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A Study on Milk and Caciocavallo Cheese from Podolica Breed in Basilicata, Italy

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Abstract: A study was undertaken on milk and caciocavallo cheese from Podolica cattle in the Basilicata Region (Southern Italy), with a view of the possible identification of specific traits useful to protect them from imitations. More than 800 individual milk samples and 29 bulk milk samples were taken in spring–early summer from cows registered in the genealogical book of the breed; moreover, 18 samples of caciocavallo cheese were taken in the same geographical area, 9 of which had been manufactured from Podolica milk. The obtained results confirmed the high aptitude of Podolica milk to cheesemaking, even though the exceptional dry weather in the period of sampling decreased the fat content with respect to the literature data. The presence of the variant A of α-lactalbumin, a characteristic trait of Podolica milk, was ascertained in only 14% of the animals considered in the study, indicating that this feature is disappearing in the population under study. The results on caciocavallo gave useful indications, because some possible peculiar characteristics were identified, such as the lower protein to fat ratio and some aroma descriptors. More research is needed to assess if these characteristics can be used for developing a multi-functional protocol, to be extended to all Italian Podolica populations, able to discriminate the cheese from imitations. In this perspective, the application of selection strategies for increasing the frequency of the variant A of α-lactalbumin should be carefully evaluated.

Keywords: Podolica breed; milk composition; coagulation properties; caciocavallo Podolico

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1. Introduction

A heated debate on the sustainability of intensive dairy cattle farming has been active for a few decades. Actually, scientific evidence exists that both intensive and extensive systems have points of strengths and weaknesses, which can be suitably managed by applying a precision approach [1–3]. Theoretically, extensive dairy farming with local breeds is considered to be the right approach in marginal areas, because it maximizes the efficiency in the use of territory resources, when compared to the high-input breeding systems. In central East Europe, cattle farming is historically connected with the large group of Podolian gray cattle [4]; for centuries, they have been reared as working animals in the wild or semi-wild state, until mechanization in agriculture threatened their survival. This group includes Podolica, a breed that survives in some marginal areas of Southern Italy, where the poor pastures and unfavorable climatic conditions do not allow for the rearing of specialized breeds [5]. To date, about 130,000 heads exist in Italy, 37,000 of which are registered in the genealogical herdbook. The leading region in terms of reared heads is Basilicata, followed by Calabria and, at quite a significant distance, other regions [6]. Although today Podolica is mostly reared for preservation purposes, the farmers are highly engaged in valorizing the animals in farm-tourism activities and marketing their meat and dairy products. Dairy products are obtained seasonally, because the milk production persists about 6–8 months from end-winter to late summer [7], with average production of a few liters a day. The cows are mostly milked in spring–early summer, when production reaches the maximum peak, and the milk is mostly used for making caciocavallo Podolico, a semi-hard to hard pasta filata cheese ripened for 6–12 months or longer. It has the shape of a pear with smooth rind, a weight from 2 to 3 kg and presents an intense pleasant flavor when fully ripened. Actually, a dozen different types of caciocavallo with different sub-names are manufactured in Italy, both at artisanal and industrial levels, whose total production cannot be exactly quantified [8]. Among them, caciocavallo Podolico has the highest reputation and fetches the highest prices on the market, because is obtained in limited amounts and the consumers appreciate the fact that it is obtained from an indigenous breed reared in a semi-wild state. Unfortunately, the cheese is often counterfeited with a similar product made with milk from specialized breeds. In this context, finding distinctive traits for discriminating it from imitations should discourage the fraud and help the farmers to preserve the breed. Unfortunately, poor scientific information is available about Podolica milk and cheese. According to Cosentino et al., Perna et al. and Pistoia et al. [7,9,10], the milk is characterized by high fat and protein content; Pieragostini et al. [11] reported that a particular trait of Podolica milk is the presence of the A variant of α-lactalbumin, a typical zebuine protein that is totally absent in the taurine breeds, except for the gray cattle group. This variant contains a Glu at position 10 of the protein, whereas the B variant has an Arg substitution at that position; a third variant (C) was identified in Bali cattle (*Bos javanicus*) [12]. Regarding caciocavallo Podolico, the production technology is based on a few cornerstones that are the production at farm level, use of raw milk, addition of autochthonous starter prepared by the backslopping method and ripening in "natural" rooms. The other parameters of cheesemaking (type of vat, temperature of milk coagulation and curd grain scalding, type of rennet, brining procedure, etc.) may vary from farm to farm [8,13]. Details about the quality characteristics are not available, except for the compositional data reported by Quinto et al. [14] and the sensory surveys performed by Cammarota et al. [15]. The former found significant differences in the profiles of total and free fatty acids and free amino acids with respect to caciocavallo obtained from Friesian cows, but the authors ascribed such differences to several factors, not only the breed. The latter reported that the most discriminating sensory traits of caciocavallo Podolico were the level of yellowness and, to a lesser extent, the thickness of the sub-rind area (the "nail"), the sweet taste and dried fruit aroma.

The aim of the present research was to widen and update the information on Podolica milk and caciocavallo by making an overall picture in Basilicata, the major Italian region in terms of heads reared. The study was performed with a view of the possible identification of specific traits useful to discriminate the cheese from imitations.

