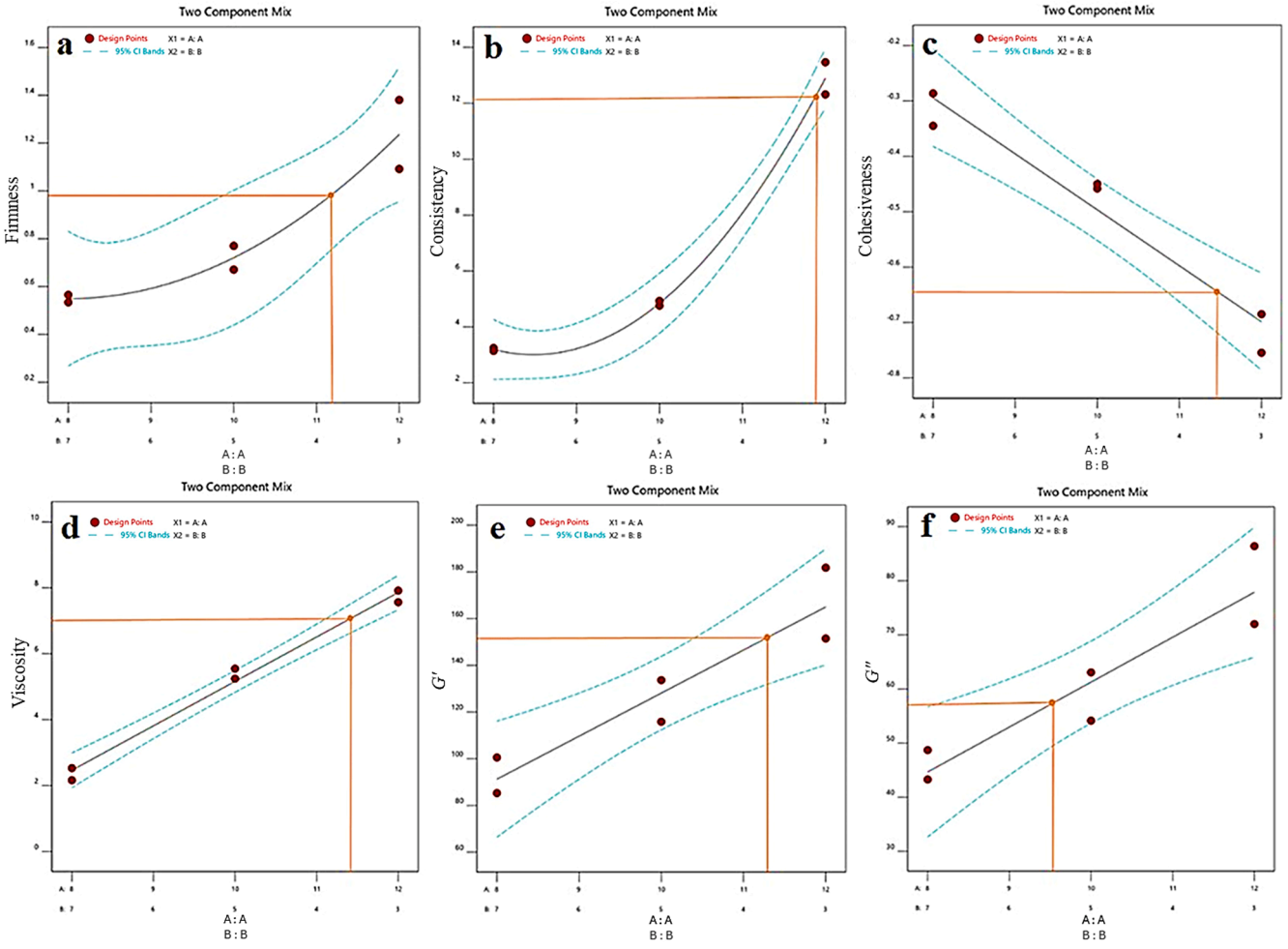

Fig. 1. Diagram of mixture design (**a**) firmness, (**b**) consistency, (**c**) cohesiveness, (**d**) viscosity, (**e**) elastic modulus (*G'*), and (**f**) viscous modulus (*G''*).

2.2.3. Sensory profiling for FDMD samples

Sensory analysis of FDMD samples was performed by a semi-trained sensory panel composed of ten assessors, following the ethical guidelines of the laboratory of Food Science and Technology of the Department of Soil, Plant and Food Science (DISSPA) of Bari University (Italy). A quantitative-descriptive analysis was done, in which assessors were asked to score the samples for specific odor, taste and texture attributes using a 5 points scale, ranging from 1 to 5. The attributes had been established throughout 3 training sessions of 2 h each, in which the assessors tasted different samples of fermented milk dessert: 3 appearance descriptors (glossiness, color uniformity, black spots), 4 olfactory and taste-olfactory sensations (sourness, cereal, chocolate, fruitiness), 3 flavor attributes (sweetness, acidity, astringency) and 3 texture descriptors (creaminess, jelly, texture uniformity) were defined.

2.2.4. Microbiological analysis

Microbiological analyses were performed in collaboration with an accredited external laboratory (EuroQuality Lab, Gioia del Colle, Italy). Counts of mesophilic lactic acid bacteria (ISO 15,214), *Escherichia coli* β-glucuronidase-positive (ISO 16,649–2), *Enterobacteriaceae* at 37 °C (ISO 21,528–2), *Listeria monocytogenes* (ISO 11,290–1, ISO 11,290–2) and *Salmonella* spp (ISO 6579) were performed.

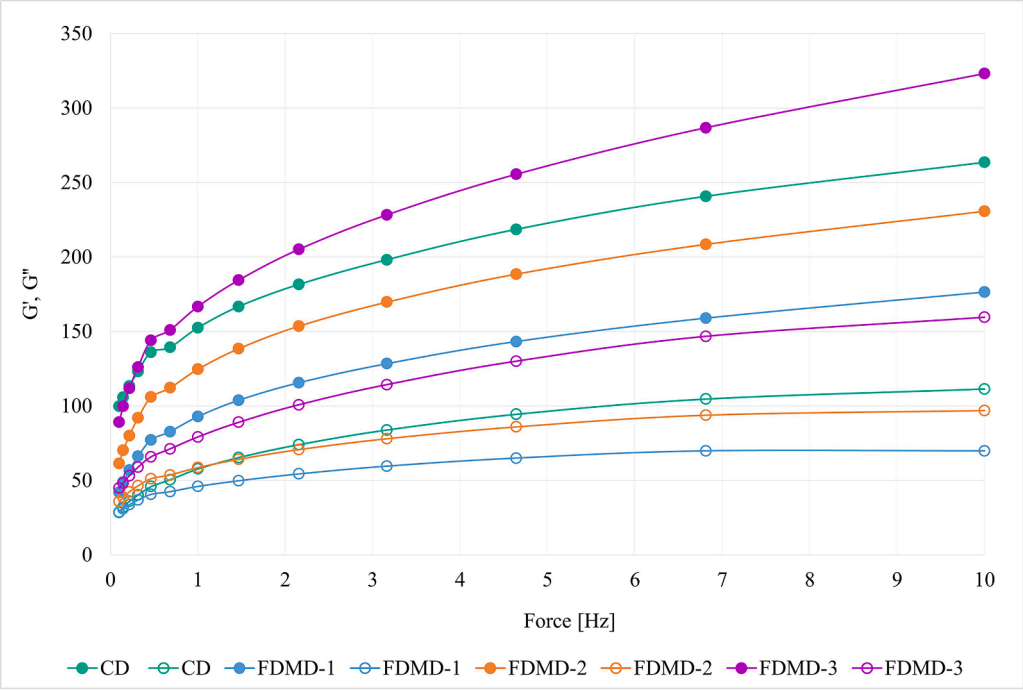

Fig. 2. Rheological properties of samples (FDMD-1: fermented donkey milk dessert with 8 % oat flour and 7 % wheat germ oil; FDMD-2: fermented donkey milk dessert with 10 % oat flour and 5 % wheat germ oil; FDMD-3: fermented donkey milk dessert with 12 % oat flour and 3 % wheat germ oil; CD: commercial oat dessert). Elastic modulus (G', filled symbol) and viscous modulus (G'', blank symbols).

