International Dairy Journal 149 (2024) 105830

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

Effect of ultrafiltration on the cheesemaking properties of donkey milk

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ARTICLE INFO

Article history:
Received 1 September 2023
Received in revised form
13 October 2023
Accepted 14 October 2023
Available online 29 October 2023

ABSTRACT

The effect of ultrafiltration on the cheesemaking properties of donkey milk was assessed. Milk was coagulated by rennet with or without modification of some technological parameters, i.e., pre-acidification with lactic acid or EPS-producing starter, addition of small amount of bovine milk. After assessing the gross composition, the milk samples were processed and coagulation was monitored with a viscosimeter. The obtained cheeses were subjected to chemical analyses, calculation of the yield and electrophoretic characterisation of the protein profile. The results indicated that the milk protein concentration was the main limiting factor for coagulation and that pre-acidification played a minor role. The most satisfactory results were obtained for milk with added EPS-producing starter, since the cheese showed the highest yield (about 11%) and the firmest texture. The outcomes of the study could be easily transferred to the dairy level, after suitable economic evaluation.

1. Introduction

Interest in donkey rearing declined over the years as industrialisation progressed and the function as an agricultural animal was lost. Recently, donkey enjoyed renewed relevance due to the biodiversity and marginal area preservation projects; in addition, the properties of its products (milk and meat) have been reconsidered and valorised. Donkey milk showed hypo-allergenicity effect on children with cow milk protein allergy, higher digestibility of fat and proteins, antitumour and anti-inflammatory effects (Martini, Altomonte, Licitra, & Salari, 2018). However, as reported by De Palo, Auclair-Ronzaud, & Maggiolino (2022), the milk yield and consequently its availability is rather scarce, thereby its economical value is proportional to its rarity.

To date, donkey milk is marketed as is (raw) or heat treated (Aspri, Economou, & Papademas, 2017), while producing cheese from donkey milk is troublesome because of its poor aptitude to rennet coagulation (Bittante et al., 2022), whose causes have not been fully clarified. Uniacke-Lowe, Chevalier, Hem, Fox, and Mulvihill (2013) reported that equid milk cannot be coagulated by rennet due to the scarce presence of κ-casein and/or its resistance to hydrolysis, even though a difference exists between species: donkey milk sometimes gives rise to weak localised clots, whereas mare milk does not coagulate at all (Uniacke-Lowe & Fox, 2012; Uniacke-Lowe et al., 2013). Actually, Egito et al. (2001) found that isolated equine κ-casein was chymosin-sensitive at the peptide bond Phe_{97}—Ile_{98} and hypothesised that failure to rennet coagulation depends on inaccessibility of κ-casein due to the way it is embedded in the micelle; another possible explanation is that micellar stabilisation depends on different mechanisms, mostly regulated by β-casein (Uniacke-Lowe & Fox, 2012). According to Malacarne et al. (2017), it is likely that the poor susceptibility of donkey milk to enzymatic coagulation depends on the low casein content and on the high level of colloidal calcium phosphate in the casein micelle, which reduces the number of phosphorylated amino acids residues available for curd formation.

Despite difficult coagulation, some protocols have been recently developed for obtaining cheese from donkey milk using camel chymosin (Iannella, 2015), applying extreme technological parameters (Faccia, Gambacorta, Martemucci, Natrella, & D'Alessandro, 2018; Malacarne et al., 2017) or adding goat milk (Šarić et al., 2016). This latter method produces a "mixed milk" product as pule, a semi-soft Serbian cheese marketed at niche level (Carder et al., 2019), whereas the other protocols involve pre-acidification of the milk and only allow the obtainment of a very fragile, fresh cheese. In fact, careful manipulation is required during cheesemaking to convert the weak coagulum into a curd with

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https://doi.org/10.1016/j.idairyj.2023.105830

sufficient adhesiveness to be moulded. D'Alessandro, Martemucci, Loizzo, and Faccia (2019) and D'Alessandro, Martemucci, and Faccia (2021) tested the addition of transglutaminase together with rennet to improve adhesiveness, but only a slight improvement of texture was obtained. Improving adhesiveness and thickness of the coagulum is a common aim in the production of acidified soft dairy products from ruminant's milk, which is solved in different ways; one of the most recent and attractive method is the use of exopolysaccharide (EPS)-producing starters (Ahmed, El Soda, Hassan, & Frank, 2005; Ramos, Seseña, Poveda, & Palop, 2023).

