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Native and Non-Native Sheep Breed Differences in Canestrato Pugliese Cheese Quality: a Resource for a Sustainable Pastoral System

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Abstract

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Canestrato Pugliese is an Italian uncooked hard cheese made by the Protected Designation of Origin (PDO) status. In the past, it was manufactured with milk from local sheep breeds (Altamurana and Leccese) while in recent years it has almost entirely been made with milk from non-native sheep breeds (Sarda and Comisana). The aim of the study was to investigate the breed effect on the quality of Canestrato Pugliese cheese by comparing two native (Altamurana and Leccese) and two non-native (Sarda and Comisana) sheep breeds. The experiment was carried out at the experimental farm of CREA-ZOE (Apulia region, Southern Italy) using a flock set-up of four sheep breeds: Altamurana, Leccese, Sarda, and Comisana. All sheep fed pasture supplemented with 200 g/sheep/day concentrate at each milking. For each breed, three cheese-makings of Canestrato Pugliese were carried out for three consecutive days following the PDO technology. At two and four months of ripening, cheese was analysed for gross composition, fatty acid profile, nutritional indexes, and volatile organic compounds. Significant differences were found between breeds in the fatty acid profile and nutritional indexes ($P \leq 0.05$). Canestrato Pugliese from Comisana, Leccese, and Sarda had a higher dry matter and fat content than that from Altamurana breed ($P \leq 0.05$). Cheeses from Altamurana and Comisana showed a higher content of unsaturated and omega-3 fatty acids and a better omega-6/omega-3 ratio than the others ($P \leq 0.05$). The best Health Promoting Index was detected in Altamurana, Comisana, and Leccese cheeses ($P \leq 0.05$). Additionally, sheep breed affected the content of volatile organic compounds ($P \leq 0.05$). The highest value of volatile organic compounds was observed in cheeses from Leccese breed ($P \leq 0.05$). The discriminant analysis performed on cheese data shows a separation between native and non-native sheep breeds. The present study reveals that the breed has an evident effect on the fatty acid and volatile organic compound profile of Canestrato Pugliese.

Keywords: native breed; pecorino cheese; nutritional quality; volatile organic compounds

Local breeds, less frequently used in intensive systems but still preserved *in situ* in marginal areas, represent an important resource for maintaining animal biodiversity (Oldenbroek 2007). In Mediterranean regions most of the dairy sheep are found in the least favoured areas, where small ruminants are often the only possible animal production activity (de Rancourt *et al.* 2006). In these areas, local breeds represent an important resource for a sustainable pastoral system thanks to their excellent adaptation to specific environmental conditions. Furthermore, native breeds are linked to traditional products and to several practices that are part of cultural heritage. A 'typical' product is a result of several factors including raw material, transformation process, and sensory characteristics. All these peculiarities are

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closely related to the geographical origin and to the social and cultural traditions of the production area (Scintu & Piredda 2007; Di Trana *et al.* 2015).

Canestrato Pugliese is an Italian uncooked hard cheese endorsed by Protected Designation of Origin status (PDO; CEE Regulation No. 1107/96). The cheese derives its name and traditional shape from the rush basket 'Canestro' in which the curd is ripened. Canestrato Pugliese is manufactured only in Bari and Foggia provinces (Apulia region, Southern Italy) exclusively from sheep milk. In the past, this cheese was manufactured from the milk of local sheep breeds (Altamurana and Leccese). In recent years, as a result of partial substitution of native sheep breeds with non-native ones, Canestrato Pugliese cheese has almost entirely been made from the milk of Comisana and Sarda breeds, native to Sicily and Sardinia regions, respectively.

Cheese is a biochemically dynamic product and undergoes significant microbiological and biochemical changes during its ripening period. Cheese ripening clearly involves a very complex series of interrelated events, which are caused by enzymes from the milk, from the rennet and from the microorganisms associated with the cheese, resulting in the development of the flavour and texture characteristic of the cheese variety (McSweeney 2004). Dairy product composition is dependent on a large number of factors linked to the animal management system, climatic conditions, feeding strategy, and animal physiological stage (Di Trana *et al.* 2004; De La Fuente *et al.* 2009). Among the very wide and diverse range of factors that affect the composition of cheese curd and, hence, the quality of the final product, an effect of sheep breed on cheese chemical composition and sensory characteristics has also been reported (Ferreira *et al.* 2009; Esposito *et al.* 2014; Kawecka & Sosin-Bzducha 2014). Taking into account that the reduction of animal biodiversity is often due to the marginalisation of traditional production systems and to their associated breeds, it is strategic to understand if the use of milk from native or non-native breeds may affect the quality of Canestrato Pugliese cheese.

