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Cocoa byproduct inclusion in dairy sheep diet: Effects on sensory, volatile, and antioxidant properties of cheese

M. Caccamo,[1] G. Luciano,[2] T. Rapisarda,[1] V. M. Marino,[1] C. Pasta,[1] A. Natalello,[2]* G. Mangione,[2] B. Valenti,[3] A. Campione,[3] G. Marino,[1] and M. Pauselli[3]
[1]Consorzio per la Ricerca nel settore della Filiera Lattiero-Casearia e dell'agroalimentare (CoRFiLaC), Regione Siciliana, 97100 Ragusa, Italy
[2]Department of Agriculture, Food and Environment (Di3A), University of Catania, 95123 Catania, Italy
[3]Department of Agricultural, Food and Environmental Sciences (DSA3), University of Perugia, 06121 Perugia, Italy

ABSTRACT

The possibility of inclusion of agro-industrial byproducts in the diet of small ruminants represents both an economic and an environmental strategy for reducing waste management by industries and costs of feeding as well as the impact of livestock farming. Large amounts of wastes from the cocoa industry are produced annually, with a considerable part represented by cocoa bean shells, considered a suitable ingredient to be included in the diet of ruminants within the limits established by European legislation. The aim of this study was to assess the effect of including cocoa bean shells in the diet of dairy sheep on the sensory, volatile, and antioxidant properties of cheese. To this purpose, 20 lactating Comisana ewes were randomly assigned to 2 experimental groups: control (CTRL) and cocoa bean shells (CBS), and received alfalfa hay ad libitum and 800 g of conventional (CTRL) or experimental (CBS) concentrate containing 11.7% CBS to partially replace corn and barley of the CTRL concentrate. Bulk milk collected from each group was used to produce a total of 15 cheeses per group, obtained in 5 different days of cheesemaking (3 cheeses a day per group). After 60 d of aging, each cheese of each experimental group was sampled for the analyses. The results on chemical composition revealed a greater content of monounsaturated fatty acids and an increase in the nutritional indices, suggesting a favorable role of cocoa bean shell dietary inclusion on the nutritive value of cheese. The cheese sensory profile was affected by the cocoa bean shell inclusion, with more pronounced appearance, odor, aroma, and taste attributes in the product. The volatile profile showed only a few significant differences, mainly related to the cheese ripening process, and no differences were found in α-tocopherol contents in cheese fat between the 2 groups. Therefore, the inclusion of coca bean shells in the diet of dairy sheep allowed us to obtain a good-quality cheese, without altering the characteristics associated with the typical profiles of sheep cheese. Furthermore, the use of this byproduct could contribute to decreasing feed costs and waste management, representing a good practice for increasing the sustainability of dairy products.

Key words: cocoa bean shell, cheese composition, byproduct inclusion, sensory profile

INTRODUCTION

Sheep farming assumes a different relevance according to the geographical area, the herd dimension, and production systems, as well as the type of available products. In this context, sheep milk production is a noteworthy sector in Europe, with more than 2.9 million t produced in 2022 (CLAL, 2024). However, consumption in liquid form is unusual; approximately 75% of the sheep milk produced is mainly used for the manufacture of several dairy products, including cheeses, yogurt, and whey cheeses (Haenlein and Wendorff, 2006; CLAL, 2024).

Due to their quality, high yield, and nutritional proprieties, with high concentrations of proteins, fats, vitamins, and minerals, sheep dairy products have gained considerable potential in terms of market expansion (Park, 2007; Milani and Wendorff, 2011), especially for their health benefits. Among other considerations are its higher digestibility compared with cow milk, as well as better tolerance by individuals allergic to cow milk (El-Agamy, 2007; Pilbrow et al., 2016). Both the nutritional aspects and sensory characteristics of sheep cheeses have increased their consumption in the last few years (Gámbaro et al., 2017).

Several strategies have been adopted for dairy sheep farming to reduce dependence on food from abroad, feed costs, and the environmental problems related to the management of waste materials (Monllor et al., 2020;

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*Corresponding author: antonio.natalello@unict.it

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Huanca et al., 2021). Particularly vegetable and agri-food as well as food processing industry byproducts have been investigated for possible alternative feeding, or partial inclusion, becoming a common practice as a way to give possible value added to the dairy products according to their nutritional characteristics as feeds. Moreover, the use of food byproducts also represents a possible way to reduce waste and to minimize waste management costs by the industry (Jaramillo et al., 2010), representing a crucial aspect in global strategies for reducing environmental impact problems (Correddu et al., 2020). However, several byproducts are commonly underestimated due to lack of valuable alternative uses, some of them rich in nutrients and bioactive compounds; thus, their inclusion in dairy sheep diets could represent a practical and sustainable option both for their recycle and valorization (Nudda et al., 2019). Moreover, it has been amply demonstrated that the use of byproducts as alternative feeds can affect milk composition and consequently the quality of derived products (Jaramillo et al., 2009).

Among the byproducts assessed for possible alternative feeding, cocoa bean shells (**CBS**) have shown enormous potential for use in the supply of animal feed for ruminants such as cattle, goats, and sheep, to partially replace cereals (Rebollo-Hernanz et al., 2022), representing a possible strategy to reduce feed-to-food competition and feeding costs during the dry season when pasture is scarce (Cornale et al., 2022). This byproduct represents the superficial tegument covering the cocoa beans, generated during the bean roasting process, comprising about 10% to 17% of the total cocoa bean weight (Hashimoto et al., 2018) and 2.1% to 2.3% of the cocoa pod, with an annual production of approximately 700,000 t (Okiyama et al., 2017).

The CBS is mainly composed of dietary fiber, protein, and fat, ranging from 19% to 60%, 12% to 18%, and 2% to 7%, respectively (Vásquez et al., 2019), as well as considerable quantities of interesting bioactive compounds, such as tannins (Badrie et al., 2015), which modify the rumen metabolism affecting animal performance and product quality (Frutos et al., 2020). Nevertheless, the factor limiting the use of CBS in ruminants is the theobromine concentration, which varies according to cocoa bean preparation and increases during fermentation (Makinde et al., 2019). High levels of this alkaloid may produce adverse effects on animal health (Adamafio, 2013). Most disease cases are reported in monogastric animals, especially dogs, whereas for ruminants only a few potential cases have been reported in dairy cattle. Thus, the possible toxic effect of theobromine in ruminants is not clearly understood; it is supposed that a fatal outcome is possible due to cardiac arrhythmia, respiratory failure, and disturbance of the central nervous system (Klein et al., 2021).