2. Materials and Methods

2.1. Milk and Cheese Samples

The study was conducted in 2022 and considered more than 800 lactating Podolica cows reared in the most important farms located in Basilicata (13 farms in total). From each farm, both individual and bulk milk samples were taken in the period of the greatest cheese production (spring–early summer). Overall, more than 800 individual and 29 bulk milk samples were collected from the end of April to mid-July. All animals were registered in the Podolica section of the genealogical book of ANABIC (Associazione Nazionale Allevatori Bovini Italiani da Carne, San Martino in Colle, PG, Italy) and were milked manually in the presence of the calf. The samples were immediately transported in refrigerated bags to the laboratory, where they were immediately analyzed. As for cheese, 18 samples of artisanal caciocavallo (manufactured at the farm level) of different ages were collected from different farms in the same geographical area: 9 of them had been made from milk of Podolica cows, while the other 9 had been obtained from milk of specialized breeds. The samples underwent a series of analyses as reported in Figure 1.

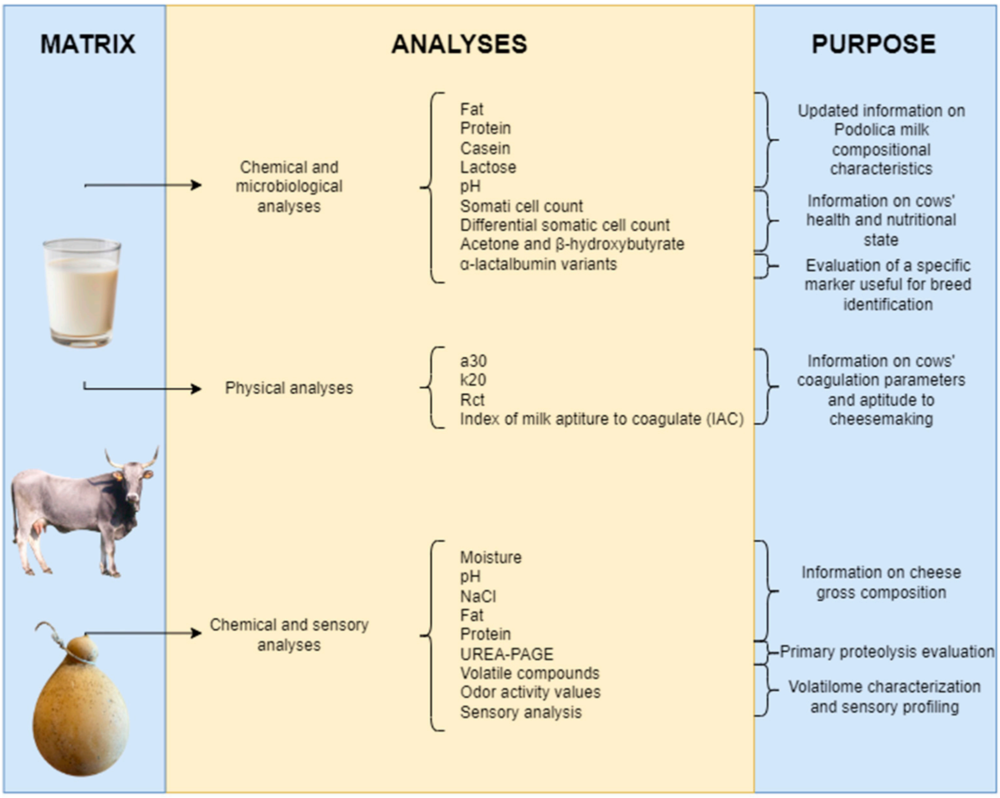


Figure 1. Scheme of the analyses conducted for each matrix and their purpose.

2.2. Chemical-Physical Analyses

The following analyses were carried out on milk: fat, total protein, casein and lactose (Milko-Scan 7RM, Foss, Hillerød, Denmark); pH (pH meter, Hanna Instruments, Woonsocket, RI, USA); somatic cell count (SCC, Fossomatic, Foss, Hillerød, Denmark); differential somatic cell count (DSCC, Fossomatic, Foss, Hillerød, Denmark); acetone and β-hydroxybutyrate (acetone, BHB, Foss Ketolab, Foss, Hillerød, Denmark); coagulation parameters (Formagraph, Foss Electric, Hillerød, Denmark) using the protocol described by Pazzola et al. [16]. Then, the index of milk aptitude to coagulate (IAC) was calculated by using the mean and standard deviation of the experimental data [17]:

$$IAC = 100 + \left[\left(\frac{a_{30} - mean_{a30}}{SD_{a30}}\right) \times 2.5\right] - \left[\left(\frac{RCT - mean_{RCT}}{SD_{RCT}}\right) \times 2.5\right]$$

where a_{30} is the curd firmness 30 min after the enzyme addition and RCT is the rennet coagulation time.

In addition to these analyses, the milks of all cows were subjected to assessment of the whey protein profile in order to evaluate the presence of the genetic variants A of α-lactalbumin. The analysis was performed by high-performance liquid chromatography (HPLC) on an Ultimate 3000 RS Dionex system apparatus with a diode array detector (ThermoFisher Scientific, Rodano, Italy), under the operating conditions reported by Pieragostini et al. [11].

The following analyses were carried out on cheese: moisture (oven drying), pH (pH meter equipped with a penetration probe, Hanna Instruments, Woonsocket, RI, USA), NaCl (chloride analyzer, Sherwood Scientific Ltd., Cambridge, UK), fat (Soxhlet method), total protein (Kjeldahl method). Moreover, the cheeses were subjected to characterization of the level of proteolysis by Urea Poly Acrylamide Gel Electrophoresis (Urea-PAGE) according to the method of Andrews [18]; the most important casein fractions (β, αS1 and αS1-I) were identified and quantified by densitometry as reported by Faccia et al. [19]. Finally, volatile organic compounds (VOC) were extracted by Solid-Phase MicroExtraction, analyzed by

Mass Spectrometry Gas Chromatography, identified by comparison with the data from the NIST library and subjected to calculation of the Odor Activity Value (OAV), following the protocol reported in previous papers [20,21].