In this context, the aim of the study was to investigate the breed effect on production quality of Canestrato Pugliese cheese (ripened two and four months), in terms of gross composition, fatty acid profile, nutritional indexes, and volatile organic compounds, by comparing two native (Altamurana and Leccese) and two non-native (Sarda and Comisana) sheep breeds.

MATERIAL AND METHODS

Animals and diets. The experiment was carried out at the experimental farm of Council for Agricultural Research and Economics Research - Unit for Extensive Husbandry (CREA-ZOE) located at an altitude of 76 m a.s.l. in the Apulia region (Southern Italy) during spring. A flock set-up of four breeds of mature sheep was used. In order to compare native and non-native breeds, the flock was set up with two native breeds, Altamurana (A) and Leccese (L), and two non-native breeds, Comisana (C) and Sarda (S). All animals were homogeneous for days in milk (90 ± 6) and body condition score (2.75). The flock grazed on native pasture during the day and was housed in shaded open pens during the night. All sheep were supplemented with 200 g/sheep/day concentrate at each milking. For each breed, three cheese-makings of Canestrato Pugliese were carried out for three consecutive days following the PDO technology. The flowchart of Canestrato Pugliese cheese manufacture is shown in Figure 1. Cheeses were ripened for two and four months in a natural cave (at 14–16°C and 75–80% relative humidity) at CREA-ZOE of Bella (Basilicata region, Southern Italy).

Milk and cheese composition. Milk samples were analysed for fat, protein, and lactose by infrared spectroscopy, and for somatic cell count (SCC) by fluoro-opto-electronic flow cytometry (Hanuš *et al.* 2011) using a CombiFoss 6000 instrument (Foss

Raw milk
↓
Heating of milk at 37°C
↓
Addition of liquid calf rennet
(20 ml/100 kg milk)
↓
Coagulation of milk after 25 min at 37°C
↓
Cutting of the curd
(size of the curd after cutting: 0.5–1.0 cm)
↓
Heating the whey (45°C for 2 min)
↓
Removal of the whey, moulding, and pressing
↓
Holding of the curd at 22°C for 24 h
↓
Salting of the curd by spreading over the surface
↓
Ripening for 2 and 4 months

Figure 1. Flowchart of Canestrato Pugliese cheese-making

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Electric, Hillerød, Denmark); pH with HI 9025 pH meter (Hanna Instruments, Ann Arbor, USA).

Cheese samples were analysed for protein (Kjeldahl method; IDF 1986), fat (Gerber method; IIRS 1955), moisture (oven drying at 102°C; IDF 1982). For pH measurement, the grated cheese samples (10 g) were mixed with equal quantities of distilled water (10 ml) in a stomacher bag and the pH of dispersion was measured potentiometrically using a pH meter (HI 9025; Hanna Instruments Inc., USA) (IDF 1989).

Fatty acid profile and nutritional indexes. Lipid fraction of cheese was extracted according to Bligh & Dyer (1959). Fatty acid methyl esters (FAME) were separated and quantified as reported by Di Trana *et al*. (2004) using a gas chromatograph (Varian model 3800) fitted with an automatic sampler (CP 8410) for a multiple injection and equipped with a flame ionisation detector. A fused silica capillary column coated with 100% cyanopropyl polysiloxane [60 m, 0.25 mm (i.d.), 0.25 μm film; DB 23, J and W; Supelco] was used to analyse the FAME content. FAME peaks were identified with reference to the retention time of FAME standards (Sigma-Aldrich, St. Louis, USA; Larodan Fine Chemicals, Malmo, Sweden).

The quality of the fatty acid profile of cheese samples was evaluated using some nutritional indexes. Thrombogenic Index (TI) was calculated as described by Ulbricht and Southgate (1991). The Health Promoting Index (HPI) was calculated as suggested by Chen *et al*. (2004): total unsaturated FA/[C12:0 + (4 × C14:0) C16:0].