Despite different studies revealing positive effects of CBS inclusion in animals' diets, only a few works have investigated the effects of dietary CBS inclusion on milk and cheese composition in dairy sheep (Carta et al., 2020, 2022; Campione et al., 2021). The bioactive components of CBS could affect the nutrient characteristics and the sensory properties of animal-origin products (Vasta and Luciano, 2011). It is well known that dietary composition affects ruminal fermentation, and thus milk compositions could be changed when byproducts are included in the diet (Chilliard et al., 2003; Romero-Huelva et al., 2017) also affecting the antioxidant activity and sensory profile of dairy products (Martin et al., 2005).

However only a few studies related to the use of byproducts in the diet of dairy small ruminants have evaluated the effect on cheese sensory characteristics. Particularly, Jaramillo et al. (2009) evaluated the effect of substitution of cereal grain and sugar beet pulp by citrus fruits (30% DM basis) in ewes' diet, revealing no adverse effect on the overall sensory quality of cheeses. Caccamo et al. (2019) investigated the sensory and volatile profiles of cheese produced with milk from dairy sheep fed with hazelnut skin, revealing that this byproduct significantly affected the fatty acids and sensory profiles of the cheeses. Recently Huanca et al. (2021) investigated the inclusion of lemon leaves and rice straw byproducts in the diet of dairy goats on the sensory profile of the derived matured cheese, showing no negative attributes.

In Campione et al. (2021), the effects of CBS inclusion in the diet of dairy sheep on animal performance, fatty acid composition of rumen contents, and milk and cheese compositions were investigated. The results revealed lower urea levels in milk, probably related to the phenolic content of CBS, as well as lower protein and higher fat content in cheese. As part of the same experiment, the present manuscript focuses on assessment of the effects of including CBS byproduct in the diet of dairy sheep on the sensory, volatile, and antioxidant properties of cheese obtained from the experiment of Campione et al. (2021).

MATERIALS AND METHODS

Experimental Design, Animals, Diets, and Cheesemaking

The experimental design for this study was the same as that previously described by Campione et al. (2021). Briefly, 20 multiparous lactating Comisana ewes with 80 ± 8 DIM, balanced for BW (65 ± 8 kg), were randomly assigned to 2 experimental groups (n = 10), namely control (**CTRL**) or CBS-supplemented (CBS) group and confined in multiple pens. The trial lasted 35 d in total. After 14 d of adaptation, each animal received chopped alfalfa hay ad libitum (particle size >4 cm in length) and

Table 1. Chemical composition of the experimental feeds, adapted from Campione et al. (2021)

			Experimental concentrate[1]	
Item (g/kg DM)	Hay	Cocoa bean shell	CTRL	CBS
CP	78.4	120.3	163.2	166.2
Ether extract	16.3	204.2	21.7	31.6
NDF	562.8	330.8	181	217.9
ADF	409	273.2	90	132.7
ADL	62.9	137	22.7	38.5
NFC	302	350.6	602.1	553.9
Ash	58.3	46.3	50.1	57.6
Total extractable phenols	8.7	43.93	1.94	5.2
Total extractable tannins	3.92	30.3	0.98	3.64

[1]CTRL = control cheese; CBS = cheese from milk of sheep fed experimental diet containing cocoa bean shell.

800 g/d per ewe of a conventional concentrate with corn and barley for the CTRL group, while the CBS group received an experimental concentrate containing CBS to partially replace corn and barley. Table 1 reports the chemical composition of the experimental feeds, which were analyzed as detailed in Campione et al. (2021). Offered feeds and orts were weighed daily per pen, and individual milk production was recorded weekly.

Cheesemaking was carried out as reported in the previous paper (Campione et al., 2021). In short, bulk milk from each of the 2 feeding groups was collected daily during the experimental period and stored at −30°C until the quantity of ~45 kg was reached and used to make 3 cheeses per day for each group. A total of 15 cheeses per group were obtained in 5 different days of cheesemaking across the experimental period. After thawing at 4°C, the milk was heated at 39°C and enriched with a mixed-strain starter culture (Lyofast MW039S SACCO, Como, Italy) consisting of strains of *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. *lactis* biovar diacetylactis. At 10 min from the addition of starter culture, liquid calf-lamb rennet (strength 235 international milk clotting units [IMCU]/mL; Crerici SPA, Italy) was added (22 g/100 L). The curd was broken after 25 min until small grain dimension (~4-mm diameter) was achieved. Afterward, the curd was removed and filled into a perforated plastic basket to drain the whey until the pH reached 5.5. After 24 h at 7°C, all the cheeses were put in brine (salt 20% wt/vol) for 12 h and aged in a cold room for 60 d at a temperature ranging from 8 to 9°C and 70% humidity. The scheme of the conducted experimental cheesemaking is reported in Figure 1.

Cheese Sampling and Analyses

Chemical Analyses. After aging, individual cheeses were sampled for analyses, and aliquots were vacuum stored at −20°C. Determinations of moisture, lipid, and protein contents of the cheese samples were performed as reported by Bradley and Vanderwarn (2001), using the Gerber–Van Gulik method (ISO, 1975), and via the Kjeldahl method (total nitrogen × 6.38), respectively. Cheese fatty acid methyl ester (**FAME**) preparation was performed according to Nudda et al. (2005). Individual FAME of cheese were separated and quantified as described for feeds. Fatty acid profiles of cheeses were then determined through transesterification using a combined basic and acid methylation, as proposed by Cruz-Hernandez et al. (2004). Briefly, 0.5 mL of lipid extract was incubated at 50°C for 15 min with 1.5 mL sodium methoxide in methanol (0.5 *M*). After cooling at room temperature, 1 mL of 5% methanolic HCl was added, and the mixture was incubated at 50°C for 30 min. Then, 1 mL of 6% aqueous K_2CO_3 was added, and a triple refrigerated extraction with 3 mL of hexane at 1,500 × *g* for 10 min was performed. The extract was evaporated under N_2 flow at 37°C and then dissolved in 1 mL of GC-grade hexane. Gas chromatograph setting for FAME identification was the same as described for feedstuff analysis. Moreover, the separation of C18:1 isomers was achieved by isothermal analysis at 165°C. Individual fatty acids were expressed as grams per 100 g of cheese.