2.3. Statistical analysis

Minitab19 (Minitab Inc., State College, PA, USA) was used for the statistical analysis of all results, reported as mean ± standard deviation (SD) of three replications. To evaluate the differences between samples, one-way and two-way ANOVA followed by Tukey's HSD test for multiple comparisons was applied.

3. Results and discussion

3.1. Structural optimization of FDMD

Table 2 shows the results obtained from the structural and rheological analyses, as well as the mathematical model selected to describe the behavior of each response together with the coefficients of determination and the results of the ANOVA, while Fig. 1 depicts the responses surfaces. The structural and rheological results of the CD are highlighted in orange in the graphs in Fig. 1. The structural characteristics of the experimental formulations were greatly influenced by the proportion of the studied ingredients and, from Fig. 1, it is easily possible to understand the effect on the product's characteristics as the concentrations of OF and WGO changed. In particular, considering the back extrusion results, a quadratic trend for firmness and consistency was observed (Fig. 1a and 1b). In contrast, a decreasing and increasing linear pattern was observed for cohesiveness and complex viscosity, respectively (Fig. 1c and 1d). The models were found significant according to ANOVA and the statistics were very satisfactory, with values of R^2 between 0.92 and 0.99. The R^2_p suffered for a consistent decrease in the case of firmness which, indeed, was likely related to the great experimental variability observed, in particular when the OF ratio increased (as it could be seen from the datapoints in Fig. 1a). The other responses presented higher values of R^2_p that could be considered as excellent.

The values of all textural parameters were affected by the increasing amount of A (oat flour). This result could be due to the fiber content of OF, including β-glucans, exerted a positive impact on the texture and complex viscosity of the samples. These properties could be explained by the interaction of oat β-glucans with milk caseins, potentially stabilizing the protein network created during the coagulation process. Alqahtani et al. (2021) found similar trends in texture analysis of a goat yogurt added with 1, 2, and 3% w/w of OF. Considering the results obtained from back extrusion analysis, the comparison between CD and FDMD-3 showed greater similarity.

As shown in Fig. 1e and Fig. 1f, the response surfaces showed a clear linear trend for both moduli (G' and G'') as the OF ratio increased. Thus, it is possible to deduce a direct proportionality of A (OF) and an inverse proportionality of B (WGO) with the rheological values. Also in this case the models were found to be significant according to ANOVA (Table 2). However, the responses presented a higher variability that is reflected in the lower values of R^2 and R^2_p, although these still could be considered acceptable and suitable to describe the responses behavior.

The results for the entire frequency range (0.1–10 Hz) of the rheological analysis are reported in Fig. 2. In all samples a value of G' greater than G'' was shown, thus indicating a gel-like behavior of the samples (G' > G''), i.e., a greater contribution of elastic properties and a solid-elastic nature of the samples. This trend in G' and G'' values is recognized as typical of weak gel structure (Nunes et al., 2006) and can be attributed to a lack of constituents, e.g., gluten, that are capable of creating complex and stable binding structures. Similar results were reported by Mohammadi et al. (2022) and Zarzycki et al. (2019) who obtained whole and skim milk desserts with rice bran protein and OF, respectively. Moreover, when the concentration of OF increased, and the amount of WGO decreased, the elasticity and complex viscosity moduli increased. The highest values of G' and G'' were shown in the FDMD-3 formulation. In particular, the increase in G' as the amount of OF increases can be related to the increase in gel strength, which makes the product harder and more consistent, as confirmed by texture analysis (Piska & Štětina, 2004). By analyzing the G', G'' and tan δ values at the reference frequency of 1 Hz, it is possible to observe that there was a greater elastic contribution than viscous contribution. Indeed, the values of tan δ for all samples are less than 1 (Table 2).

In comparison to CD, the viscolestic behavior exhibited a trend similar to the experimental formulations. Specifically, at the frequency

Table 3
Proximate composition (%) of samples.

Parameters	FDMD-A	FDMD-B
Kcal	157.57[a]	163.54[a]
Moisture	68.58 ± 0.35[a]	66.99 ± 0.07[b]
Fat	8.10 ± 0.03[a]	8.07 ± 0.34[a]
Protein	3.87 ± 0.13[a]	3.34 ± 0.06[a]
Carbohydrate	17.15 ± 0.08[b]	18.98 ± 0.12[a]
Fiber	0.38 ± 0.01[a]	0.41 ± 0.02[a]
Ash	1.97 ± 0.06[a]	2.19 ± 0.11[a]

[a,b]Values in the same row bearing different letters are different at $P < 0.05$. Values shown are mean ± SD.
Abbreviation: FDMD-A: fermented donkey milk dessert with 0.5 % (w/w) pomegranate extract; FDMD-B: fermented donkey milk dessert with 1 % (w/w) pomegranate extract.

of 1 Hz, CD presented a G' with values similar to those of sample FDMD-3. In contrast, the G'' most similar to the commercial product was that of formulation FDMD-2.

Overall, considering the great importance of firmness (Fig. 1a) and elasticity (Fig 1e) for this kind of product, a close inspection of the response surfaces suggested that values between 11 and 12 gs of OF and between 3 and 4 gs of WGO were suitable to reach characteristics similar to CD. Thus, the final formulation was as follows: 11.5% of OF and 3.5% of WGO.

3.2. Finalization of formulation and safety and quality evaluation during storage

3.2.1. Physicochemical characterization

The proximate composition shown in Table 3 evidenced a strong similarity between samples FDMD-A and FDMD-B. Indeed, no differences were shown between the two samples, exception of moisture, which was lower in FDMD-B (66.9%), and a less concentration of carbohydrates in FDMD-A (17.15%). The results are in agreement with Frühbauerová et al. (2020) who added increasing percentages of grape skin extract (1 and 2% w/w) in a dairy product spread. Thus, a higher amount of extract in the product formulation results in an increase in dry matter content with a consequent reduction in moisture.

Analyzing the pH trend over time (Table 4), a decrease in pH up to day 20 and subsequent stabilization up to day 30 was observed. The decrease in pH during the storage period can be attributed to the progressive conversion of lactose to lactic acid by lactic acid bacteria. Significant differences were observed between the two samples, with a slightly lower pH value in FDMD-B samples during storage that could derive from the greater quantity of pomegranate extract (Sandhya et al., 2018). These results are in agreement with Al-Hindi et al. (2020) who produced a fermented milk beverages supplemented with 150 and 300 mg/L of pomegranate peel extract. Shiby et al. (2013) also obtained similar results in the production of whey-pomegranate energy drinks.

Statistical analysis of a_w values showed no significant differences between the two samples, but an influence of storage time was highlighted ($P < 0.001$). In contrast, the concentration of the PE in the two distinct samples and the time of storage significantly ($P < 0.05$) impacted the moisture trends. The highest moisture value was found in freshly made FDMD-A with a gradual decrease during 30 days of storage in all samples. In particular, FDMD-B presented lower values than FDMD-A until day 20 of storage, presenting almost similar values to FDMD-A at day 30. The decrease in moisture content during the storage period could be due to evaporative loss of small amount of moisture, in agreement with Mahajan et al. (2015).