2.3. Sensory Analyses

Sensory evaluation of the cheese samples was performed by a panel of seven trained assessors from the staff of the Section of Food Science and Technology at the Department of Soil, Plant and Food Sciences of the University of Bari (Bari, Italy). They were selected following international standards and trained as reported by Trani et al. [22]. The panelists evaluated the samples by quantitative descriptive analysis as reported by Faccia et al. [23,24]. Two preliminary sessions were dedicated to the development of descriptors, which were selected based on weight percentage (frequency of citations × perceived intensity); only descriptors with a weight percentage greater than 30% were considered. Based on the results obtained, a sheet was prepared based on a 6-point scale and applied to the 18 samples of caciocavallo cheese.

2.4. Statistical Analysis

The data were statistically processed by XLSTAT software (version 2020.1.3, Addinsoft, New York, NY, USA). Discrete variables were described by their mode values and continuous variables by their means. Data from the chemical analyses underwent one-way ANOVA followed by Tukey's honestly significant difference test at a critical value for significance of $p < 0.05$; data from the sensory analysis were compared by using the Kruskal–Wallis test. Milk samples' parameters were shown by using a grouped marginal plot by OriginPro 2022 (OriginLab Corporation, Northampton, MA, USA).

3. Results and Discussion

3.1. Milk

3.1.1. Compositional Parameters

Figure 2 shows the gross composition (fat, protein and lactose) of the individual (A) and bulk milk (B) samples, respectively. The distribution is visible in the scatter plot, whereas data (min, average, median, max, lower quartile, upper quartile and outliers) are in the boxplots and the curve of the sample size is on the top. The farms are identified with a progressive number on the abscissa axis (ID Farm). For many parameters, the standard deviation in the individual milk dataset was very high, due to the high number of samples (the highest in the scientific literature for this breed) and the well-known great variability connected with the lactation stage, state of health, rearing system and pasture quality [7,9,10]. The study did not consider all these aspects, as the purpose was to obtain information about milk independently from any variable, except for the registration in the herdbook. In contrast, the variability in the bulk milk dataset was very low, for two reasons: (a) the dataset consisted only of 29 samples; (b) the milk quality was highly influenced by the most productive animals that overwhelmed the less productive ones, more prone to a non-balanced composition.

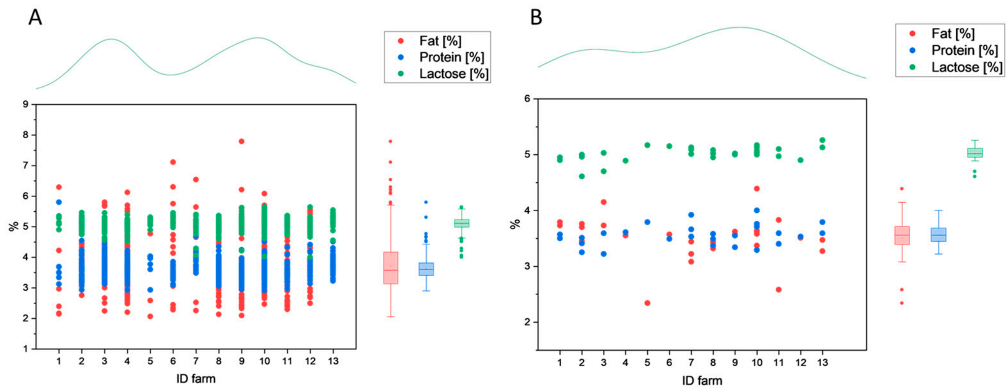


Figure 2. Grouped marginal plot of the gross composition (fat, protein and lactose) of individual (**A**) and bulk milk (**B**) samples.

Regarding the single parameters, the fat content in the individual samples varied from a minimum of 2.06% to a maximum of 5.71%, with an average value of 3.88%. The presence of many outliers was probably also due to the way the milk samples were taken, because fat is the milk macroconstituent that is most affected by the milking procedure. In fact, Podolica cows can only be milked manually in the presence of the calf that often attaches to one nipple and makes the milking operation difficult. The average protein content (3.66%) was not very different from that of fat, but the variation range was narrower (2.9–4.43%), while the average content of lactose was 5.09%. For these two latter parameters, fewer outliers were observed with respect to fat. Regarding bulk milk, the fat content ranged from 3.08 to 4.15%, the average value was 3.61% and only a few outliers were present. Protein varied from a minimum of 3.22% to a maximum of 4.00% and the average value was 3.61%. For both fat and protein, the average values were slightly lower than those observed in the individual dataset, whereas the lactose content (5.02 ± 0.14%) was similar. Compared to the results reported by Perna et al. [9] in a study carried out in the same geographical area but over a wider period of the year, the average contents of protein and lactose found in the present study were significantly higher, whereas that of fat was lower (3.88% vs. 4.27%). The differences probably depended on the weather in the period of sampling, which in 2022 was extremely dry in Basilicata. In fact, the results are more similar to those reported by Quinto et al. [14] in a one-year study conducted in the spring–summer period in a neighboring region. Regarding the casein content, on average it corresponded to 80% of total protein, which is slightly higher than reported for bovine bulk milk (around 78%) [25–27]. This result indicates that in the investigation period the milk was potentially highly suitable for cheesemaking.

3.1.2. Health Parameters

Figure 3 shows the values for acetone and β-hydroxybutyrate (BHB) and for somatic (SCC) and differential somatic cell counts (DSCCs).