Odor active volatile compounds (**OAC**) were extracted by using a static solid-phase micro-extraction technique as reported by Carpino et al. (2004), with some modifications, as detailed below. Divinylbenzene/carbowax/polydimethylsiloxilane-coated fibers (DVB/CAR/PDMS; 50/30 µm; Supelco, Bellefonte, PA) were used to adsorb OAC from headspace samples. Ten grams of cheese were conditioned at 40°C for 40 min. An additional 40 min was required for the fiber exposition to establish the volatile compound equilibrium between headspace and fiber solid phase samples. The fiber was conditioned for 1 h at 225°C before the initial use and for 5 min between each analysis. For the GC-MS analysis and the identification of OAC, a 7890A Series GC system (Agilent Technologies, Santa Clara, CA) coupled with an Agilent 5975C Mass Selective Detector (triple axis) was

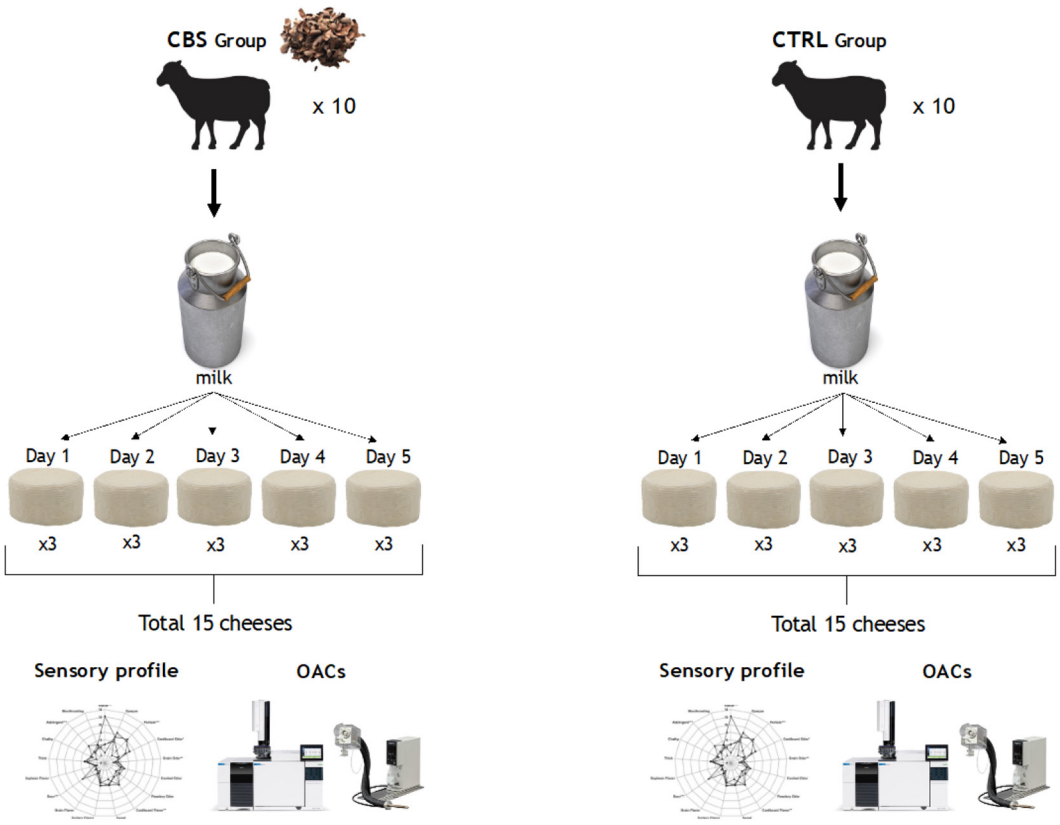

Figure 1. Schematic of the experimental cheesemaking.

used. The HP-5 capillary column (30 m × 0.25-mm i.d. × 0.25-µm film thickness; Agilent Technologies, Santa Clara, CA) was used to separate the volatile components. The chromatographic conditions were as follows: splitless injector at 220°C; oven program conditions: 35°C for 3 min, 6°C/min to 200°C, and 30°C/min to 240°C for 3 min. Helium pressure (carrier gas) was set at 93.77 MPa, and the gas flow was 1.0 mL/min. The mass selective detector operated in scan mode (5.15 scan/s) with 70 electron volts (eV) electron ionization. Peak identification was carried out by comparison of mass spectra with the bibliographic data from the Wiley 175 library (Wiley and Sons Inc., Germany), and with the linear retention indices of authentic standards (Sigma-Aldrich) calculated by running a paraffin series (from C5 to C20) under the same working conditions. The OAC data were expressed as arbitrary units of chromatograph area.

For GC olfactometry analysis, an HP 6890 Series GC system (Agilent Technologies, Santa Clara, CA) gas chromatograph coupled with an olfactometer was used. Column, injection, and oven setting were the same as reported for GC-MS. Trained human nose (sniffer) was used as final detector simultaneously with a mass detector (Rapisarda et al., 2014). The eluted compounds were mixed with humidified air, and the sniffer was continuously exposed to this source for 30 min. During the olfactometric analysis, the sniffer described the perceptions and duration of odors.

The OAC recognition was performed using the single-sniff method, and the sniffer was trained with 7 standard aroma compounds used to evaluate olfactory acuity (Marin et al., 1988). These compounds were selected for study because they are all naturally occurring food constituents, and specific anosmia has been reported for some of them. The sniffer had no specific anosmia for these standards.

The determination of cheese α-tocopherol and cholesterol was as described by Marino et al. (2010) and Oh et al. (2001), respectively. Both α-tocopherol and cholesterol were determined by an HPLC method using an SB-C18 column (5-µm particle size, 4.6-nm i.d. × 250 nm, Agilent Zorbax, Agilent Technologies, Santa Clara, CA). The HPLC system (Waters 2695; Waters, Milford, MA) was equipped with a multiwavelength (λ) fluorescence detector (Waters 2475) using an excitation wavelength of 297 nm and an emission wavelength of 340 nm for the detection of α-tocopherol, equipped with a dual λ absorbance detector (Waters 2487) using a wavelength of 203

Table 2. Descriptive attributes and definitions used to evaluate sheep cheese, adapted from Bozzetti et al. (2004)