Determination of volatile components. Volatile organic compounds (VOCs) were determined by dynamic headspace (DHS) analysis (Ciccioli *et al*. 2004) using capillary gas chromatography with a mass selective detector (GC/MS). A sample of 3 g of cheese was weighed into a 25 ml glass container. Subsequently, samples were purged with 50 ml/min pure helium gas at 40°C in a water bath for 1 h to isolate headspace volatiles. VOCs were trapped in a glass tube packed with Tenax TA, 60/80 mesh, and Carbopack B, 60/80 mesh (both Supelco, Bella Fonte, USA) at a ratio of 2 : 1, respectively. The trapped compounds were thermally desorbed at 220°C for 5 min into an automatic thermal desorption system (TDS2; Gerstel GmbH & Co.KG, Mülheim an der Ruhr, Germany) and cryofocused at –150°C before being injected by heating at 250°C in the PTV inlet of CIS4 injector (Agilent Technologies, Wilmington, USA). VOCs were separated with Agilent HP-1MS fused silica capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness) in Agilent model 6890 N GC in the following conditions: helium flow rate 2.2 ml/min; splitless injection mode; oven temperature programme: 40°C for 10 min, then 10°C/min to 150°C held for 12 minutes. The GC column was connected to the ion source (250°C) of an Agilent MSD 5973 quadrupole mass spectrometer (interface line 280°C), operating in the scan mode (40–400 amu). Ionisation was done by electronic impact at 70 eV.

The compounds were identified by comparing their mass spectra with those in the Wiley 7N Mass Spectral Database and their retention time. Data were expressed as arbitrary unit (a.u. = peak area × 10^{-6}).

Statistical analysis. The statistical analysis of chemical composition, FA profile, and VOC profile data was performed by ANOVA procedure (Systat 13, 2009) with sheep breed (Altamurana, Leccese, Comisana, and Sarda) as the main factor. Differences between means were tested using Fisher's LSD test. Statistical differences were considered significant at $P \leq 0.05$.

The data concerning the fatty acid profile, nutritional indexes, and the VOC profile of cheeses were pooled per breed before submitting them to the canonical discriminant analysis to ascertain the discriminant effect of the above-mentioned variables at two and four months of ripening. Wilk's Λ was used as the statistical selection criterion to determine the addition or removal of variables in the discriminant function. For better visualisation, the canonical scores were plotted in the discriminant space.

RESULTS AND DISCUSSION

Milk and cheese chemical composition. As shown in Table 1, sheep breed affected the pH value and protein content of the milk used for cheese manufacture ($P \leq 0.05$). The native Altamurana and Leccese breeds showed a higher protein percentage than Comisana and Sarda breeds (6.03 and 6.02% vs 5.83 and 5.76%, respectively; $P \leq 0.05$). Milk from Leccese, Altamurana, and Comisana breeds was characterised by the higher pH value compared to Sarda breed (6.57, 6.54, and 6.50, respectively; $P \leq 0.05$). A significant breed effect on the milk chemical composition was previously reported by Kawecka and Sosin-Bzducha (2014), using two indigenous Polish breeds reared under the same environmental conditions.

The physicochemical characteristics of Canestrato Pugliese cheeses from different breeds at two and four months of ripening are shown in Figure 2. Cheeses

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Table 1. Mean values[$] for the gross composition parameters of milk of different breeds

	Altamurana	Comisana	Leccese	Sarda	SEM	*P*
pH	6.54^{a}	6.50^{a}	6.57^{a}	6.45	0.02	**
Fat (%)	7.51	8.06	8.11	7.91	0.24	ns
Protein (%)	6.03^{a}	5.83^{b}	6.02^{a}	5.76	0.10	*
Lactose (%)	4.49	4.40	4.53	4.55	0.08	ns
SCC[&] (10^3/ml)	1162	1865	1260	1436	56	ns