The individual samples evidenced strong variability and many outliers: for acetone, the average value was 0.14 mM, whereas for BHB it was 0.054 mM, with the minimum and maximum value falling within the normal range of healthy animals. In bulk milk, no outliers were found and the variability was much lower than in the individual dataset, as well as the average values (0.06 mM for acetone and 0.02 mM for BHB). A strong correlation was observed for these two parameters (r = 0.877, $p < 0.0001$) and it can be concluded that the animals were not significantly affected by ketosis. SCC is a general indicator of udder health in dairy animals and gives a rough indication about the udder inflammation state that leads to decreased milk coagulation ability and reduced cheese yield [28–31]. DSCC detects polymorphonuclear leukocytes and lymphocytes that play a key role in inflammatory responses and has been proposed as a biomarker to reveal subclinical mastitis even at low SCC levels [32,33]. A critical threshold value for DSCC (good udder health < 68.5% > subclinical mastitis) was proposed by Zecconi et al. [34]. Individual milk samples showed a high variability, as the scarce hygienic condition of grazing animals or manual milking deeply affects the somatic cell count [14]. The majority of the samples had an SCC value varying between 2000 and 170,000 CFU/mL, few samples were between 200,000 and 400,000 CFU/mL and only one individual had an SCC > 400,000 CFU/mL. Concerning DSCC results, the mean (58.12%) was below the critical threshold value. Bulk milk samples (Figure 3B bottom) showed SCC varying from 34,000 to 400,000 CFU/mL, but most of the data fell within 167,000 and 309,000 CFU/mL (interquartile range); DSCC showed a narrowed distribution if compared to individual samples (interquartile range between 55 and 77%), with a mean value of 65.66%. Overall, these values indicated animals with good health.

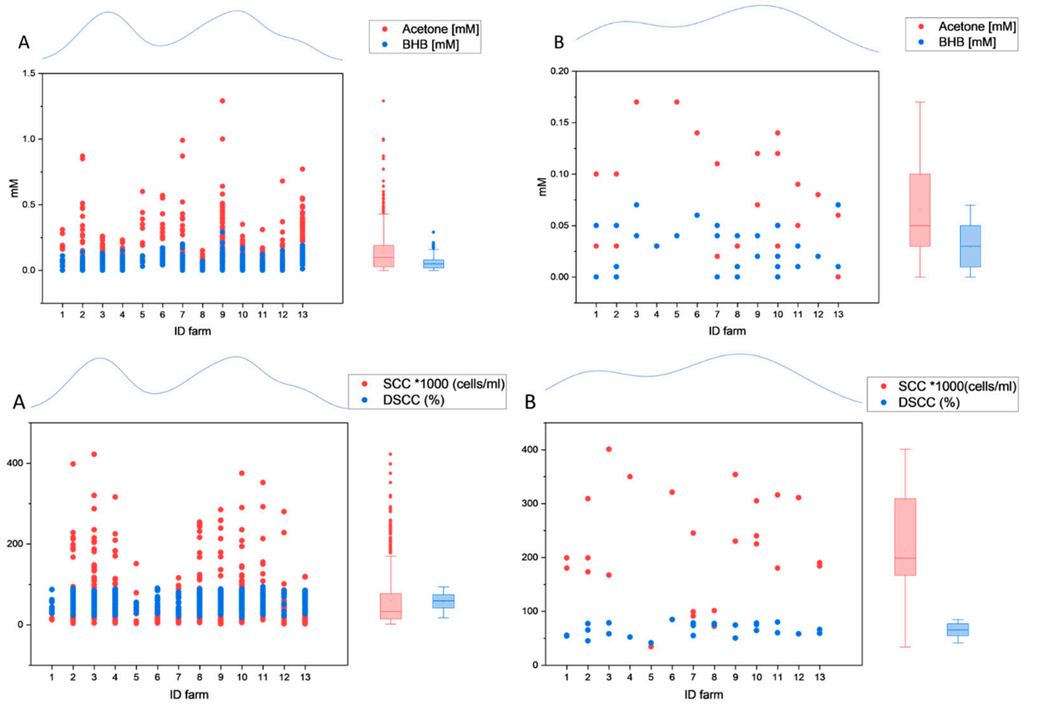


Figure 3. Grouped marginal plot of acetone and β-hydroxybutyrate (BHB) (**upper graphs**) and of somatic cell count (SCC) and differential somatic cell count (DSCC) (**bottom graphs**) of individual (**A**) and bulk milk (**B**) samples.

The pH values of both individual and bulk milk all fell within the normal range of fresh milk (6.69–6.77), while the urea content ranged from very high to very low concentrations. Urea is considered an index of correct animal nutrition and should be in the range from 21 to 34 mg/dL [35]. Our data showed an average value slightly below the lower value of the range for both individual and bulk milk samples (20.9 mg/dL and 20.6 mg/dL, respectively). These results suggest a possible inadequacy of animal feeding plans (protein deficient diet), even though seasons, stage of lactation, presence of pasture and other factors also influence the urea content in milk [36]. Because our samples were collected in late spring–summer, the poor pasture might be an important cause of the low urea content.

3.1.3. Technological Parameters

The coagulation properties of individual and bulk milk samples are shown in Table 1. Formagraph analysis registers the behavior of small-loop pendulums immersed in linearly oscillating cuvettes containing rennet-added milk maintained at a fixed temperature. Before gel formation, the pendulums do not move, but as gel formation begins, the increase in viscosity causes synchronous motion of the pendulums. The registration of such movements is translated into the measures of the coagulating parameters RCT (rennet coagulation time), K20 (time to curd firmness of 20 mm) and a30 (curd firmness 30 min after addition of enzyme). In cheese production, milk that aggregates quickly (low RCT) and forms a firm curd soon after the addition of the clotting enzyme (low K20 and high a30 values) is desirable [37]. The observed values were compared with those recently reported by Niero et al. [38] in a study considering four different cattle breeds (Brown Swiss, Holstein Friesian, Alpine Gray and Simmental).