Sensory item	Definition
Appearance	
Rind color	Color intensity of the rind
Paste color	Color intensity of the paste
Presence/absence of holes or slits	Evaluation of the uniformity in structure
Dimension of holes/slits	Evaluation of the uniformity in structure
Odor	
Lactic	Odor associated with cooked milk
Animal/sheep	Odor associated with lamb rennet
Spicy	Odor associated with pepper
Toasted	Odor associated with nutty, caramelized, browned character of Maillard browned starches and sugar
Smoked	Odor associated with smoked cheese
Taste	
Sweet	Fundamental taste associated with sucrose
Acid	Fundamental taste associated with citric acid
Savory	Fundamental taste associated with sodium chloride
Bitter	Fundamental taste associated with quinine
Astringent	Complex taste associated with shrinking, drawing, or puckering of the skin or mucous surface in the mouth
Aroma	
Yeast	Aroma associated with fresh compressed yeast
Animal/sheep	Aroma associated with lamb rennet
Toasted	Aroma associated with toasted nuts
Smoked	Aroma associated with smoked cheese
Tactile sensations/perception	
Soft/hard	Resistance or not to a given deformation; force required to compress sample when placed between fingers
Oily	Presence of oily residue on the skin when sample manipulated between the fingers
Elasticity	Ability of a substance to recover its initial shape and dimension after being submitted to pressure
Plasticity	Power to undergo a permanent change in shape
Mouth sensations/perception	
Moisture	Perception of the degree of moisture in the cheese sample
Soft/hard	Resistance or not to chewing
Mellow	Sensation produced by sweet solutions, such as sucrose or fructose
Soluble	A sensation that emerges when the sample melts extremely quickly in the saliva
Dispersion	Degree to which sample breaks into the mouth during chewing

nm for the detection of cholesterol. The mobile phases were methanol 100% vol/vol and acetonitrile/methanol/2- propanol (7:3:1, vol/vol/vol) for α-tocopherol and cholesterol, respectively. All reagents used were HPLC-grade with a proven purity between 95% and 99.9% and were obtained from Sigma-Aldrich (St. Louis, MO). Identification and quantification of α-tocopherol and cholesterol were based on external standards obtained from Sigma-Aldrich, with purity ≥99.6%. All chemical analyses were performed in duplicate.

The degree of antioxidant protection (**DAP**), used to evaluate the antioxidant protection of foods (Pizzoferrato et al., 2007), was calculated as the molar ratio between tocopherols and cholesterol contents in cheese.

Sensory Analyses. Seventeen assessors (8 women and 9 men) aged 38 to 54 years, who each had 3 years of experience in the sensory evaluation of sheep cheeses, were involved, and the significance level was set at 0.05 to define sensory profiles of both CBS and CTRL cheeses. The pieces of cheese were served at room temperature using white plastic dishes, each marked using a random 3-digit code. The tasting station was lighted to prevent the perception of differences in colors of the samples. The samples were described by qualitative descriptive analysis, according to Stone et al. (1974). Attribute terms for evaluation of cheeses were developed by the 17 panelists using qualitative descriptive analysis methodology. Briefly, ballot development and panelist training were accomplished during 7 working sessions lasting 30 to 50 min. The descriptive terms developed for each major sensory attribute category are reported in Table 2. Each attribute was presented as a separate unstructured line scale that recorded panelist responses in increments of 0.1 between 1 (leftmost position) and 15 (rightmost position). The cheese samples were cubed (~1 cm each side) and were presented on white paperboard plates. The panelists also had available an entire transverse slice of each cheese for evaluating appearance attributes.

Calculations and Statistics

The general liner model procedure for repeated measures was used to test the effect of dietary treatment, time of cheese production (d 1–5), and their interaction as fixed factors on the chemical and fatty acid compositions of cheese. Least square difference was used for the multiple comparisons of the means.

Table 3. Effect of the dietary treatment on cheese composition

Item	Dietary treatment[1] (D)		SEM	*P*-value[2]		
	CTRL	CBS		D	Time (T)	D × T
Cheese composition (%)						
Moisture	35.7	34.8	0.687	0.390	<0.001	0.184
Fat	30.3	33.3	0.472	<0.001	0.009	0.012
Protein	25.8	23.7	0.414	0.003	0.260	0.360
Sodium chloride	2.24	2.22	0.069	0.907	0.016	0.387
Ash	5.89	5.96	0.099	0.751	0.198	0.838
Groups of fatty acids (g/100 g of cheese)						
∑SFA	20.04	22.32	0.329	<0.001	<0.001	0.012
∑MUFA	6.63	7.82	0.156	<0.001	0.031	0.242
∑PUFA	1.44	1.39	0.021	0.046	0.011	0.018
∑OBCFA[3]	1.15	1.28	0.022	<0.001	0.002	0.223
∑PUFA n-6	0.20	0.21	0.017	0.009	0.045	0.047
∑PUFA n-3	1.11	1.04	0.003	0.718	0.075	0.006
PUFAn6/n3	5.58	5.08	0.094	0.007	0.806	0.345
AI[4]	2.99	2.80	0.031	0.001	0.528	0.977
TI[5]	1.40	1.60	0.027	<0.001	0.139	0.194
HH[6]	0.517	0.545	0.005	0.001	0.249	0.988
α-Tocopherol						
µg/100 g of cheese	201.50	224.18	17.61	0.169	0.002	0.420
µg/g of fat	6.59	6.61	0.593	0.969	0.006	0.275
Total cholesterol						
mg/100 g of cheese	45.40	38.13	1.807	0.057	0.196	0.867
mg/g of fat	1.38	1.01	0.147	0.142	0.249	0.964
DAP ($\times 10^{-3}$)	4.20	5.29	0.384	0.067	0.050	0.668

[1]Dietary treatment consisted of hay ad libitum + 800 g/head per day of a control concentrate (CTRL) or a concentrate containing 11.7% cocoa bean shell (CBS).

[2]Probability of significant effect ($P \leq 0.05$).

[3]Odd- and branched-chain fatty acids.

[4]Atherogenic index, calculated as (12:0 + 4 × 14:0 + 16:0)/(MUFA + PUFAn-6 + PUFAn-3).

[5]Thrombogenic index: (14:0 + 16:0 + 18:0)/[(0.5 × MUFA) + (0.5 × PUFAn-6) + (3 × PUFAn-3) + (PUFA n-3/PUFAn-6)].

[6]Hypocholesterolemic/hypercholesterolemic ratio (*cis* − C18:1 + ΣPUFA)/(C12:0 + C14:0 + C16:0).

For OAC data, a one-way ANOVA was applied to log-transformed values of chromatograph area to test the effect of dietary treatment.