[$]average value for the cumulative milk sample of three days of cheese-making for each breed analysed in duplicate; [&]SCC – somatic cell count; means with different letters within the same row differ at $P < 0.05$; **$P \leq 0.01$; *$P \leq 0.05$; ns – not significant

from Sarda, Comisana, and Leccese had higher dry matter content than the cheese from Altamurana, both at two and four months of ripening ($P \leq 0.001$). A breed effect was also detected on the fat content of cheese ripening for two months ($P \leq 0.001$). Cheese obtained from Sarda, Leccese, and Comisana milk had higher fat content compared to cheese made from Altamurana milk (35.83, 35.33, and 34.83 vs 29.33%, respectively; $P \leq 0.05$). The breed effect on protein content reported above for milk was not observed either in two or four months ripened cheeses. The highest pH value was detected in cheese from Altamurana breeds both at two and four months of ripening (5.47 and 5.45 respectively; $P \leq 0.05$).

The values obtained for the gross composition parameters of Canestrato Pugliese at two months of ripening are in agreement with those previously reported for the same cheese by Albenzio *et al.* (2001) (ca. 26.5% for protein, 30% for fat, and from 36.5% to 38.0% for the final moisture content).

A few studies have been published in attempt to elucidate the effect of breed on the cheese chemical composition and results are often contrasting. Comparing some hard cheeses produced in several parts of Spain from different ewe breeds, Gonzalez Vinas *et al.* (1999) observed no significant differences in the physicochemical composition. On the contrary, using two indigenous Polish sheep breeds (Coloured Mountain Sheep and Podhale Zackel), Kawecka and Sosin-Bzducha (2014) detected a breed effect on the chemical composition of 'oscypek', a traditional cheese dried for 24 h and smoked for four days. Recently, Esposito *et al.* (2014) reported a negligible breed effect on the chemical composition of Pecorino cheese, ripened for 106 days, comparing Appenninica and Sarda sheep breeds.

Fatty acid profile and nutritional indexes. The fatty acid profile and nutritional indexes of Canestrato Pugliese cheeses from the milk of different breeds are shown in Table 2.

The fatty acid (FA) profile of Canestrato Pugliese cheese at two months of ripening was significantly influenced by breed, except for the content of total *trans* FA. The short-chain FA were higher in Leccese cheese than in the others (34.82 g/100 g FA; $P \leq 0.05$). The highest medium-chain FA content was found in cheese from Sarda milk (35.31 g/100 g FA; $P \leq 0.05$) while the highest long-chain FA were detected in Comisana cheese (35.39 g/100 g FA; $P \leq 0.05$). The saturated FA (SFA) content was higher in cheese from Sarda milk than in the others (75.80 g/100 g FA; $P \leq 0.05$). The monounsaturated FA (MUFA) value was higher in cheeses from Comisana, Altamurana, and Leccese breeds compared to Sarda breed (22.46,

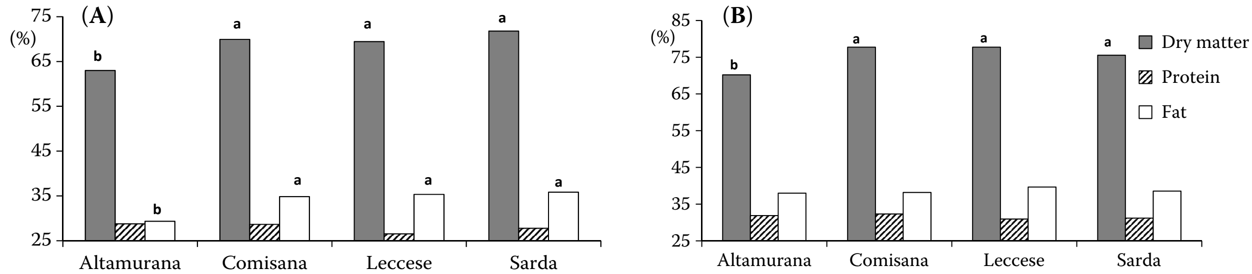

Figure 2. Mean values for the gross composition parameters of Canestrato Pugliese cheese at two months (**A**) and four months (**B**) of ripening

Mean values marked by different superscript letters differ statistically ($P \leq 0.05$)

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Table 2. Effect of sheep breed on the fatty acid profile (g/100 g of FA) of Canestrato Pugliese cheese ripened two and four months