Fig. 3 shows the SDS-PAGE electropherograms of the samples during 30 days of storage. It was possible to clearly observe the main casein fractions (α_{s1}, α_{s2} and β casein) and two main whey proteins, positioned in the area below 20 KD. β-casein was the most abundant protein, followed by α_s-caseins (the sum of α_{s1} and α_{s2} caseins) and β-lactoglobulin.

Table 4
pH, a_w, moisture, total phenols content (TPC), antioxidant activity (ABTS and DPPH), tocopherols and tocotrienols content of samples during 30 days of storage.

	Days	FDMD-A	FDMD-B	*P*-value S	*P* -value T	*P* -value S*T
a_w	0	0.9983 ± 0.001[a]	0.9977 ± 0.003[a]	ns	< 0.001	ns
	10	0.9677 ± 0.001[d]	0.9645 ± 0.001[d]			
	20	0.9877 ± 0.001[c]	0.9891 ± 0.001[bc]			
	30	0.9912 ± 0.002[bc]	0.9943 ± 0.002[ab]			
pH	0	4.32 ± 0.01[a]	4.30 ± 0.02[a]	< 0.001	< 0.001	< 0.001
	10	4.25 ± 0.01[b]	4.21 ± 0.01[c]			
	20	4.17 ± 0.03[c]	4.10 ± 0.01[d]			
	30	4.19 ± 0.02[c]	4.18 ± 0.02[c]			
Moisture	0	68.58 ± 0.35[a]	66.993 ± 0.07[b]	< 0.05	< 0.05	< 0.05
	10	67.20 ± 0.05[b]	66.91 ± 0.06[b]			
	20	67.47 ± 0.01[ab]	67.15 ± 0.03[b]			
	30	67.18 ± 0.04[b]	67.19 ± 0.29[b]			
TPC (mg GAE/g)	0	1.20 ± 0.04[b]	1.71 ± 0.03[a]	< 0.001	< 0.001	< 0.001
	10	0.99 ± 0.05[c]	1.06 ± 0.05[c]			
	20	0.64 ± 0.01[e]	0.77 ± 0.00[d]			
	30	0.62 ± 0.02[e]	0.66 ± 0.01[e]			
ABTS (μmol TE/g)	0	5.54 ± 0.24[bc]	6.89 ± 0.28[a]	< 0.001	< 0.001	ns
	10	4.28 ± 0.24[de]	6.02 ± 0.95[ab]			
	20	4.08 ± 0.38[de]	4.78 ± 0.11[cd]			
	30	3.56 ± 0.18[e]	4.08 ± 0.09[de]			
DPPH (μmol TE/g)	0	3.70 ± 0.26[b]	4.73 ± 0.15[a]	< 0.001	< 0.001	< 0.001
	10	1.99 ± 0.09[d]	4.79 ± 0.55[a]			
	20	2.90 ± 0.06[c]	3.09 ± 0.17[bc]			
	30	2.17 ± 0.08[d]	2.60 ± 0.10[cd]			
α-Tocopherol (mg/100 g)	0	0.26 ± 0.03[c]	0.23 ± 0.02[c]	< 0.001	< 0.001	< 0.001
	10	0.25 ± 0.01[c]	0.38 ± 0.05[a]			
	20	0.35 ± 0.01[b]	0.21 ± 0.02[c]			
	30	0.25 ± 0.01[c]	0.24 ± 0.01[c]			
α-Tocotrienol (mg/100 g)	0	0.47 ± 0.01[e]	0.54 ± 0.01[c]	< 0.001	< 0.001	< 0.001
	10	0.51 ± 0.01[cd]	0.62 ± 0.01[a]			
	20	0.58 ± 0.02[b]	0.43 ± 0.01[f]			
	30	0.48 ± 0.02[de]	0.50 ± 0.03[d]			
β-Tocopherol (mg/100 g)	0	0.47 ± 0.01[e]	0.49 ± 0.01[d]	< 0.001	< 0.001	< 0.001
	10	0.51 ± 0.02[c]	0.57 ± 0.02[a]			

(*continued on next page*)

Table 4 (*continued*)

	Days	FDMD-A	FDMD-B	*P*-value S	*P* -value T	*P* -value S*T
	20	0.55 ± 0.03^{b}	0.38 ± 0.01^{f}			
	30	0.47 ± 0.01^{e}	0.52 ± 0.05^{c}			
β-Tocotrienol (mg/100 g)	0	0.53 ± 0.01^{cd}	0.57 ± 0.02^{b}	< 0.001	< 0.001	< 0.001
	10	0.52 ± 0.01^{e}	0.61 ± 0.01^{a}			
	20	0.56 ± 0.02^{b}	0.42 ± 0.03^{f}			
	30	0.52 ± 0.01^{de}	0.55 ± 0.01^{c}			

^{a,f} Values for each parameter in all samples analyzed (type and time) bearing different letters are different at $P < 0.05$. Values shown are mean ± SD.
Abbreviation: FDMD-A: fermented donkey milk dessert with 0.5 % (w/w) pomegranate extract; FDMD-B: fermented donkey milk dessert with 1 % (w/w) pomegranate extract; S: type of sample; T: different time of storage; ns: not significant.

The band with weak intensity below *β*-lactoglobulin should be lysozyme. The absence of *α*-lactalbumin should be the consequence of the heat treatment at 80 °C for 10 min; in fact, according to Miao et al. (2020) *α*-lactalbumin is less heat-stable than lysozyme. Some weak bands in the high molecular weight zone of the gel were tentatively identified as lactoferrin, serum albumin and immunoglobulins. The patterns revealed that the addition of different percentage of PE did not affect the protein profile in FDMD samples. In addition, the absence of noticeable differences in the intensity of bands over time indicated the absence of proteolysis.

Table 4 shows the antioxidant properties of the samples during storage. In particular, there was a significant difference depending on the sample, time and their ratio in all the parameters analyzed. Indeed, considering the TPC values found in the various samples, it is possible to show a higher concentration in FDMD-B than in FDMD-A samples, having a lower percentage of added PE. Moreover, there was a gradual decrease during the storage period in both samples. Freshly FDMD-B was found to be the sample with the highest concentration of total polyphenols (1.71±0.03 mg GAE/g). These results are in agreement with Al-Hindi & Abd El Ghani (2020) and Lashkari et al. (2020) who have added varying percentages of pomegranate juice and extract into fermented milk products and evaluated antioxidant activity during storage. In addition, high values of TPC were accompanied by high values of ABTS and DPPH.

Regarding tocopherols and tocotrienols, their concentration, although statistically different in the various samples, remained stable in FDMD-A and FDMD-B over the 30 days of storage, considering the low concentrations found. Due to a higher concentration of *α* and *β*-tocotrienols in the starting oil used (Squeo et al., 2022) these were shown to be higher than *α* and *β*-tocopherols in the samples analyzed. Tocotrienols and tocopherols are recognized as natural antioxidants typical of vegetable oils and are used as additives by food industries to address the low oxidative stability of PUFAs (Saini et al., 2021) and, in particular, some studies have suggested that tocotrienols exert greater antioxidant activity than tocopherols and have more relevant health benefits (Peh et al., 2016). Thus, a good presence of tocotrienols and tocopherols in new FDMD formulations contribute to oxidative stability over time, along with the contribution made by PE and OF. Ellagic acid present in PE is able to react with caseins and milk serum proteins, positively influencing their antioxidant characteristics (Lashkari et al., 2020). No less important is the contribution of total polyphenols derived from flour (0.55 mg GAE/g of OF, data not show), which contribute to the increased functionality and health benefits of FDMD samples.