A general linear model was used to test the effect of dietary treatment on cheese sensory profile, with dietary treatment considered as fixed effect, whereas panelists and time were considered as random effects. Assessors to define cheese profiles were continuous bipolar from 1 (absent or nothing) to 15 (a lot), with the exception of both color paste and rind, in which 1 = white and 15 = yellow, and for dimensions of holes and slits, in which 1 = small and 15 = big. Least squares means were compared with test the effect of the treatment one-way ANOVA.

Principal components analysis was applied to the matrix (assessor × treatment × replicate rows; 27 columns) of original standardized sensory data using JMP 12 software (SAS Institute Inc.) to study the main tendencies in variation between cheeses. The most significant 2 principal components were analyzed using a factorial analysis.

Significance was declared when $P \leq 0.05$. Analyses were performed using JMP statistical software (SAS Institute Inc.) v.12.0.1.

RESULTS

Cheese Composition

Results for cheese composition, previously provided by Campione et al. (2021), who found that the feeding treatment with CBS inclusion affected the chemical composition of cheese, were reported in grams per 100 g of cheese (Table 3).

α-Tocopherol and cholesterol contents in ewe cheeses of both feeding groups were measured (Table 3). To evaluate the effects of diet, α-tocopherol and cholesterol were referred to as fat content. The diet did not affect either α-tocopherol or cholesterol contents of cheese fat. In cheese fat of both feeding groups, the α-tocopherol content was on average 6.6 µg/g, whereas the cholesterol content was on average 1.2 mg/g.

Table 4. Effect of the dietary treatment on cheese sensory profile (observations, n = 104)

Sensory item[1]	Treatment				Adjusted R^2	Treatment (F-ratio)	P-value
	CONTROL		CBS				
	LSM	SE	LSM	SE			
Appearance							
Rind color[2]	6.99	0.32	7.04	0.32	0.73	0.12	0.720
Paste color[2]	5.25	0.30	5.73	0.30	0.45	5.33	0.023*
Presence/absence either of holes or slits	2.44	0.41	2.81	0.41	0.56	3.67	0.059†±
Dimension of holes/slits[3]	2.07	0.32	2.38	0.32	0.67	5.59	0.020*
Odor							
Lactic	4.77	0.28	5.00	0.28	0.46	1.43	0.234
Animal/sheep	3.14	0.30	3.29	0.30	0.62	0.78	0.378
Spicy	2.87	0.41	3.20	0.41	0.63	3.35	0.071
Toasted	3.24	0.44	3.68	0.44	0.72	7.39	0.008**
Smoked	4.01	0.48	4.51	0.48	0.55	4.82	0.031*
Taste							
Sweet	3.63	0.33	3.06	0.33	0.66	11.89	0.001**
Acid	5.25	0.45	5.81	0.45	0.49	5.60	0.020*
Savory	3.52	0.30	3.77	0.30	0.64	3.05	0.085
Bitter	4.29	0.70	4.26	0.70	0.66	0.01	0.912
Astringent	4.58	0.38	4.74	0.38	0.41	0.43	0.5144
Aroma							
Yeast	4.67	0.44	4.90	0.44	0.56	1.16	0.284
Animal/sheep	2.95	0.29	3.08	0.29	0.73	0.89	0.348
Toasted	2.75	0.27	3.55	0.27	0.44	13.76	<0.000**
Smoked	3.01	0.30	3.63	0.30	0.37	8.52	0.011*
Tactile sensations/perception							
Soft/hard	4.95	0.34	5.44	0.34	0.39	4.03	0.048†
Oily	5.66	0.45	5.82	0.45	0.58	0.53	0.468
Elasticity	3.29	0.39	3.40	0.39	0.58	0.27	0.602
Plasticity	5.58	0.38	6.22	0.38	0.49	7.60	0.007**
Mouth sensations/perception							
Moisture	5.58	0.48	4.92	0.48	0.69	12.88	<0.001**
Soft/hard	5.18	0.29	5.27	0.29	0.34	0.29	0.591
Mellow	6.25	0.43	5.95	0.43	0.61	2.22	0.139
Soluble	5.74	0.30	5.50	0.30	0.60	1.90	0.172
Dispersion	5.16	0.32	4.84	0.32	0.52	2.76	0.100

[1]Except as indicated, assessments were scored from 1 = nothing to 15 = a lot.

[2]Assessor scale: 1 = white to 15 = yellow.

[3]Assessor scale: 1 = small to 15 = big.

†$P = 0.05$, marginally significant; *$P < 0.05$; **$P < 0.01$.

The DAP values showed the highest values in CBS cheese samples compared with CTRL, 5.29 versus 4.2, respectively.

Sensory Profile

The sensory profiles of both CBS and CTRL cheeses are reported in Table 4. Data showed an effect for treatment variable in different descriptors of the scale used to define the profile. Cheese's appearance was affected by the CBS inclusion in the diet. Particularly, the paste color of cheese was significantly more yellow for CBS compared with CTRL cheeses. Similarly, the dimensions of the holes in the paste were bigger in CBS compared with CTRL ($P = 0.02$), and a tendency was detectable for the presence of holes and slits to be slightly higher for CBS than for CTRL cheese ($P = 0.06$).

Within odor attributes, only toasted and smoked were perceived as significantly higher for CBS rather than CTRL cheese ($P < 0.01$).

Most of the taste attributes did not show significant differences between the 2 cheeses. The CBS cheese showed a significantly higher acid ($P = 0.02$) and lesser sweet ($P < 0.01$) taste compared with the CTRL.

All aroma attributes confirmed the same trends of odor attributes, with toasted and smoked aromas significantly higher in CBS cheese (toasted $P < 0.01$; smoked $P = 0.01$). Among tactile sensations, CBS cheese reported significantly higher plasticity ($P = 0.007$) and soft/hard perception ($P = 0.048$) than CTRL. Mouth sensations did not show significant differences between the cheese except for moisture, the only descriptor that was significantly lower ($P < 0.001$) in CBS compared with CTRL cheese.