	Ripening period											
	two months						four months					
	Altamurana	Comisana	Leccese	Sarda	SEM	*P*	Altamurana	Comisana	Leccese	Sarda	SEM	*P*
SCFA	33.25^{ab}	32.33^{b}	34.82^{a}	33.65^{ab}	0.47	**	23.844	26.928	24.413	26.626	1.00	ns
MCFA	32.24^{b}	32.29^{b}	31.29^{c}	35.31^{a}	0.21	***	37.107^{ab}	35.426^{ab}	33.954^{b}	38.887^{a}	0.99	**
LCFA	34.51^{ab}	35.39^{a}	33.899^{b}	31.04^{c}	0.38	***	39.051	37.652	36.077	34.49	1.27	ns
SFA	73.78^{b}	73.14^{b}	74.39^{ab}	75.80^{a}	0.37	***	70.23^{b}	71.66^{ba}	71.24^{ab}	73.44^{a}	0.62	*
MUFA	21.89^{a}	22.46^{a}	21.70^{a}	20.36^{b}	0.29	***	25.20^{a}	23.80^{ab}	24.33^{ab}	22.32^{b}	0.54	**
PUFA	4.32^{ab}	4.39^{a}	3.91^{bc}	3.83^{c}	0.16	*	4.56	4.54	4.43	4.24	0.14	ns
ω-3 PUFA	0.88^{ab}	0.90^{a}	0.69^{b}	0.73^{ab}	0.05	*	0.76	0.85	0.73	0.72	0.05	ns
ω-6 PUFA	2.45^{a}	2.44^{a}	2.25^{ab}	2.12^{b}	0.09	*	2.63	2.58	2.60	2.37	0.09	ns
ω-6/ω-3	2.84^{b}	2.74^{b}	3.28^{a}	3.00^{ab}	0.11	**	3.462	3.128	3.537	3.315	0.13	ns
CLA	0.47^{b}	0.55^{a}	0.46^{b}	0.49^{b}	0.01	***	0.57^{a}	0.57^{a}	0.52^{b}	0.57^{a}	0.01	**
Total Trans	1.04	1.07	1.02	1.17	0.04	ns	0.98^{b}	1.11^{a}	0.93^{b}	0.98^{b}	0.03	***
C18:2 n6	1.71^{a}	1.65^{a}	1.64^{a}	1.44^{b}	0.04	***	1.90^{a}	1.68^{b}	1.85^{a}	1.66^{b}	0.05	**
C18:3 n3	0.41^{b}	0.47^{a}	0.41^{b}	0.36^{b}	0.01	***	0.46	0.46	0.45	0.42	0.02	ns
TI	2.26^{b}	2.21^{b}	2.32^{b}	2.61^{a}	0.05	***	2.385^{b}	2.348^{b}	2.4^{b}	2.667^{a}	0.04	***
HPI	0.50^{a}	0.52^{a}	0.51^{a}	0.40^{b}	0.01	***	0.49^{a}	0.50^{a}	0.49^{a}	0.41^{b}	0.01	***

SCFA – short-chain fatty acids (C4-C10:1); MCFA – medium-chain fatty acids (C11-C16:1); LCFA – long-chain fatty acids (C17-C22:6 n3); SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; ω-3 – omega-3 fatty acids; ω-6 – omega-6 fatty acids; CLA – conjugated linoleic acid; means with different letters within the same row differ at $P < 0.05$; ***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$; ns – not significant

21.89, and 21.70 g/100 g FA vs 75.80 g/100 g FA, respectively; $P \leq 0.05$). The highest polyunsaturated fatty acid (PUFA) content was detected in the Canestrato Pugliese cheese derived from Comisana milk (4.39 g/100 g FA; $P \leq 0.05$). Significant differences in SFA, unsaturated FA and MUFA content were previously reported by Kawecka and Sosin-Bzducha (2014) in cheese from two indigenous Polish sheep breeds.

From a nutritional point of view, the omega-6 PUFA/omega-3 PUFA ratio (Σω-6/Σω-3) is of considerable importance. Indeed, a recent investigation indicates that excessive amounts of ω-6 and a very high Σω-6/Σω-3 ratio, as found in today's Western diets, promote the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases (Simopoulos 2011). Opportunities to enhance omega-3 FA in many foods, including dairy products, are still being explored. The optimal Σω-6/Σω-3 ratio varies from 1/1 to 4/1 depending on the disease under consideration (Simopoulos 2011). In our experiment, the optimal Σω-6/Σω-3 ratio was detected in cheeses from Altamurana and Comisana milk (2.84 and 2.74; $P \leq 0.05$).