To the best of our knowledge, no attempt has been made to improve the cheesemaking properties of donkey milk by adjusting the gross composition. To this aim, a well-established technique that can be applied is membrane filtration, which allows the selective concentration of milk constituents by filtering the liquid throughout a porous membrane of variable pore size; the liquid moves under pressure over the surface of the membrane, through which only the molecules with lower size than the pores can pass. The process can be used for different purposes (debacterisation, standardisation, concentration, demineralisation, etc.), by choosing the suitable filtration approach: reverse osmosis, nanofiltration, ultrafiltration, microfiltration.

From a technological point of view, a weak point of donkey milk is the protein content, in fact, according to Aspri et al. (2017) the total protein content of bovine milk ranges between 3.1 and 3.8% versus 1.5 and 1.8% for donkey milk. Thus, to mimic bovine milk, ultrafiltration (UF) might be considered, since it allows to concentrate the proteins based on their molecular weight and shape. The selectivity of the process depends on several parameters, the most important of which is the pore size.

Commonly, UF is applied to cow milk to bring the fat/protein ratio to the desired value to improve the cheese yield and standardise the cheesemaking process. It is normally performed throughout 10 kDa pore size membranes that allow the retention of the fat globules, casein micelles and whey proteins, which are the key-compounds of the cheese yield. Maubois and Mocquot (1971) were the first researchers to demonstrate the affordability of milk ultrafiltration in cheesemaking, and adapted the process to different types of cheese (Maubois & Mocquot, 1975). Concentration by UF can be also applied as a preparative step before freezing the milk for long-term storage and is very useful to overcome the problem of seasonal production of milk from minor species (Voutsinas, Katsiari, Pappas, & Mallatou, 1995). Unfortunately, information about the application of membrane filtration to donkey milk is lacking; in our opinion, the process could improve the coagulation properties and make easier the cheesemaking process. Based on this hypothesis, an investigation was undertaken to assess the effect of ultrafiltration, alone or in combination with the variation of some technological parameters, on the cheesemaking properties of this type of milk.

2. Materials and methods

2.1. Milk collection and ultrafiltration treatment

Masseria Lamacarvotta, located in Laterza (Apulia Region, Southern Italy), offered the milk for the experimentation. Bulk milk was collected three times from end-May to early-June by mechanical milking. Each time, 12 L of milk were taken and transported to the laboratory under refrigerated conditions. At the arrival, the milk was divided into two portions of 4 and 8 L, respectively: the former was not ultrafiltered, the latter was concentrated about two-folds (reaching half of the initial volume) by ultrafiltration for approaching the protein content of bovine milk. A SartoJet ultrafiltration system equipped with a Hydrosart 10 kDa cut-off membrane (Sartorius Stedim Biotech, Sweden) was used, with transmembrane pressure of 1.5 bar. The UF process was coupled with continuous diafiltration with pot water and stopped when the volume of the retentate was around 4 L.

2.2. Experimental design for the cheesemaking trials

The experimental design for the cheesemaking trials is summarised in Fig. 1. The milk samples were designed by considering the two main aspects that have been hypothesised to be responsible of the poor coagulability of donkey milk: the low protein concentration and the scarce presence of κ-casein. In addition, two further aspects were considered: milk pre-acidification by addition of lactic acid and the use of EPS-producing starter. Thus, following are reported the