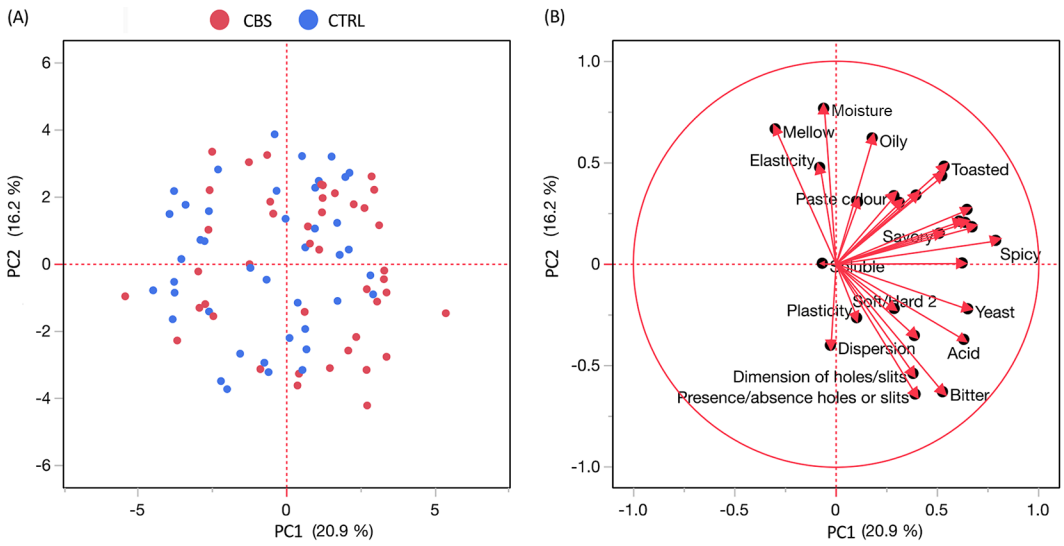

Figure 2. Principal components analysis plots: PC1 vs. PC2 for the dietary treatments (A) and for all 29 sensory attributes (B). CTRL = control cheese; CBS = cheese from milk of sheep fed experimental diet containing cocoa bean shell.

Principal components analysis on sensory attributes of both CBS and CTRL cheeses evaluated by panelists, is reported in Figure 2. The plots from the principal components analysis applied to sensory attributes explained 37.1% (principal component [**PC**]1, 20.9%; PC2, 16.2%) of the total variation. Oily, paste color, toasted, savory, and spicy attributes were the main factors in the positive PC1, whereas moisture, mellowness, and elasticity were the dominating factors along the negative PC2 zone. The remaining attributes were located in the negative PC1 zone.

The score plot showed that most of the CBS cheeses were located within the negative PC1 zone, displaying a higher acid bitter taste and toasted, spicy, and yeast aromas, as well as higher perception of holes presence and dimension.

The CTRL cheeses, mainly located in the PC2 zone, were described with more mellow and moist mouth sensations, and more plastic tactile sensations.

OAC Profile

A total of 6 CTRL and BCS cheese batches were analyzed by gas chromatography/olfactometry (**GC/O**) and GC/mass spectrometry. Generally, CTRL and CBS cheese samples showed poor and very similar volatile profiles (Supplemental Table S1, see Notes). No significant differences in number and type of volatile compounds between CTRL and CBS groups were found. As shown by MS, all samples showed a high number of non-odorant volatile compounds.

Up to 47 different OAC in cheeses were detected for the experiment, as revealed by MS, belonging to the following chemical classes: acids (8 compounds), alcohols (5 compounds), aldehydes (3 compounds), alkane (1 compound), aromatic hydrocarbons (7 compounds), ester (11 compounds), ketone (4 compounds), lactone (1 compound), pyrazine (2 compounds), and terpene (5 compounds). Not all of these OAC were detected in each cheese sample, and only some were found in all cheeses. In Supplemental Table S2 (see Notes), OAC detected by both GC/O and MS are presented in detail for each batch of cheeses (1–5) analyzed per CTRL and CBS.

For the acids chemical class, the CBS cheeses compared with CTRL presented the decanoic acid OAC with a fat rancid odor perception in all batches. For alcohol, the phenylethyl alcohol OAC was mostly present in CTRL cheese samples, meaning a major presence of rose odor perception. For aldehydes, the CBS cheeses compared with CTRL presented the OAC nonanal in fewer batches, meaning a lesser presence of green odor perception. The ester chemical class was low in the octanoic acid methyl ester, implying an orange presence in both CBS and CTRL, and is even less in CBS; meanwhile the decanoic acid ethyl ester (meaning fruit) is present in both CTRL and in all batches of the CBS treatment. For the ketone chemical class, 2-octanone OAC was more abundant in batches of the CBS cheeses than CTRL cheeses, indicating a soap odor perception. On the contrary, for 2-undecanone, meaning orange odor, perception was consistently present in CBS batches. In the sulfur chemical class, the OAC dimethyl

Table 5. Means of ln (x + 1) area of odor-active compounds grouped by chemical families in tested CTRL and CBS cheeses

				Treatment[1]			
Chemical family	LRI[2]	RT	Observation	CTRL	CBS	*F*-ratio	*P*-value
Acids	1,123	20.32	78	18.5	17.84	4.54	0.04*
Alcohol	1,118	13.72	20	16.27	16.19	0.14	0.72
Aldehyde	1,032	11.39	25	15.5	15.2	0.27	0.61
Ester	1,089	13.17	85	17.79	17.35	2.04	0.15
Ketone	1,107	13.48	53	15.61	15.12	1.4	0.24
Sulfur	925	8.42	6	14.93	14.51	26.21	<0.01**
Terpene	986	10.1	20	16.1	15.64	0.2	0.65

[1]CTRL = control cheese; CBS = cheese from milk of sheep fed experimental diet containing cocoa bean shell.
[2]LRI = linear retention index.
*$P < 0.05$; **$P < 0.0$.

sulfone was present in only a few batches of the CBS cheeses. Finally, the terpene chemical class was more present in CBS batches, with α-pinene (green and fresh perception). β-Myrcene and d-limonene, spicy and fresh lemon, respectively, were present as unique compound in CTRL batches. All the other OAC were present in similar amounts in both dietary treatments.

By transforming data of area (Table 5), we found the OAC to be very similar among the 2 groups, with few exceptions. Among the acid chemical class, decanoic acid (fat, rancid odor perception) was marginally significant in the control rather than in the treatment group ($P = 0.05$). For the sulfur chemical class, dimethyl sulfone was significantly higher in the control group rather than in the treatment group ($P < 0.01$). These 2 were the only OAC that presented differences between the 2 groups.