A significant effect of breed was also found on the conjugated linoleic acid (CLA) isomer content and on the level of its precursors, linoleic acid (LA), and α-linolenic acid (LNA) ($P \leq 0.001$). The highest CLA and LNA values were detected in the Canestrato Pugliese cheese derived from Comisana milk (0.55 and 0.47 g/100 g FA, respectively; $P \leq 0.05$). LA content was higher in Altamurana, Comisana, and Leccese cheeses than in Sarda cheese (1.71, 1.65, and 1.64 vs 1.44 g/100 g FA, respectively; $P \leq 0.05$). The differences observed in the CLA content are very important because in the last years CLA has aroused much attention due to its anticarcinogenic activity, antiatherogenic, anti-obesity, anti-diabetes, and immune-stimulating properties (Crumb & Vattem 2011). Previous studies were focused mainly on the effect of sheep breed on the milk fatty acid profile (Signorelli *et al.* 2008; Talpur *et al.* 2009; Mierlita *et al.* 2011), whereas the effect of breed on cheese quality was investigated to a lesser extent. Secchiari *et al.* (2001) observed the breed effect

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on milk's total CLA isomer content, with a higher CLA content in local Garfagnina and Massese breeds compared to Sarda breed. The study conducted by Kawecka and Sosin-Bzducha (2014) is one of the few studies carried out simultaneously on milk and cheese made from different sheep breeds in Poland; they observed the breed effect on some isomers of CLA (CLA *c*9, *t*11; CLA *t*10, *c*12, and CLA *c*9, *c*11) in "oscypek" cheese manufactured from milk of different Polish breeds.

In our study, the sheep breed affected the value of nutritional indexes ($P \leq 0.001$). Health Promoting Index was proposed by Chen *et al*. (2004) as an indicator of the health value of dietary fat. A dairy product with high Health Promoting Index (HPI) or low Thrombogenic Index (TI; Ulbricht & Southgate 1991) value is assumed more beneficial to human health. The relative proportion of FA having antiatherogenic (PUFA, CLA) and pro- (saturated and *trans* FA) effects appears to vary in cheese fat, with consequent changes in the HPI value. In our study, the HPI value was higher in Canestrato Pugliese from Comisana, Leccese, and Altamurana breeds than from Sarda breed (0.52, 0.51, and 0.50 vs 0.40, respectively; $P \leq 0.05$). A slight effect of breed on nutritional indexes of cheese was reported by Di Trana *et al*. (2009) comparing Canestrato Pugliese obtained from three different sheep breeds (Altamurana, Comisana, and Gentile di Puglia) maintained in the same rearing conditions. The FA profile of Canestrato Pugliese cheeses at four months of ripening was also affected by breed (Table 2). Comparing the fatty acid profile of cheese ripened two and four months, we can observe that the differences between breeds are less evident after four months of ripening. At four months of ripening, the medium-chain FA were higher in cheese from Sarda breed than from the others (38.89 g/100 g FA; $P \leq 0.05$). The lowest content of SFA (70.23 g/100 g FA; $P \leq 0.05$) and the highest level of MUFA (25.20 g/100 g FA; $P \leq 0.05$) were observed in cheese obtained from Altamurana milk. The CLA value was higher in cheese from Altamurana, Comisana, and Sarda than in Leccese cheese (0.57 vs 0.52 g/100 g FA; $P \leq 0.05$). The LA value was higher in Altamurana and Leccese cheese than in the others (1.90 and 1.85 g/100 g FA, respectively; $P \leq 0.05$). In contrast to the cheese ripened two months, the effect of breed was detected on the content of total trans FA, with the highest value in Comisana cheese (1.11 g/100 g FA; $P \leq 0.05$). A reduction in the effect of sheep breed on the FA profile of Canestrato Pugliese could be due to the long-time of ripening. In a recent study, Esposito *et al*. (2014) reported few significant differences between sheep breeds in cheese FA content after 106 days of ripening.