Table 1. Coagulation properties of individual and bulk milk and values found in the literature.

	Individual Milk	Bulk Milk	Literature Range *
RCT	23.42 ± 5.5 [a]	22.47 ± 2.52 [a]	21.32–21.82
k20	5.17 ± 1.55 [a]	5.23 ± 0.77 [a]	5.28–6.49
a30	38.94 ± 11.07 [a]	37.48 ± 5.18 [a]	14.15–17.76
IAC	100.84 ± 3.65 [a]	102.48 ± 3.00 [a]	100.32–100.97

RCT: rennet coagulation time; k20: time to curd firmness of 20 mm; a30: curd firmness 30 min after addition of enzyme; IAC: index of milk aptitude to coagulate; * Niero et al. [38]. Values in the same row with different superscripts are different at $p < 0.05$.

In general, individual and bulk milk showed similar coagulation properties: RCT was slightly above the range found in the above study, whereas k20 and a30 showed better values; in particular, the latter was more than double if compared to the literature. To sum up, Podolica milk in this study slowly reacted to rennet, but the coagulum had very good properties. The index of milk aptitude to coagulate (IAC) is a parameter considering RCT and a30 with equal importance (50/50%), which has been recently proposed for summarizing the milk performance during cheesemaking. Values above 100 indicate favorable milk coagulation properties and vice versa [39]. Our results showed no differences between individual and bulk milk, but the values of individual milk approached the upper range reported by Niero et al. [38]. In general, coagulation properties of milk are influenced by season and the milk collected during summer tends to have scarce technological quality [40,41]. Considering that in the present study the samples were collected in the hot season, the IAC values are fair, because other authors found lower values for Brown Swiss, Holstein Friesan and Simmental breeds in different periods of the year [42].

3.1.4. Whey Protein Profile

Aschaffenburg et al. [43] reported that variant A of α-lactoalbumin is exclusive to Indian and African Zebu (Bos indicus), while variant B is common to all the European dairy breeds, except for the group of Podolian gray cattle. This sign of hybridization that probably took place in ancient times was investigated by Pieragostini et al. [11], who confirmed the presence of variant A in Podolica cattle but did not indicate the frequency of the protein in the population they considered.

Our study aimed to quantify the number of cows that preserved this variant in the population under study. Figure 4 shows the two types of chromatograms obtained: the first one contains both A and B variants (the expected heterozygous status) and the second only the B variant, the one common to all modern European breeds. Variant A was found in only 14% of the animals, indicating that this particular genetic trait is disappearing. This result leads to a series of questions that are worth consideration from the genetic and zootechnic point of view: why did selection penalize these animals? Was it a sort of natural selection? Or are they less productive or less competitive in the specific environmental context? Could the inversion of this trend be a possible strategy for valorizing and protecting the breed?