DISCUSSION

Cheese Chemical Composition

The inclusion of CBS in dairy sheep diet affected the chemical composition of cheese, as previously described in Campione et al. (2021). Particularly, a greater percentage of fat content was found in CBS cheese compared with CTRL samples. It is well known that animal diet is one of the main important features influencing the quality of ruminant products. Specifically, it is amply reported that a diet higher in fat usually increases the fat content in milk and cheese, influencing their physicochemical and nutritional properties (Ashes et al., 1997).

Likewise, the fatty acid composition of ruminant-derived products can also be influenced by the diet (Chilliard et al., 2007). The hypocholesterolemic/hypercholesterolemic (**H**/**H**) ratio was higher in the CBS cheese than in the CTRL samples; both values were within the range of the results published by Chen and Liu (2020) and Bodnár et al. (2021). Higher values of the H/H ratio are considered more beneficial for human health (Paszczyk et al., 2020).

Concerning the vitamin content, although the presence of vitamin E in the cocoa shells was reported by other authors (Rojo-Poveda et al., 2020), no diet effect was found on cheese α-tocopherol contents. However, in contrast to CBS, the inclusion of other byproducts such as hazelnut peels in ovine feed formulation, as reported by Marino et al. (2021), significantly increased α-tocopherol contents in cheese. In addition, there were no significant differences in cholesterol contents in cheese fat between animal feeding groups. Compared with cocoa beans, Agus et al. (2018) found a lower content of cholesterol in cocoa bean shells, and stigmasterol was the predominant phytosterol. However, the effect of CBS supplementation on ewe cholesterol metabolism is unknown. Duong et al. (2019) reported that high-phytosterol feeds consumed by cattle had an insignificant effect on the phytosterol and cholesterol contents in milk. However, the cholesterol content found in cheese of both feeding groups in this study was half that found by other authors in Pecorino cheese (Marino et al., 2021; Martini et al., 2021). Losses and degradation of cholesterol during cheesemaking could be one explanation of these results. The use of nonhomogenized thawed milk may have affected the stability of fat globules and tocopherol during cheese manufacture, which could also explain the variability found among the cheeses from the same batch. It is known that the freezing process could damage the milk fat globule membrane, which may become more susceptible to oxidation (Bottiroli et al., 2020). In this regard, the potential cholesterol oxidative stability was also investigated by calculating the DAP as reported by Pizzoferrato et al. (2007). We found no differences in the DAP values between animal feeding groups. The DAP values were 4.2 and 5.3×10^{-3} in both CTRL and CBS cheese samples, respectively. On the basis of the minimum threshold level of 7×10^{-3} for cholesterol oxi-

dative stability suggested by Pizzoferrato et al. (2007), it cannot be excluded that oxidation may also have occurred in both CTRL and CBS cheese samples.

Effect on Sensory Profile

Regarding the sensory profile, the statistical analysis of the attribute ratings collected from 17 trained panelists evidenced that sheep feeding had some effects on several descriptors of the 2 groups' cheeses.

The cheese paste color was the appearance parameter that showed a higher score in CBS cheese than CTRL, with more yellowness. Color is a very important attribute of foods and serves as an index of quality (Fox et al., 2017), since consumers associate specific colors with certain flavors (Wadhwani and McMahon, 2012). Usually, cheeses made from sheep milk are whiter than similar cheeses made from bovine milk, since cattle transfers carotenoids to adipose tissue differently from sheep (Fox et al., 2017). Particularly, β-carotene, due to its cleavage, catalyzed by enzyme activity to retinal in the sheep liver, is not measurable in sheep milk, and therefore does not affect color changes in the resulting Pecorino cheese (Cardinault et al., 2006; Serrapica et al., 2020). This yellow color could be related to the chemical changes during ripening, such as proteolysis of casein, becoming less white, and the prevalence of reflecting components (Johnson, 1999). However, the fat composition can also affect the color of cheese. According to Rohm and Jaros (1997) the higher total MUFA content shown by CBS cheese than CTRL can be regarded as the most important descriptor of cheese body color, and the increase of fat lipolysis with ripening progress could be responsible for the increase in the yellow color (González-Martín et al., 2020).

Appearance attributes related to holes were perceived as greater in CBS cheese, as a possible consequence of microbial activity from the aging process, normally related to slight gas production (Fox et al., 2000).

It was also observed that cheeses from the CBS diet presented greater intensity of odor, aroma, and taste, and the characteristics of feeding CBS were reflected in toasted and smoked sensations. This could be related to the fact that the secondary compounds that derive from the feeding can be easily transferred to milk or cheese (Wiedenhoeft and Barton, 1995).

Regarding taste attributes, although acid and sweet parameters were found to be significantly higher in CBS than CTRL cheese, the scores were numerically similar between the 2 groups.

The higher tactile plasticity and hard sensation as well as the lesser mouth sensation of moisture in CBS cheese could be related to lesser moisture content (34.8% vs. 35.7) and also to differences related to the aging process. Indeed, during ripening, several biochemical processes occur in cheese, which give rise to important changes in the texture and sensory characteristic of cheeses, varying from batch to batch (McSweeney 2004). Thus, it is reasonable to hypothesize that CBS dietary inclusion affected the cheese sensory profile during the aging process, without altering the acceptance of the product.

It is amply reported that dietary composition, when byproducts are included, affects ruminal fermentation, and milk composition could be modified to a lesser or greater extent (Chilliard et al., 2003; Romero-Huelva et al., 2017), as well as its technological properties and the sensory quality of the derived dairy products (Coulon et al., 2004). Several studies have described the influence of the use of byproducts in the diet of sheep on the characteristics of cheeses; however, only a few of these studies included the effect on sensory profile. Caccamo et al. (2019) evaluated how the inclusion of hazelnut skin in the diets of dairy ewes can affect the chemical and sensory characteristics of ovine cheeses, showing that this byproduct had significant effects, and revealed a minor production of off-flavors associated with spicy and acid characteristics. Also, the inclusion of artichoke silage in sheep's diets had a positive effect on the sensory characteristics of 60-d-ripened cheese (Jaramillo et al., 2010), as did inclusion of grape pomace on the sensory profile in cheeses until 120 d of ripening (Bennato et al., 2023). Instead, no significant differences were found in cheese sensory profiles when using levels up to 30% of citrus byproducts (Jaramillo et al., 2009) or spray-dried olive mill wastewater (Branciari et al., 2020) as part of the diet in dairy ewes.