The nutritional indexes at four months of ripening showed the same trend reported after two months of ripening with the highest value of TI (2.7; $P \leq 0.05$) and the lowest value of HPI (0.41; $P \leq 0.05$) detected in the Canestrato Pugliese from Sarda breed.

Volatile components. The flavour of cheese originates from microbial, enzymatic, and chemical transformations. The breakdown of milk proteins, fat, lactose, and citrate during ripening gives rise to a series of volatile and non-volatile compounds which may contribute to cheese flavour (Engels *et al*. 1997). The physicochemical characteristics of cheeses from different breeds are linked to the development of volatile flavour compounds, particularly from lipid degradation during ripening (McSweeney 2004). In

Table 3. Effect of sheep breed on the volatile organic compound (VOCs) content (a.u. = peak area × 10^6) of Canestrato Pugliese cheese ripened two and four months

	Ripening period											
	two months						four months					
	Altamurana	Comisana	Leccese	Sarda	SEM	P	Altamurana	Comisana	Leccese	Sarda	SEM	P
Aldehydes	4.97	7.14	4.11	5.25	1.82	ns	6.21	5.95	4.77	4.78	1.43	ns
Ketones	15.54	16.03	16.08	15.94	3.46	ns	11.91^{c}	68.78^{a}	42.91^{b}	34.51^{b}	4.65	***
Acids	1514.60^{b}	1335.00^{bc}	2025.20^{a}	1051.20^{c}	137.70	**	1345.70^{b}	2212.20^{a}	2107.80^{a}	1455.10^{b}	88.66	***
Esters	168.80^{ba}	163.50^{ba}	117.70^{b}	231.60^{a}	21.14	*	248.60^{a}	188.40^{b}	270.60^{a}	152.10^{b}	17.71	**
Alcohols	135.90	204.20	125.10	139.00	38.60	ns	195.40^{a}	75.60^{bc}	178.80^{ab}	12.60^{c}	33.06	**
Monoterpenes	13.11	15.22	9.73	10.04	2.09	ns	6.10	7.80	6.90	4.20	1.31	ns
Total VOCs	1852.9^{ba}	1741.8^{b}	2297.9^{a}	1452.9^{b}	139.7	*	1813.9^{b}	2558.7^{a}	2611.8^{a}	1659.1^{b}	93.0	***

Means with different letters within the same row differ at $P < 0.05$; *** $P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$; ns – not significant

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our study, sheep breed affected the content of volatile organic compounds in Canestrato Pugliese cheese ripened two and four months (Table 3).

At two months of ripening, the total VOC value was highest in cheese from Leccese (2,297.9 a.u.; $P \leq 0.05$) that also showed the highest content of acids (2,025.2 a.u.; $P \leq 0.05$). Acids were the largest class of volatile components detected in all cheeses both at two and four months of ripening. The highest content of the acid class of VOC found in Canestrato Pugliese cheese does not agree with a previous study performed on the same cheese (Di Cagno *et al.* 2003). These differences may be due to the use of non-starter lactic acid bacteria culture and different procedure to measure the VOC profile. In our study, ester content was also different in cheeses from different breeds ($P \leq 0.01$), Sarda cheese having the highest value (231.6 a.u., $P \leq 0.05$).

At four months of ripening, a significant effect of breed was observed in almost all classes. The highest value of total VOC as well as the highest content of acids was observed in cheeses from Leccese (2,611.8 and 2,107.8 a.u., respectively; $P \leq 0.05$) and Comisana (2,558.7 and 2,212.2 a.u., respectively; $P \leq 0.05$) breeds. In cheese from Comisana breed the highest ketone content was observed (68.78 a.u.; $P \leq 0.05$). Ester content was higher in cheese from Leccese (270.6 a.u.; $P \leq 0.05$) and Altamurana (248.6 a.u.; $P \leq 0.05$) native breeds than in the others. In Altamurana cheese was also detected the highest alcohol content (195.4 a.u.; $P \leq 0.05$). The breed effect on the VOC profile was also proved by Ferreira *et al.* (2009) on Portuguese 'Castelo Branco' PDO cheeses manufactured with milk from different sheep breeds (Merino da Beira Baixa, Assaf, and Crusade). Recently, a different VOC profile was found in Pecorino produced by two different Italian breeds, Appenninica and Sarda (Esposito *et al.* 2014).