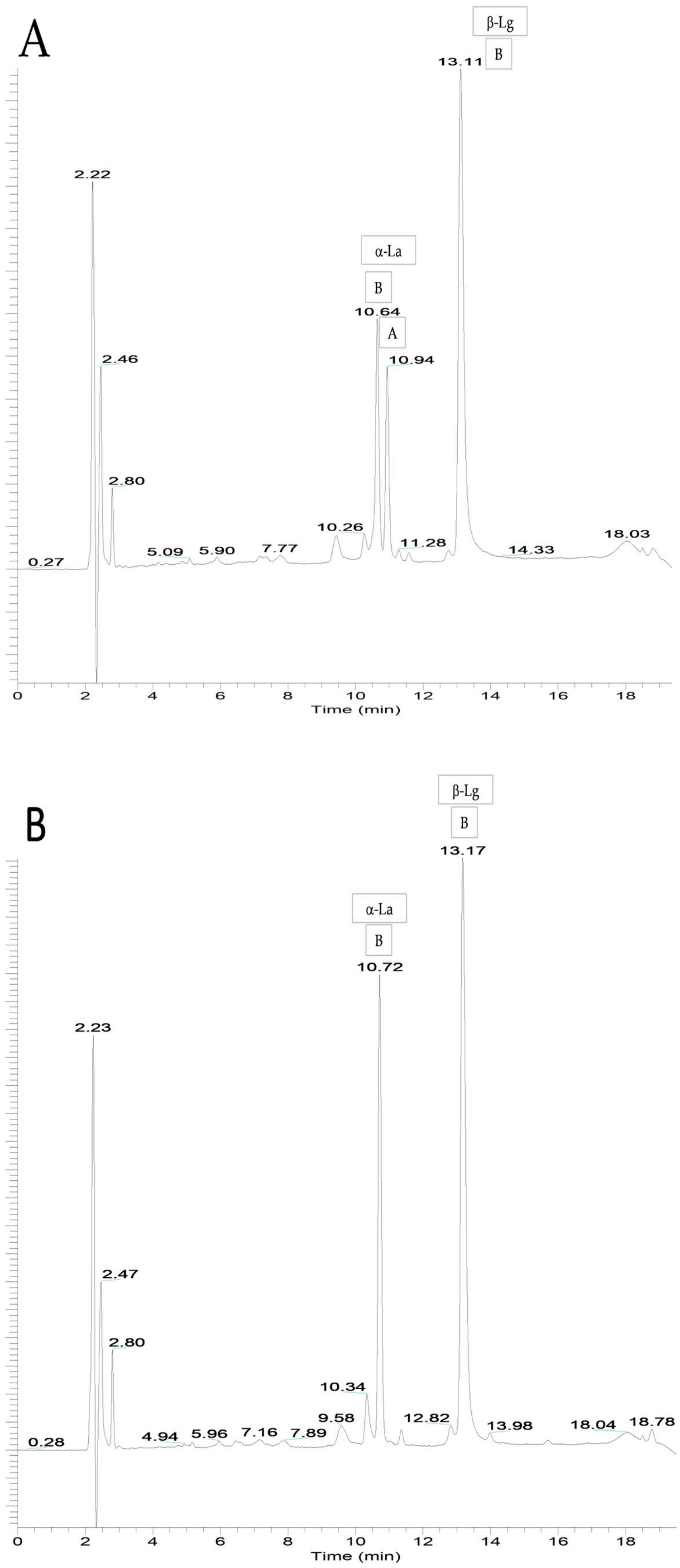


Figure 4. Chromatograms of the whey proteins form individual milk samples evidencing the presence of genetic variants (**A**,**B**) of α-lactoalbumin.

3.2. *Caciocavallo Podolico*

3.2.1. Gross Composition

Due to the different ages of the samples, the gross composition of the cheeses was very variable (Table 2). Nevertheless, some differences between caciocavallo obtained from Podolica and specialized breeds were observed. In particular, the former contained more NaCl and had a lower value of the protein to fat ratio. The higher salting level could be traced back to the ancient cheesemaking protocols, to which Podolica farmers are very close, that involve intense salting of the cheese in order to keep undesired fermentations under control. On the other hand, the lower protein to fat ratio is in good agreement with the very high fat content typical of Podolica milk, as reported by several researchers [7,9,10]. In this regard, it is worth repeating that the milk fat concentration observed in the present study was only slightly higher than that of protein, probably because of the exceptionally dry weather that took place in end spring–summer in 2022.

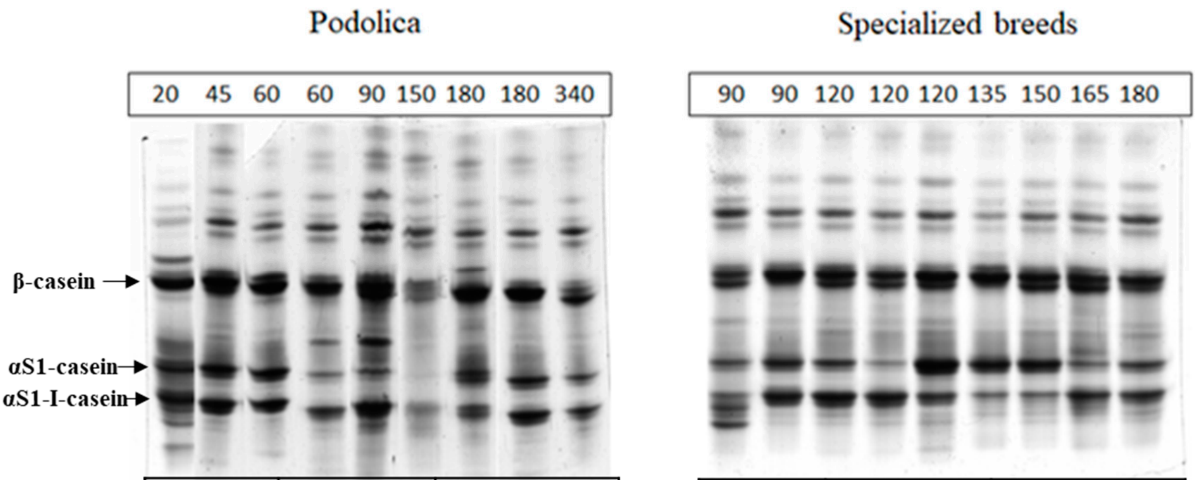
Table 2. Gross composition of caciocavallo cheese from Podolica and specialized breeds.

Ripening Time (Days)	pH	Moisture %	NaCl %	Protein %	Fat %	Protein to Fat Ratio
			Podolica			
20	5.07 ^{b}	40.8 ^{f}	3.0 ^{a}	23.9 ^{a}	28.1 ^{a}	0.85 ^{a}
45	5.30 ^{d}	32.8 ^{c}	3.4 ^{b}	27.5 ^{b}	30.9 ^{b}	0.89 ^{b}
60	5.19 ^{c}	36.5 ^{d}	3.5 ^{b}	25.1 ^{a}	29.7 ^{b}	0.85 ^{a}
60	5.32 ^{d}	35.3 ^{d}	3.6 ^{bc}	26.5 ^{b}	29.5 ^{b}	0.90 ^{b}
90	5.15 ^{c}	38.7 ^{e}	3.5 ^{b}	25.4 ^{ab}	28.3 ^{ab}	0.90 ^{b}
150	5.40 ^{e}	27.8 ^{a}	3.8 ^{cd}	29.7 ^{c}	34.0 ^{c}	0.87 ^{ab}
180	5.30 ^{d}	28.2 ^{a}	3.8 ^{cd}	29.5 ^{c}	32.8 ^{c}	0.90 ^{b}
180	4.93 ^{a}	32.3 ^{bc}	3.7 ^{c}	27.9 ^{b}	30.4 ^{b}	0.92 ^{c}
340	5.28 ^{d}	29.4 ^{ab}	4.0 ^{d}	29.0 ^{c}	32.5 ^{bc}	0.89 ^{b}
			Specialized breeds			
90	5.33 ^{d}	41.1 ^{f}	3.1 ^{a}	24.6 ^{a}	26.5 ^{a}	0.93 ^{c}
90	5.23 ^{cd}	37.5 ^{e}	2.9 ^{a}	27.5 ^{b}	28.8 ^{ab}	0.95 ^{c}
120	4.97 ^{a}	42.0 ^{f}	3.2 ^{ab}	24.6 ^{a}	26.0 ^{a}	0.95 ^{c}
120	5.16 ^{c}	33.7 ^{c}	3.2 ^{a}	27.8 ^{b}	29.7 ^{c}	0.94 ^{c}
120	5.10 ^{b}	39.8 ^{ef}	3.5 ^{c}	25.1 ^{ab}	27.9 ^{ab}	0.90 ^{bc}
135	5.06 ^{b}	31.1 ^{b}	3.5 ^{c}	29.3 ^{c}	29.9 ^{b}	0.98 ^{d}
150	5.09 ^{b}	31.3 ^{b}	3.2 ^{ab}	29.6 ^{c}	30.8 ^{b}	0.96 ^{c}
165	5.16 ^{c}	35.6 ^{d}	3.3 ^{b}	27.2 ^{b}	28.2 ^{a}	0.96 ^{cd}
180	5.29 ^{d}	34.1 ^{c}	3.4 ^{b}	28.3 ^{bc}	29.4 ^{b}	0.96 ^{cd}