During the production and storage process, the stability of the coloring compounds in dairy products is affected by their structure, concentration, presence of light and oxygen, sugars, enzymes, pH value and storage temperature (Antonino et al., 2024). In particular, the color change between the two FDMD samples is certainly due to the interaction of PE phenolic compounds with the other ingredients used. The results shown in Table 5 highlighted higher values of L* in FDMD-B at all times of analysis ($P < 0.001$) and an increase in brightness up to day 30 of storage. In contrast, the red value (a*) was significantly higher in FDMD-A ($P < 0.001$) throughout the preservation time, and a less linear trend was found for the b* parameter. El-shafei et al. (2017) also showed this trend, enriching a goat cream cheese with the same percentages of PE used in our study.

The results shown in Table 5 support the recognized ability of plant extracts in changing the structural profile of fermented milks and other milk-based desserts compared to formulations without this constituent (Szwajgier & Gustaw, 2015). Specifically, higher values were shown in both samples of firmness, consistency and complex viscosity at 0 days, which gradually decreased until day 30 of storage. The FDMD-B showed higher values throughout the analysis period. In contrast, the cohesiveness increased during the storage time in all samples, being lower in FDMD-B. These results are in agreement with the moisture values shown in Table 4 because, water molecules and fat globules are located within the three-dimensional protein networks; if the amount of water is higher, the network structure is weaker, becoming more susceptible to deformation during compression (Lashkari et al., 2020). Based on the results, it can be stated that a higher amount of PE in FDMD resulted in higher dry matter content and lower water content, requiring more

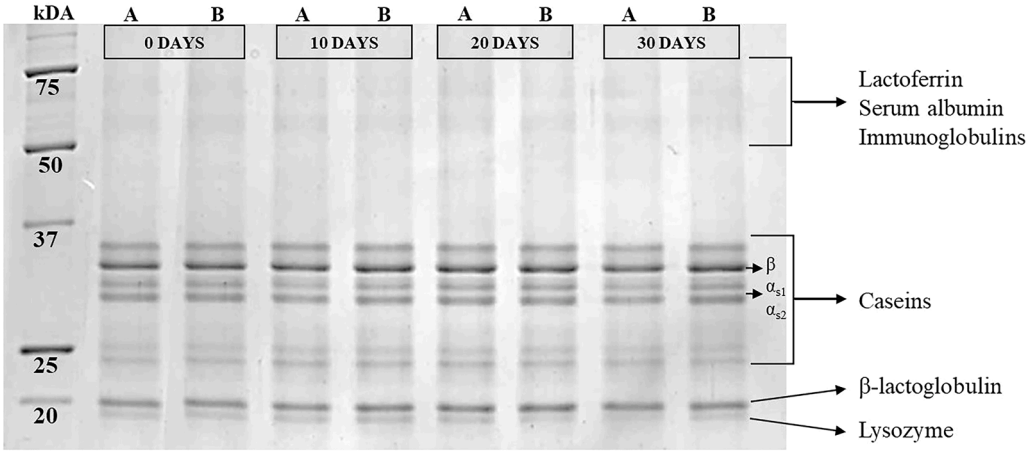

Fig. 3. SDS-PAGE of fermented donkey milk dessert with 0.5 % and 1 % (w/w) of pomegranate extract (**A** and **B**, respectively) during 30 days of storage.

Table 5
Color, textural, and rheological parameters (elastic modulus *G'*, viscous modulus *G''*, and tanγ) of samples during 30 days of storage.

	Days	FDMD-A	FDMD-B	*P*-value S	*P*-value T	*P*-value S*T
L*	0	36.79 ± 0.04^{d}	36.61 ± 0.13^{d}	ns	< 0.001	ns
	10	37.30 ± 0.17^{cd}	38.40 ± 0.05^{b}			
	20	36.95 ± 0.04^{cd}	37.82 ± 0.07^{bc}			
	30	37.31 ± 0.07^{cd}	41.48 ± 0.83^{a}			
a*	0	10.91 ± 0.04^{a}	10.47 ± 0.04^{d}	ns	ns	ns
	10	10.67 ± 0.06^{c}	10.08 ± 0.02^{f}			
	20	10.79 ± 0.02^{b}	10.19 ± 0.02^{ef}			
	30	10.75 ± 0.02^{bc}	10.25 ± 0.03^{e}			
b*	0	17.79 ± 0.05^{bc}	17.94 ± 0.03^{ab}	< 0.05	< 0.05	< 0.05
	10	17.55 ± 0.15^{cd}	17.36 ± 0.05^{de}			
	20	18.20 ± 0.02^{a}	17.11 ± 0.03^{e}			
	30	17.88 ± 0.04^{b}	17.74 ± 0.21^{bc}			
Firmness (N)	0	1.59 ± 0.01^{a}	1.64 ± 0.02^{a}	< 0.001	< 0.001	< 0.001
	10	0.91 ± 0.08^{c}	1.33 ± 0.12^{b}			
	20	0.93 ± 0.05^{c}	1.28 ± 0.07^{b}			
	30	0.90 ± 0.06^{c}	0.99 ± 0.14^{c}			
Consistency (mJ)	0	11.48 ± 1.25^{a}	12.59 ± 0.83^{a}	< 0.001	< 0.001	ns
	10	11.66 ± 0.71^{a}	9.45 ± 2.17^{a}			
	20	11.40 ± 0.70^{a}	10.02 ± 0.88^{a}			
	30	10.48 ± 1.26^{a}	9.50 ± 1.04^{a}			
Cohesiveness (N)	0	-1.10 ± 0.02^{cd}	-1.15 ± 0.01^{d}	< 0.001	< 0.001	< 0.001
	10	-0.94 ± 0.04^{bc}	-0.98 ± 0.04^{bcd}			
	20	-0.48 ± 0.06^{a}	-0.88 ± 0.12^{b}			
	30	-0.60 ± 5.73^{a}	-0.67 ± 0.11^{a}			
Viscosity (mJ)	0	9.08 ± 0.60^{ab}	9.79 ± 0.28^{a}	< 0.001	< 0.001	< 0.001
	10	8.73 ± 0.23^{abc}	8.04 ± 0.76^{bcd}			
	20	7.40 ± 0.17^{cd}	7.04 ± 0.06^{d}			
	30	6.97 ± 0.79^{d}	7.16 ± 0.95^{cd}			
G'	0	453.00 ± 36.77^{b}	514.06 ± 6.38^{a}	< 0.001	< 0.001	< 0.001
	10	267.33 ± 19.24^{c}	273.80 ± 32.09^{c}			
	20	189.06 ± 13.62^{d}	182.83 ± 6.57^{d}			
	30	198.03 ± 2.44^{d}	178.83 ± 14.40^{d}			
G''	0	188.96 ± 13.29^{a}	250.53 ± 34.29^{b}	< 0.001	< 0.001	< 0.001
	10	133.43 ± 7.81^{c}	137.76 ± 11.60^{c}			
	20	104.57 ± 7.39^{c}	114.67 ± 25.16^{c}			
	30	109.80 ± 1.75^{c}	101.06 ± 6.40^{c}			
tan γ	0	0.41 ± 0.01^{c}	0.48 ± 0.07^{bc}	< 0.001	< 0.001	< 0.001
	10	0.49 ± 0.01^{abc}	0.50 ± 0.01^{abc}			
	20	0.55 ± 0.01^{abc}	0.62 ± 0.11^{a}			
	30	0.55 ± 0.01^{abc}	0.56 ± 0.01^{ab}			

[a,f] Values for each parameter in all samples analyzed (type and time) bearing different letters are different at $P < 0.05$. Values shown are mean ± SD.
Abbreviation: FDMD-A: fermented donkey milk dessert with 0.5 % (w/w) pomegranate extract; FDMD-B: fermented donkey milk dessert with 1 % (w/w) pomegranate extract; S: type of sample; T: different time of storage; ns: not significant.

energy for deformation. However, values of the texture parameters obtained were close to those predicted by the experimental design, considering the percentages of OF and WGO used to obtain the product (Fig. 1). Thus, it can be affirmed that the application of the experimental design was useful in that, its mathematical prediction proved to be reliable in practice.