Therefore, although several studies on the sensory properties of ripening Pecorino cheese were conducted, our results are the first that report the effect on the sensory profile of cheese affected by CBS diet inclusion in dairy sheep. The significant differences reported did not negatively affect the overall acceptability of the product, confirming how this diet inclusion can be suitable as an alternative feed resource in ruminants' nutrition.

The evaluation of these byproducts demonstrated that diets supplemented with these alternative feeding resources supply the animal nutritional requirements, without compromising milk and cheese quality in small ruminants (Vasta et al., 2008).

The sensory characteristics reported for CBS cheese, such as higher hardness and more intense toasted and smoked taste and aroma, could improve the acceptability of the product by consumers who particularly appreciate tasty and flavored cheese (Bennato et al., 2023), making this alternative feeding a suitable strategy for the reuse and thus the enhancement of CBS.

A further step could be the development and evaluation of a preference map to understand consumer behavior

regarding CBS cheese, by using a hedonic scale provided to consumers. This can help to understand which are the best attributes that particularly fit consumer preferences (Worch and Piqueras-Fiszman, 2015; Qannari 2017).

Effect on OAC Profile

Dietary CBS inclusion in dairy sheep partially exerted some effect on the OAC profile of cheeses.

To the best of our knowledge, no information can be found in the literature regarding the effect of a diet containing CBS on the volatile compounds of cheese. The OAC analysis identified compounds belonging to 7 different chemical families: acids, alcohols, aldehydes, esters, ketones, sulfur, and terpene, most of which are composed of esters, which, in dairy products, originate from lactose fermentation or from amino acid catabolism acids (Bertuzzi et al., 2018). However only acids and sulfur compounds were significantly different between the 2 cheese groups, with lower value perception in CBS cheese.

Sulfur compounds, widely detected in surface-ripened cheese, in the correct balance are major contributors to the characteristic strong flavor of different types of cheeses, derived from the catabolism of free fatty acids (Fox et al., 2017; Bertuzzi et al., 2018).

Acids were the most abundant volatile compounds in both 2 cheese treatments; these usually strongly contribute to the aroma of different cheese varieties, including Pecorino, Manchego, and Cheddar (Frank et al., 2004; Barron et al., 2005).

Particularly, butanoic acid abundance was lower in CBS than in CTRL, resulting in a lesser perception of cheesy or putrid odors in CBS than in CTRL samples (Thomsen et al., 2012). Several authors have attributed this flavor to an increase in concentration of short-chain free fatty acids or to unbalanced proteolysis during cheese ripening (Fox et al., 2017; McSweeney, 2017).

In the present study, the difference between the 2 cheese groups could be related to the significant difference reported in fatty acid contents, with lower PUFA content in CBS compared with CTRL cheese. According to the high content of fatty acids detected in both the cheese groups, lipolysis could be the major pathway responsible for flavor generation (Zabaleta et al., 2016).

Literature shows that the volatile odor difference in cheese samples could be due to the different fatty acid composition of cheeses, since the strong relation between milk fat and flavor development in cheese is known (Hassan et al., 2013). At the same time, the variation in milk composition, including fat content, induced by dietary management can be reflected in milk and cheese flavors, according to the mechanism involved in aroma compound development (Marilley et al., 2004).

In accordance with other studies that evaluated the effects of byproduct inclusion in dairy sheep diet on the OAC of cheese, our work revealed that CBS dietary inclusion exerted a partial effect on odor compound development in cheese, without altering the acceptability of the product, as also confirmed by the sensory analysis.

The findings of this study are useful both for the sensory and animal feeding fields. Indeed, the inclusion of CBS byproducts in the diets of dairy sheep did not produce unequivocal results.

The role of the small ruminant industry is important, mainly in the rural communities of Mediterranean countries (Pulina et al., 2018). The use of CBS byproducts in dairy sheep feeding, discounting some issues related to theobromine in the literature, easily resolved by following a limit (maximum of 300 mg/kg in feedstuff; EFSA, 2008), can be considered a useful strategy to enhance this waste by using them in animal feeds.

The slight differences observed in the sensorial properties between CBS and CTRL cheeses did not alter the overall acceptability of the products as well as organoleptic characteristics, revealing how this dietary inclusion can be useful in dairy sheep farming without altering the quality of the final products.

The use of human-inedible feeds for ruminants, which do not require arable land, should be supported to further improve the sustainability of food manufacture (Halmemies-Beauchet-Filleau et al., 2018) including dairy production. Moreover, improving knowledge of the effects of the use of byproducts in ruminant feeding on milk composition and related products could help cheesemakers understand the nature of the milk components that influence the cheesemaking properties and overall qualities of sheep dairy products (Cabiddu et al., 2006).

Cheese from dairy sheep fed with CBS did not show any negative differences compared with CTRL samples, with attributes in line with consumers' preferences. Thus, the use of CBS as alternative feedstuffs can be compatible with the growing sustainable livestock systems.

CONCLUSIONS

The present study evaluated the effects of the inclusion of cocoa bean shells in the diet of dairy sheep on the composition, volatile, antioxidant, and sensory characteristics of cheese. The higher MUFA content and increased nutritional indices suggest a favorable role of cocoa bean shell dietary inclusion on the nutritive value of the cheese. In terms of cheese sensory profile, cocoa bean shells affected appearance, odor, aroma, and taste without altering the acceptance of the product. The volatile profile showed only a few significant differences, mainly related to the cheese ripening process, and no differences were found in α-tocopherol contents in cheese

fat between the 2 groups. Hence, incorporating cocoa bean shells into the diets of dairy sheep enabled the production of high-quality cheese without compromising its characteristic traits, aligning well with the traditional profiles of sheep cheese. Furthermore, the use of this byproduct could contribute to decreasing feed costs and waste management, representing a good practice for increasing the sustainability of dairy products.

NOTES

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Nonstandard abbreviations used: CBS = cocoa bean shells; CTRL = control diet; DAP = degree of antioxidant protection; FAME = fatty acid methyl ester; GC/O = gas chromatography/olfactometry; H/H ratio = hypocholesterolemic/hypercholesterolemic ratio; OAC = odor active volatile compounds; PC = principal component.

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ORCIDS

M. Caccamo https://orcid.org/0000-0003-3500-0724
V. M. Marino https://orcid.org/0000-0002-5612-899X
C. Pasta https://orcid.org/0009-0005-2388-4771
A. Natalello https://orcid.org/0000-0002-9802-2501
G. Mangione https://orcid.org/0000-0001-7200-341X
B. Valenti https://orcid.org/0000-0002-5737-9862