Values in the same column with different superscripts are different at $p < 0.05$.

3.2.2. Urea-PAGE

Strong variability was also found for primary proteolysis, as assessed by Urea-PAGE (Figure 5). The expected strict correlation with the ripening time was not always found because cheeses of the same age had different patterns and sometimes younger cheeses were more proteolyzed than older ones. For instance, the Podolica cheese sample having 340 days of ripening was less proteolyzed than that ripened for 150 days, and the 150-day-ripened sample from specialized breeds was less proteolyzed than one of the two 90-day-ripened samples. It is likely that this result depended on the different moisture and NaCl contents, which indicate a very scarce level of standardization of the cheesemaking process and of the ripening phase. Poor standardization is a typical feature of artisanal cheeses manufactured at the farm level from unpasteurized milk, where the measurements during manufacturing, if any, are empirically conducted based on the experience of the cheesemakers. In addition, unpasteurized milk implies different microbial populations with different enzyme and proteolysis activity; moreover, according to Stobnicka-Kupie [44], the microbial contamination of the dairies (air and surfaces) changes from dairy to dairy and thus could also affect the proteolysis phenomena. Regarding the rate of casein degradation, the αS1 fraction was more hydrolyzed than the β-fraction, in agreement with the outcomes of Gobbetti et al. [45], but it only totally disappeared in one sample (150-day-ripened caciocavallo Podolico). Overall, it can be concluded that no discriminant trait was observed by Urea-PAGE between the two types of caciocavallo cheese.



Days	Intensity of residual β-cn	Intensity of residual αS1-cn
20	average	average
45	high	average
60	high	average
60	average	low
90	high	low
150	very low	absent
180	average	average
180	average	average
360	low	low

Days	Intensity of residual β-cn	Intensity of residual αS1-cn
90	low	low
90	high	average
120	high	low
120	average	very low
120	high	high
135	high	average
150	high	average
165	high	very low
180	average	low

Figure 5. Urea Polyacrilamyde Gel Electrophoresis (Urea-PAGE) of caciocavallo samples obtained from Podolica and specialized breeds and level of intensity of the bands of the two main casein fractions.

3.2.3. VOC Analysis

Overall, 57 VOCs were identified from the entire set of samples, belonging to seven different chemical groups. Acids were by far the most important one (63% of total VOC), followed by esters (13%), alcohols (11%), ketones (7%), other compounds (5%), aldehydes and sulfur compounds (1%). The primary role of acids in the VOC fraction of caciocavallo cheese was in agreement with the results reported by Gobbetti et al. [45]. From the statistical point of view, three chemical groups and 31 compounds were connected to specific features of the sample: moisture, ripening time, level of proteolysis and breed (Table 3). Moisture and ripening time exerted the deepest influence, whereas the breed seemed to be less important. Acids and esters were found to be more abundant in caciocavallo Podolico with respect to the counterpart obtained from specialized breeds that, in turn, evidenced a higher level of ketones. This result must be considered with caution for two main reasons: (a) the formation of acids and esters in cheese is highly connected to the activity of microorganisms and to the biochemical events of ripening (proteolysis and lipolysis), which are not breed-dependent phenomena [46]; (b) the number of cheeses considered in the study was rather low.

Table 3. List of VOCs significantly linked to specific features of the caciocavallo samples under study (moisture content, ripening time, level of proteolysis and breed).

	Moisture	**Ripening Time**	**Proteolysis**	**Breed**
Groups				
Acids	**		*	** (Podolica)
Alcohols	**			
Esters	**		**	** (Podolica)
Aldehydes				
Ketones				** (specialized breeds)
Single compounds				
Acetic acid	**	*		
Acetoin	**		**	
1-butanol		**		
2-butanol	**		**	
1-butanol, 2-methyl	**			
2-butanone	**		**	
Butyl butanoate		**		** (Podolica)
Butyl hexanoate		*		
Ethanol		**		
Ethylacetate	**	*		
Ethyldecanoate		**		
Ethylpentanoate		**		
Ethylpropionate	**			
2-hexanone			**	
2-heptanol		**		
2-heptanone	**	**	**	** (specialized breeds)
Hexanal			**	
Hexanoic acid			**	
Isobutyric acid	**			
D-limonene		*		
Methylbutyl butanoate		*		
2-nonanone	**			
8-nonen-2-one	**			
2-octanone	*			
1-pentanol	**			
2-pentanol		**		
3-pentanol,2-metil			**	
Phenylethyl alcohol		*		
Propyl butanoate		*		** (Podolica)
Propyl hexanoate		**		
2-undecanone	*			

* = statistical difference at $p < 0.1$; ** = statistical difference at $p < 0.05$.