On rheological analysis (Table 5), the different PE concentration did not affect the modulus of elasticity and with a significance $P < 0.05$ for the viscosity modulus. The viscoelastic behavior of FDMD-A and FDMD-B was in line with what was obtained during the structural optimization of the product, thus a structure behavior typical of a weak gel, although with higher values than previously obtained. As a matter, the samples presented higher G' and G'' values. This phenomenon may be attributed to the extended homogenization time used for the incorporation of chocolate and PE, which allowed for stronger interactions between the various constituents of the samples. In addition, during the 30-day storage period, the structure was quite stable, with a limited decrease in the viscoelatic behavior elasticity over time (Fig. 4).

On analysis of volatile compounds, the samples exhibited a profile characterized by 30 volatile compounds. The highest concentration of volatile compounds was found after 10 days of storage in both samples (868.20 μg/gr for FDMD-A and 794.03 μg/gr for FDMD-B), followed by a gradual decrease until the 30th day of storage, with a predominant concentration of volatile compounds in the FDMD-A formulation. Moreover, statistical analysis showed differences between the two samples, an influence of storage time ($P < 0.001$) and a correlation between the two variables. As shown in Table 6, the volatiles fingerprint of the two samples shows the same complexity in terms of number of compounds, but differs in concentration. Specifically, the volatile compound profiles of FDMD samples were characterized by higher presence of aldehydes and ketones, possibly resulting from the β-oxidation reaction of unsaturated fatty acids (Vincenzetti et al., 2018). Among the ketones, high concentrations of 2,3-butanedione and 2,3-pentanedione were identified in the two samples, with an increase until day 20 and then a subsequent decrease, in agreement with Mantzourani et al. (2018). Within the class of aldehydes, 2-methyl-butanal and 3-methyl-butanal were identified as the most abundant, with a higher concentration in FDMD-B throughout the storage period. Their presence was identified in donkey milk cheese and fermented pomegranate drinks (Faccia et al., 2020; Mantzourani et al., 2020). These theoretically impart butter and green odors and derive from lactic acid bacteria fermentation (Dan et al., 2017). Hexanal and nonanal also showed high concentrations, with significant differences for hexanal, major in FDMD-B, and similar trend in the two samples for nonanal. Thus, their high concentration is ascribable to microbial metabolism and lipid oxidation. Hydrocarbons, as well as alcohols, they make little

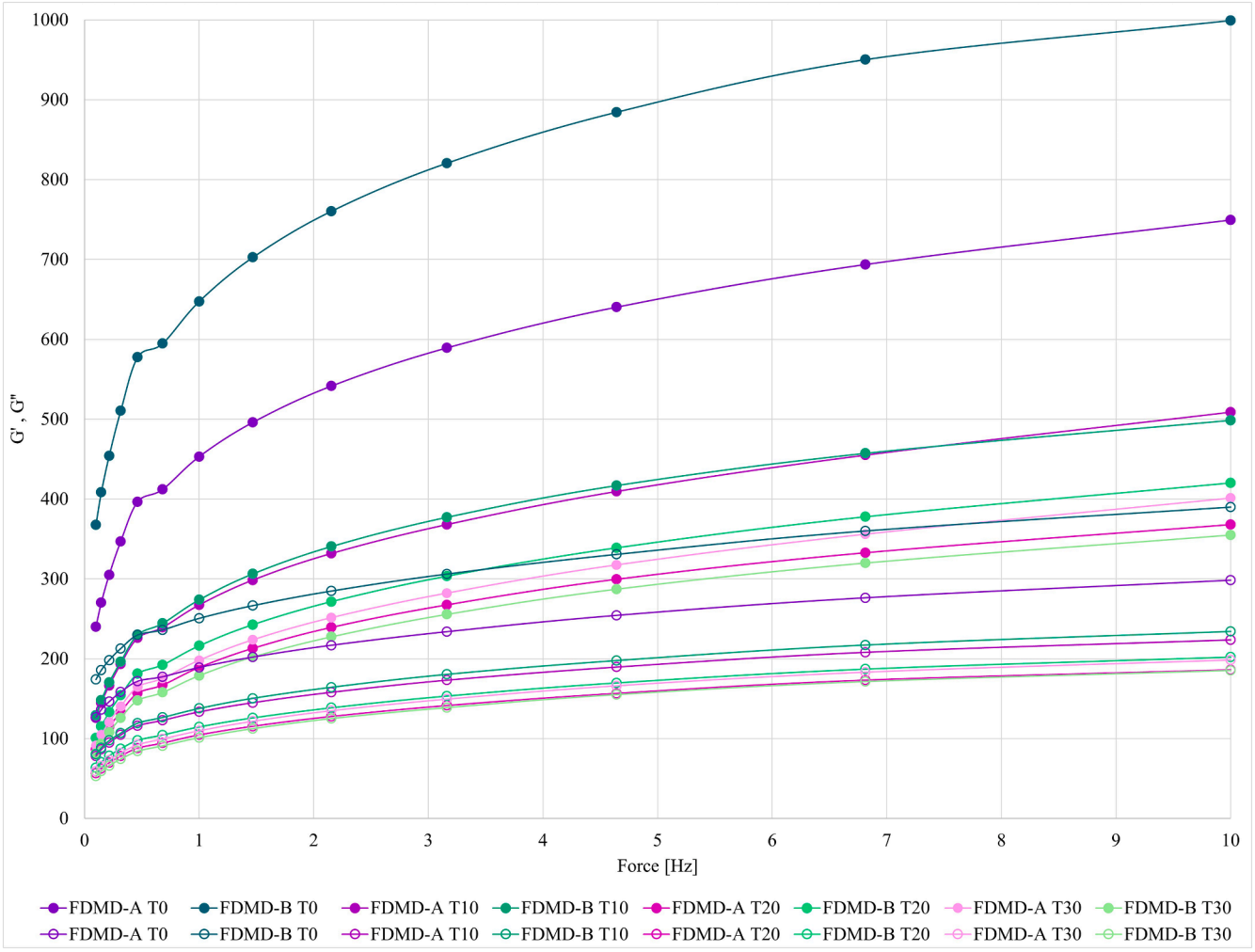

Fig. 4. Rheological properties of FDMD-A (fermented donkey milk dessert with 0.5 % pomegranate extract) and FDMD-B (fermented donkey milk dessert with 1 % pomegranate extract) during 30 days of storage. Elastic modulus (G', filled symbol) and viscous modulus (G'', blank symbols).

contribution at the aromatic level as they have high threshold values, but can contribute to the formation of other aromatic compounds by acting as precursors. In particular, pyrazines, generated by the Maillard reaction, are characterized by hazelnut and toasted flavors, whose aroma contribution is derivate from chocolate (Pieracci et al., 2021). The most abundant volatile compound in FDMD samples was acetic acid, previously reported in donkey milk (Faccia et al., 2018). The elevated concentrations of acetic acid are likely attributed to the fermentation process conducted by the microorganisms inoculated during FDMD production.