Discriminant analysis. Canonical discriminant analysis, performed simultaneously on fatty acid profile, nutritional indexes, and VOC profile, clearly differentiated the Canestrato Pugliese cheese produced from milk of different sheep breeds. A plot of the first canonical variable (*x*-axis) and the second canonical variable (*y*-axis) is shown for each ripening period (Figure 3). At two months of ripening, the discriminant analysis highlights that Altamurana and Leccese native breeds were well discriminated from Comisana and Sarda breeds. The variables contributing most to the separation were some main classes of FAs (UFA and omega-3), some SFAs (C8:0, C10:0), aldehydes, acids, and alcohols. The plot of Canestrato Pugliese cheese at two months of ripening shows how much closer the two native breeds are compared to the non-native breeds (Figure 3A). After four months of ripening (Figure 3B), the differences between the native and non-native breeds are reduced and the latter approach the native ones. Nevertheless, all cheeses at four months of ripening remain well discriminated.

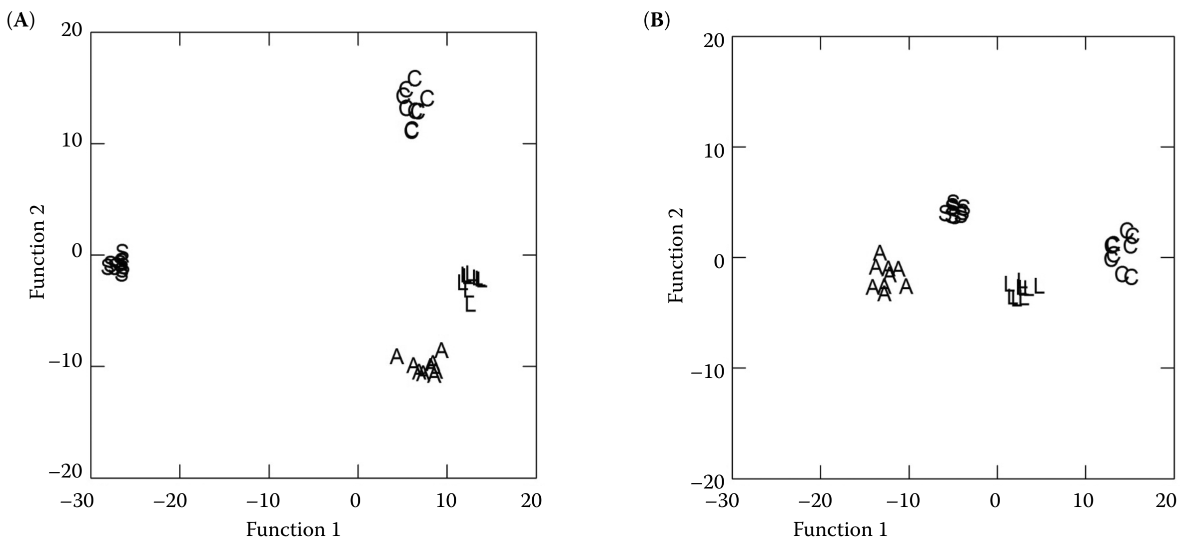

Figure 3. Cheese distribution by breed using the canonical 1 and canonical 2 discriminant functions at two (**A**) and four (**B**) months of ripening

A – Altamurana, C – Comisana, L – Leccese, S – Sarda

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CONCLUSION

In the present study it was shown that the breed significantly affected differences in fatty acid profile, nutritional indexes, and volatile organic compounds of Canestrato Pugliese produced from the milk of sheep that received an identical diet and were housed under the same conditions. Among the breeds investigated, Altamurana and Comisana produced a cheese characterised by some peculiarities that are considered beneficial to human health. Additionally, the content of volatile organic compounds was significantly different between breeds, the highest value being recorded for Leccese.

The highlighting of differences between sheep breeds and their products is a valuable tool to support the sheep breeds still existing in the Mediterranean and in marginal areas of Southern Italy. Therefore, every effort to add value to native breeds is important, especially as a contribution to their conservation through sustainable use.

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