Table 4 shows the VOCs with an odor activity value (OAV) higher than 1 found in the samples. OAV is defined as the ratio between the concentration of the volatile compound in the headspace of the sample and its odor threshold in water. Basically, an OAV value higher than 1 indicates a potentially "aroma-active" compound that can contribute to the aroma perception of the product under study. From the table, it can be observed that the compounds were common to both types of caciocavallo (Podolico and specialized breeds) and that five of them played a major role: butanoic acid, responsible for the typical animal sweat and dirty socks odor; hexanoic and acetic acids (pungent, goaty, vinegar odors); and two esters, ethyl butyrate and ethyl hexanoate (fruity odors). It is worth highlighting that among the listed compounds, only hexanoic and propionic acids had statistically different OAV values in the two groups of cheese.

Table 4. Potentially "aroma-active" VOCs (OAV > 1) in caciocavallo cheese obtained from Podolica and specialized breeds.

Compound	Odor Threshold in Water (ppb)	OAV in Cheese (Average Value) Podolico	OAV in Cheese (Average Value) Specialized Breeds	Descriptors
Butanoic acid	1	780 ^{a}	720 ^{a}	sweat, dirty socks
Hexanoic acid	5	93 ^{a}	81 ^{b}	pungent, goaty
Acetic acid	10	38 ^{a}	41 ^{a}	vinegar
Ethyl butyrate	7,5	18 ^{a}	22 ^{a}	fruity, pineapple
Ethyl hexanoate	6	13 ^{a}	16 ^{a}	fruity, apple peel
Limonene	38	3 ^{a}	2 ^{a}	lemon-like
Propyl butyrate	11	3 ^{a}	2 ^{a}	fruity, pear
Butyl butyrate	5	1 ^{a}	2 ^{a}	fruity, pineapple
Propionic acid	6	2 ^{b}	3 ^{a}	pungent, sweat, dairy
1-hexanol	6	1 ^{a}	1 ^{a}	fresh grass
2-nonanone	80	1 ^{a}	1 ^{a}	green, earthy, musty

Values in the same row with different superscripts are different at $p < 0.05$.

3.2.4. Sensory Analysis

The descriptors developed by the panel and the scores attributed to the cheeses (modal values) are shown in Table 5. Of course, the different ages of the samples influenced the variability of the scores; nevertheless, it was possible to detect some peculiarities for caciocavallo Podolico. In fact, one texture descriptor (adhesive), three odor descriptors (cowy/barny, smoky and solvent) and one taste descriptor (salty) were significantly different with respect to the group of cheeses obtained from specialized breeds. The higher adhesivity matched well with the results of the gross composition (lower protein to fat ratio), as well as the salty taste (higher NaCl concentration). In contrast, the aroma descriptors were not clearly linked to the VOC profiles, because the three descriptors have been reported to derive from molecules [47] that were not found at an OAV level > 1 or were not detected at all. It is not surprising, because it is well known that the aroma characteristics of milk and milk products not only depend on specific aroma-active molecules but also on the interaction of different compounds that act in a synergetic way, even if present at very low concentrations [48].

Table 5. Sensory attributes (modal values on a 6-point scale from 0 to 5) of caciocavallo cheese obtained from Podolica and specialized breeds.

Descriptor	Podolica	Min–Max	Specialize Breeds	Min–Max	Sig
TEXTURE					
Adhesive	2	1–2	1	0–1	*
Crumbly	2	1–3	2	1–3	
Eyes	1	0–1	1	0–1	
Hard	3	2–4	3	3–4	
Soluble	2	0–3	2	1–3	
AROMA					
Butter	1	0–1	1	0–1	
Boiled cabbage	2	1–3	3	2–3	
Cowy/barn	4	3–5	3	1–3	*
Smoky	2	2–3	1	0–1	*
Solvent	2	2–3	1	0–1	*
TASTE					
Salty	3	3–4	2	1–3	*
Bitter	0	0	0	0–1	
Pungent	2	0–3	2	0–2	
Acid	1	0–2	1	0–2	
Umami	2	1–3	2	0–3	

Asterisk indicates a significant difference between the two groups of cheese at $p < 0.05$.

4. Conclusions

The first outcome of the present study was the chemical and technological characterization of Podolica breed milk in a specific geographic area during the warm season. The obtained dataset indicated that although the chemical quality was good for cheesemaking, the exceptional dry weather in the period of sampling lowered the fat content with respect to the literature data. The microbiological quality of milk was acceptable for most of the samples tested (only one sample exceeded the EU regulation limit), and the high variability of SCC could be attributable to the traditional hand milking process and different farming conditions. In addition to this, it was ascertained that a characteristic trait of Podolica milk, which is the presence of the variant A of α-lactalbumin, is disappearing in the population under study and cannot be used as a molecular marker of authenticity for milk and cheese. A second interesting result was derived from the study on caciocavallo, because some possible specific traits for the cheese obtained from the Podolica breed were identified, such as the lower protein to fat ratio and some aroma descriptors. Although more research is needed on a wider set of cheese samples, they represent a promising discriminant index. In fact, they could be used in connection with other specific traits of the breed for developing a multi-functional authentication approach that can be extended to all Italian Podolica populations. In this perspective, the possibility of increasing the number of animals carrying variant A of α-lactalbumin should be carefully evaluated.

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