3.2.2. *Microbiological analyses*

Microbiological results of the samples during the 30-day storage period (Table 7) showed no evidence of *Listeria monocytogenes* and *Salmonella* spp. In addition, *β-glucuronidase-positive E. coli* counts showed values within the limits defined by Regulation (EC) No. 1441/2007. In DFMD-A samples, a slightly higher level of *Enterobacteriaceae* was observed, with a tendency for a modest decrease over time, which actually affected both formulations. Finally, the number of mesophilic lactic acid bacteria tended to slightly decrease over time and with increasing PE content. Overall, these results demonstrated that the samples were microbiologically safe during the whole storage period. Moreover, the addition of PE did not limit the survival of mesophilic lactic acid bacteria.

3.2.3. *Sensory evaluation*

The results of the sensory analysis are shown in Fig. 5. On visual analysis, significant differences were shown between the two samples only for color uniformity, which increased from day 10 to day 30. The scores given to structural descriptors showed strong similarity between the two samples (P not significant). Texture was very uniform at all times of analysis, confirming that the increased presence of PE did not change the texture of FDMD samples. In addition, the jelly and creamy attributes increased over time. The scores for taste and flavor attributes showed significant differences ($P < 0.05$) as a function of samples, time and their relationship. In particular, the fruity descriptor (identified as "red fruits") was found to be slightly higher in FDMD-B in the first 10 days of storage, in agreement with the higher concentration of added PE. However, at 20 and 30 days, the red fruit sensation increased equally in the two samples. This aroma might be connected to the aldehydes, alcohols and terpenes content in PE that are responsible of fruity and floral notes (Van Nieuwenhove et al., 2019). This olfactory perception was found to be partly related to sourness, which was higher in FDMD-B. The increase in acidity in the samples up to day 20 is due to the progressive conversion of lactose to lactic acid, in agreement with Mahajan et al. (2015). In contrast, cereal and chocolate sensations decreased ($P < 0.05$) during storage. Generally, there was no evidence of a strong change in the product over the 30 days of storage, with an overall appreciation of the two samples.

Table 6
Volatile organic compounds content (μg/gr) of samples during 30 days of storage.

	Days	FDMD-A	FDMD-B	*P*-value S	*P*-value T	*P*-value S*T[2]
			Ketones			
2-Butanone	0	1.47 ± 0.12^{de}	1.41 ± 0.06^{e}	< 0.001	< 0.001	< 0.001
	10	1.82 ± 0.06^{bc}	1.95 ± 0.10^{b}			
	20	2.23 ± 0.10^{a}	1.50 ± 0.04^{de}			
	30	1.69 ± 0.08^{cd}	1.79 ± 0.06^{bc}			
3-Hydroxy-2-butanone	0	57.51 ± 9.45^{b}	31.01 ± 0.88^{c}	< 0.001	< 0.001	< 0.001
	10	106.84 ± 14.45^{a}	43.97 ± 5.03^{bc}			
	20	49.07 ± 0.58^{bc}	28.77 ± 0.71^{c}			
	30	43.63 ± 8.54^{bc}	38.93 ± 9.12^{bc}			
2,3-Butanedione	0	74.60 ± 3.13^{ef}	70.86 ± 2.93^{f}	< 0.001	< 0.001	< 0.05
	10	94.84 ± 1.89^{bc}	103.20 ± 4.03^{a}			
	20	101.63 ± 2.16^{ab}	106.91 ± 4.62^{a}			
	30	80.80 ± 0.75^{de}	88.13 ± 1.72^{cd}			
2-Heptanone	0	4.95 ± 0.63^{c}	5.30 ± 0.45^{c}	< 0.05	< 0.001	ns
	10	24.52 ± 2.44^{a}	25.39 ± 2.56^{a}			
	20	19.40 ± 0.78^{b}	22.73 ± 4.05^{ab}			
	30	18.66 ± 0.77^{b}	23.56 ± 1.54^{ab}			
2-Nonanone	0	2.26 ± 2.04^{c}	1.99 ± 0.10^{cd}	< 0.05	< 0.001	< 0.001
	10	14.58 ± 3.58^{a}	5.73 ± 0.15^{bc}			
	20	8.79 ± 1.41^{b}	7.76 ± 1.71^{b}			
	30	7.87 ± 0.66^{b}	9.53 ± 1.03^{b}			
2,3-Pentanedione	0	50.01 ± 2.81^{d}	49.94 ± 0.8^{d}	< 0.05	< 0.001	< 0.05
	10	103.89 ± 2.78^{ab}	109.59 ± 3.9^{a}			
	20	112.07 ± 3.37^{a}	111.10 ± 4.99^{a}			
	30	90.25 ± 2.57^{c}	99.33 ± 2.59^{b}			
2-Pentanone	0	0.60 ± 0.19^{d}	0.65 ± 0.03^{e}	ns	< 0.001	ns
	10	1.42 ± 0.15^{cd}	1.69 ± 0.09^{b}			
	20	1.73 ± 0.22^{a}	1.72 ± 0.12^{a}			
	30	1.64 ± 0.21^{bc}	1.50 ± 0.04^{c}			
			Aldehydes			
Benzaldehyde	0	7.20 ± 0.64^{d}	5.17 ± 0.46^{de}	< 0.001	< 0.001	< 0.001
	10	18.28 ± 1.73^{a}	16.95 ± 2.06^{ab}			
	20	13.78 ± 1.51^{c}	14.18 ± 0.83^{c}			
	30	14.58 ± 0.68^{c}	16.46 ± 2.09^{ab}			
2-Methyl-butanal	0	23.86 ± 2.14^{d}	23.99 ± 1.00^{d}	ns	< 0.001	ns
	10	49.17 ± 1.77^{ab}	51.31 ± 0.13^{a}			
	20	34.47 ± 9.56^{c}	38.99 ± 1.72^{bc}			
	30	36.18 ± 1.54^{c}	37.18 ± 2.08^{c}			
3-Methyl-butanal	0	27.28 ± 2.39^{d}	32.18 ± 0.69^{cd}	< 0.001	< 0.001	ns
	10	53.11 ± 1.43^{b}	62.40 ± 2.95^{ab}			
	20	42.20 ± 6.18^{c}	51.20 ± 1.79^{ab}			
	30	53.34 ± 2.27^{ab}	63.36 ± 3.94^{a}			
Hexanal	0	7.63 ± 1.01^{e}	8.58 ± 0.30^{e}	< 0.001	< 0.001	< 0.001
	10	43.39 ± 0.98^{b}	49.78 ± 2.62^{a}			
	20	30.61 ± 1.32^{d}	35.92 ± 0.57^{c}			
	30	37.66 ± 2.65^{c}	51.32 ± 0.99^{a}			
Nonanal	0	4.03 ± 0.15^{c}	2.19 ± 0.04^{c}	ns	< 0.001	ns
	10	16.48 ± 2.01^{a}	13.99 ± 2.93^{ab}			
	20	9.33 ± 1.72^{b}	10.61± 0.12^{b}			
	30	10.53 ± 2.52^{b}	12.92 ± 0.93^{ab}			
Octanal	0	1.13 ± 0.29^{de}	0.70 ± 0.15^{e}	< 0.05	< 0.001	< 0.05
	10	3.18 ± 0.66^{cd}	3.59 ± 0.56^{c}			
	20	2.37 ± 0.10^{cde}	3.67 ± 0.77^{cd}			
	30	7.76 ± 2.07^{b}	10.47 ± 0.25^{a}			
(E)-2-Octenal	0	1.41 ± 0.46^{bc}	1.21 ± 0.11^{c}	ns	< 0.001	ns
	10	4.73 ± 0.56^{a}	4.69 ± 0.60^{a}			
	20	1.97 ± 0.19^{bc}	1.65 ± 0.08^{bc}			
	30	2.07 ± 0.01^{bc}	2.39 ± 0.35^{b}			
2-Methyl-propanal	0	11.60 ± 0.74^{a}	11.18 ± 0.61^{ab}	< 0.05	< 0.001	< 0.001
	10	7.35 ± 0.39^{cd}	7.42 ± 0.68^{cd}			
	20	6.04 ± 1.40^{d}	10.02 ± 0.23^{ab}			
	30	9.18 ± 0.59^{bc}	9.50 ± 0.56^{b}			
trans-2-Heptenal	0	12.25 ± 0.89^{ab}	5.58 ± 3.19^{bc}	ns	< 0.001	< 0.05
	10	14.54 ± 4.72^{ab}	16.76 ± 6.20^{a}			
	20	9.84 ± 0.34^{bc}	10.62 ± 0.84^{abc}			
	30	8.54 ± 0.29^{bc}	12.53 ± 3.06^{ab}			
			Esters			
Acetic acid, ethyl ester	0	1.04 ± 0.05^{d}	1.12 ± 0.08^{cd}	ns	< 0.001	< 0.001
	10	1.99 ± 0.01^{a}	2.14 ± 0.12^{a}			
	20	2.04 ± 0.06^{a}	1.74 ± 0.08^{b}			
	30	1.29 ± 0.09^{c}	1.29 ± 0.07^{c}			
Acetic acid, methyl ester	0	1.36 ± 0.18^{cd}	1.28 ± 0.06^{cd}	< 0.001	< 0.001	< 0.05
	10	1.30 ± 0.05^{cd}	1.56 ± 0.17^{abc}			
	20	1.43 ± 0.12^{cd}	1.83 ± 0.07^{a}			

(*continued on next page*)

Table 6 (*continued*)

	Days	FDMD-A	FDMD-B	*P*-value S	*P*-value T	*P*-value S*T[2]
	30	1.22 ± 0.08[d]	1.67 ± 0.05[ab]			
1-Butanol, 3-methyl-, acetate	0	4.60 ± 0.72[b]	4.20 ± 0.42[b]	ns	< 0.001	ns
	10	23.23 ± 2.02[a]	19.87 ± 1.72[a]			
	20	20.24 ± 0.17[a]	20.62 ± 4.99[a]			
	30	22.55 ± 5.78[a]	23.68 ± 6.8[a]			
			Hydrocarbons			
Alpha-pinene	0	0.78 ± 0.14[c]	0.91 ± 0.02[c]	< 0.05	< 0.001	ns
	10	3.47 ± 0.07[a]	3.93 ± 0.40[a]			
	20	2.57 ± 0.17[b]	2.50 ± 0.13[b]			
	30	2.18 ± 0.12[b]	2.48 ± 0.14[b]			
Benzene	0	1.48 ± 0.19[c]	1.40 ± 0.02[c]	< 0.05	< 0.001	< 0.05
	10	2.25 ± 0.17[ab]	2.31 ± 0.28[ab]			
	20	1.81 ± 0.66[bc]	2.77 ± 0.83[a]			
	30	1.76 ± 0.10[bc]	2.02 ± 0.30[abc]			
Methyl-benzene	0	11.28 ± 1.74[d]	15.00 ± 0.44[d]	< 0.001	< 0.001	< 0.001
	10	36.34 ± 1.75[b]	52.09 ± 5.09[a]			
	20	25.83 ± 1.32[c]	32.70 ± 2.98[b]			
	30	23.76 ± 1.15[c]	35.43 ± 1.00[b]			
1-Ethyl-3-methyl- benzene	0	13.32 ± 2.16[a]	11.53 ± 0.86[ab]	< 0.001	< 0.001	< 0.001
	10	11.23 ± 0.58[ab]	5.83 ± 1.79[c]			
	20	6.91 ± 0.06[c]	6.78 ± 0.34[c]			
	30	0.19 ± 0.02[d]	7.35 ± 1.09[c]			
dl-Limonene	0	3.57 ± 0.53[bc]	1.31 ± 0.06[c]	ns	< 0.001	< 0.05
	10	5.45 ± 0.85[ab]	4.18 ± 0.38[abc]			
	20	4.75 ± 2.00[ab]	6.23 ± 0.14[ab]			
	30	3.66 ± 2.09[bc]	7.25 ± 1.10[a]			
Tetramethyl-pyrazine	0	2.42 ± 1.08[b]	0.94 ± 0.12[c]	< 0.05	< 0.001	< 0.05
	10	12.39 ± 4.68[a]	4.98 ± 1.05[b]			
	20	4.85 ± 1.57[b]	3.53 ± 1.02[b]			
	30	11.60 ± 3.46[a]	6.54 ± 0.17A[b]			
Trimethyl-pyrazine	0	1.09 ± 0.16[c]	0.73 ± 0.07[c]	ns	< 0.001	ns
	10	4.29 ± 0.63[a]	3.33 ± 1.56[ab]			
	20	2.37 ± 0.41[bc]	2.29 ± 0.09[bc]			
	30	3.07 ± 0.20[ab]	3.65 ± 0.13[ab]			
			Carboxylic acid			
Acetic acid	0	96.33 ± 16.71[ab]	69.23 ± 1.24[b]	ns	< 0.001	ns
	10	150.55 ± 20.28[a]	115.58 ± 60.64[ab]			
	20	58.96 ± 13.71[b]	68.74 ± 5.96[b]			
	30	75.08 ± 8.26[b]	76.59 ± 18.33[b]			
			Furans			
2-Ethyl-furan	0	2.42 ± 0.34[e]	2.37 ± 0.09[e]	ns	< 0.001	< 0.05
	10	7.11 ± 0.81[ab]	7.73 ± 0.84[a]			
	20	5.19 ± 0.58[cd]	4.17 ± 0.22[d]			
	30	5.25 ± 0.29[cd]	6.11 ± 0.19[bc]			
2-Pentyl-furan	0	8.33 ± 1.09[c]	8.10 ± 0.91[c]	< 0.001	< 0.001	< 0.001
	10	46.50 ± 2.56[a]	45.25 ± 5.04[ab]			
	20	31.02 ± 6.29[b]	29.10 ± 2.91[b]			
	30	30.92 ± 3.13[b]	34.49 ± 0.81[b]			
2-Furancarboxaldehyde	0	3.92 ± 0.54[ab]	3.41 ± 0.52[b]	< 0.05	< 0.05	ns
	10	3.96 ± 0.36[ab]	6.84 ± 2.15[a]			
	20	4.66 ± 1.42[ab]	5.17 ± 1.02[ab]			
	30	5.37 ± 0.56[ab]	6.25 ± 1.02[a]			

[a,f] Values for each parameter in all samples analyzed (type and time) bearing different letters are different at $P < 0.05$. Values shown are mean ± SD.
Abbreviation: FDMD-A: fermented donkey milk dessert with 0.5 % (w/w) pomegranate extract; FDMD-B: fermented donkey milk dessert with 1 % (w/w) pomegranate extract; S: type of sample; T: different time of storage; ns: not significant.

4. Conclusion

Donkey milk is gaining popularity as an alternative to breast milk and infant formulas for children allergic to cow's milk. It is also used in the manufacturing of fermented dairy goods, yielding functional products suitable for both children and the elderly. A new fermented dessert made from DM, OF, and WGO was developed using D-optimal mixture design. The formulation with 11.5% w/w of OF and 3.5% w/w of WGO was found to be the best in terms of rheological and textural parameters. The second step involved enriching the FDMD with dark chocolate (3% w/w) and two different percentages (0.5 and 1% w/w) of PE. Dark chocolate was added to enhance the flavor, while PE was added to assess its antioxidant power and polyphenolic molecules. The 30-day study showed that PE provided the formulations with polyphenols and antioxidant power, with better results with the addition of 1% (FDMD-B). The samples were microbiologically safe and stable, with limited proteolytic enzyme action during the 30-day storage period. The mathematical prediction of the Design of Experiment proved reliable in practice, with values obtained from texture and rheological analysis falling within those predicted by the theoretical DoE trend. Further studies could include an extension of the shelf-life study until quality decay to define the limit of acceptability over time. Despite limited donkey milk production in Italy, the adaptability of livestock to the Mediterranean climate and easy farm management are promising for increasing the production of this niche milk. This new formulation with excellent technological and nutritional characteristics could be a promising functional product suitable for fragile subjects, such as children and the elderly, with gastrointestinal pathologies, allergies to cow's milk proteins, and other dietary diseases. Additionally, further studies are needed to assess the acceptability of the new product to consumers.

Table 7
Microbiological growth (Log^{10} CFU/g) of samples during 30 days of storage.

	Days	FDMD-A	FDMD-B	*P*-value S	*P*-value T	*P*-value S*T
Mesophilic Lactic Acid Bacteria	0	7.32	7.23	ns	ns	ns
	10	7.38	7.32			
	20	6.76	6.84			
	30	6.86	7.04			
β-glucuronidase-positive E. coli	0	< 10	< 10	ns	ns	ns
	10	< 10	< 10			
	20	< 10	< 10			
	30	< 10	< 10			
Enterobacteriaceae	0	3.93	3.66	ns	ns	ns
	10	3.76	3.40			
	20	3.64	3.45			
	30	3.82	3.49			
Listeria monocytogenes	0	nd	nd	ns	ns	ns
	10	nd	nd			
	20	nd	nd			
	30	nd	nd			
Salmonella spp	0	nd	nd	ns	ns	ns
	10	nd	nd			
	20	nd	nd			
	30	nd	nd			

Abbreviation: FDMD-A: fermented donkey milk dessert with 0.5 % (w/w) pomegranate extract; FDMD-B: fermented donkey milk dessert with 1 % (w/w) pomegranate extract; S: type of sample; T: different time of storage; ns: not significant; nd: not detected.

Ethical statement

For the sensory evaluation, no human ethics committee or formal documentation process is available, but the appropriate protocols for protecting the rights and privacy of all participants were utilized during the execution of the research.

Participants gave informed consent via the statement "I am aware that my responses are confidential, and I agree to participate in this survey" where an affirmative reply was required to enter the survey. They were able to withdraw from the survey at any time without giving a reason. The products tested were safe for consumption."

CRediT authorship contribution statement

Claudia Antonino: Writing – original draft, Data curation. **Graziana Difonzo:** Writing – review & editing, Writing – original draft, Conceptualization. **Giuseppe Natrella:** Writing – original draft, Methodology. **Giacomo Squeo:** Writing – review & editing, Methodology. **Michele Faccia:** Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of competing interest

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

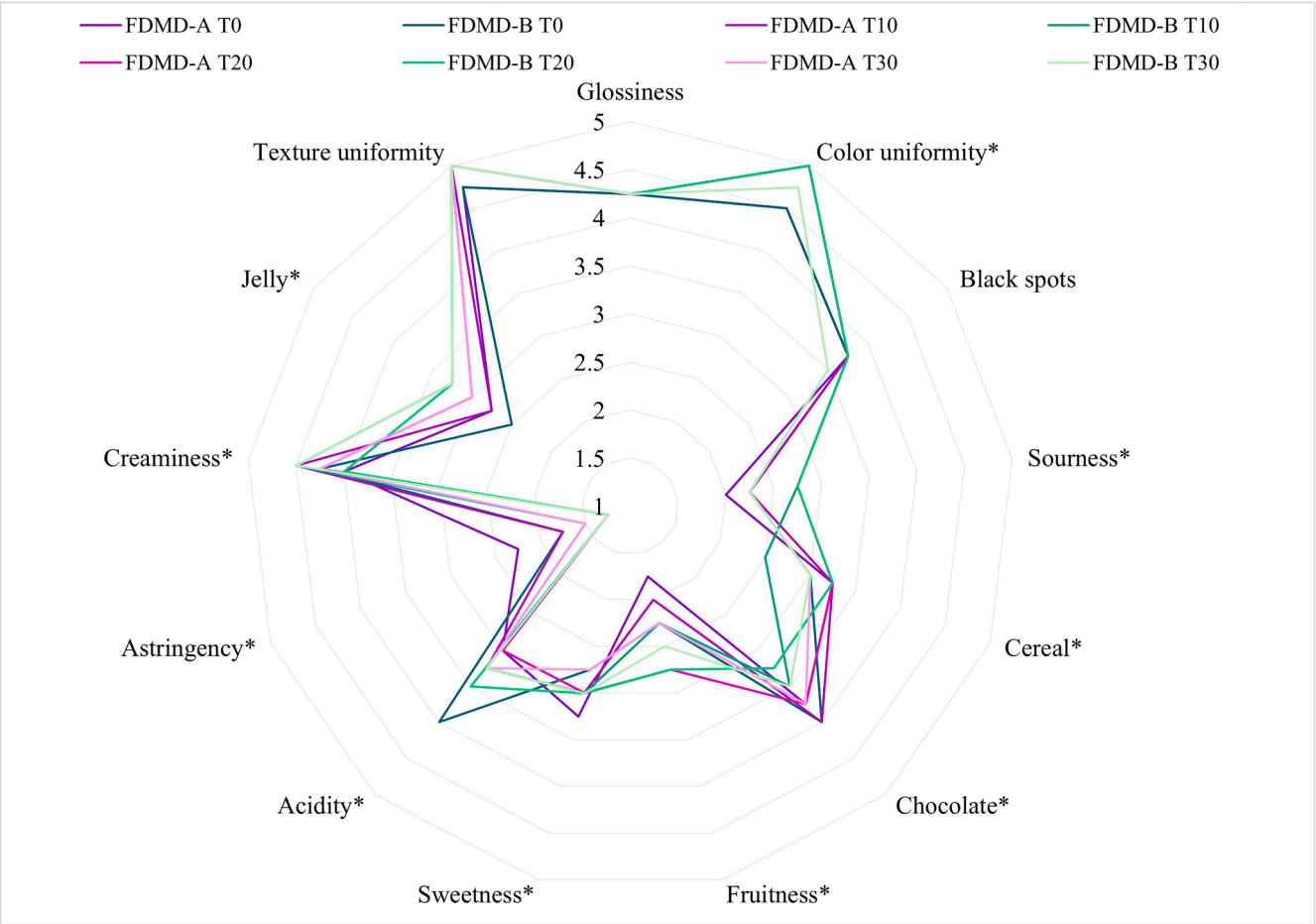

Fig. 5. Sensory profile of FDMD-A (fermented donkey milk dessert with 0.5 % pomegranate extract) and FDMD-B (fermented donkey milk dessert with 1 % pomegranate extract) during 30 days of storage.
* Values for each parameter in all samples analyzed (type and time) are different at $P < 0.05$.

Data availability

Data will be made available on request.

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