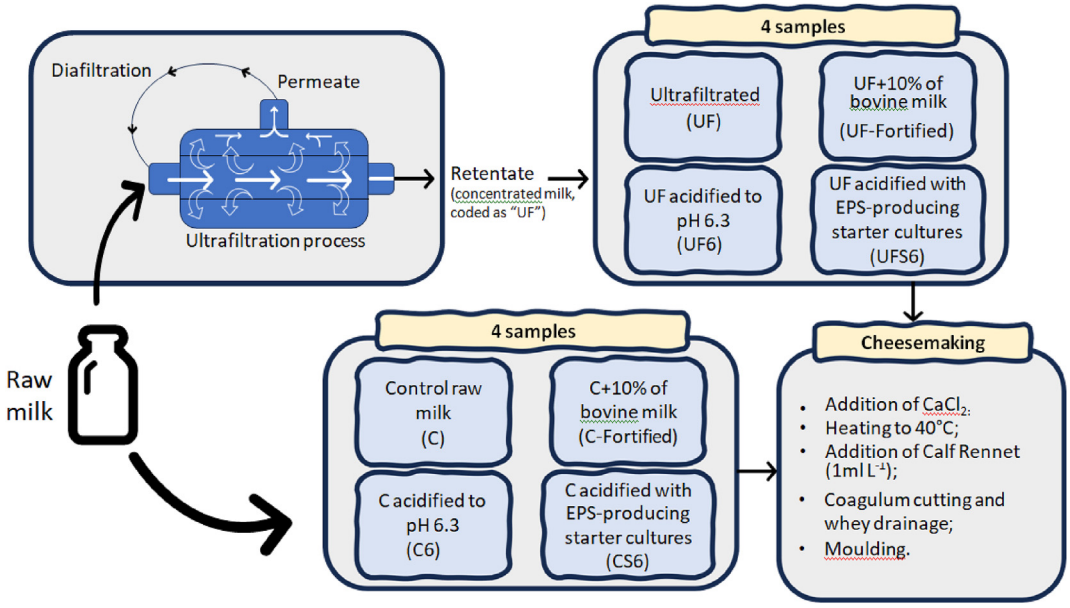

Fig. 1. Experimental design for the cheesemaking trials.

specific purpose of each sample; both non-ultrafiltered milk (C) and ultrafiltered (UF) milk were divided into 4 sub-aliquots (1 L each): (i) the non-ultrafiltered sample (C), used as control; (ii) the non-ultrafiltered sample acidified to pH 6.3 with lactic acid (C6), prepared to confirm that pre-acidification helps rennet coagulation; (iii) the non-ultrafiltered sample acidified to pH 6.3 by EPS-producing starter fermentation (CS6), prepared to assess the combined effect of pre-acidification and EPS production; (iv) the non-ultrafiltered sample fortified with 10% of skimmed cow milk (C-Fortified), prepared to verify if fortification with bovine κ-casein improves coagulation; (v) the ultrafiltered sample (UF), prepared to verify whether increasing the protein concentration improves coagulation; (vi) the ultrafiltered samples acidified to pH 6.3 with lactic acid (UF6), prepared to assess the combined effect of the increase of the protein concentration and pre-acidification; (vii) the ultrafiltered samples acidified to pH 6.3 by EPS-producing starter fermentation (UFS6), prepared to verify the combined effect of the increase of the protein concentration, acidification and EPS production; (viii) the ultrafiltered sample fortified with 10% of skimmed cow milk (UF-Fortified), prepared to verify the combined effect of the increase of the protein concentration and fortification with bovine casein.

For CS6 and UFS6, the starter used was an EPS producing strain of *Streptococcus thermophilus* (Texture-Tek, culture S352, Biochem, Rome-Italy); while concerning the fortified samples (C-Fortified and UF-Fortified), bovine skim milk containing 3.61% protein, 0.09% fat and 5.03% lactose was used. Fortification resulted in a theoretical addition of about 0.37 g bovine κ-casein, calculated according to the following formula:

$$\text{Fortification} = \text{LF}(g) \cdot 0.78 \cdot 0.13$$

where LF is amount of total protein added (3.61 g), 0.78 is the percentage weight of casein on total protein, and 0.13 is the average percentage weight of κ-casein on total casein in bovine milk.

Cheesemaking was carried out as described in previous papers (Faccia, Gambacorta, Martemucci, Difonzo, & D'Alessandro, 2019; Faccia et al., 2018). In short, after the addition of 0.3 g L^{-1} of calcium chloride, the milk was heated to 40 °C and added with 1 mL L^{-1} of calf rennet. After coagulation, the coagulum was gently cut, the whey was drained off by means of a small suction pump and the obtained curd was inserted into small plastic baskets for overnight storage under refrigerated conditions (4 ± 2 °C). All the whey expulsed from the curd cutting until cheese analysis was collected and measured in a graduated cylinder. As reported above, three replicates were carried out on different days.

2.3. Milk and cheese analyses

For milk, the pH value was measured by a pH meter with FC2020 pH edge electrode for dairy (Hanna Instrument Inc., UK), whereas the protein and fat contents were determined by Kjeldahl (ISO 17997-1|IDF 29:2004) and Gerber (ISO 488|IDF 105:2008) methods, respectively. The viscosity of control milk and ultrafiltered milk, before and after the addition of rennet, was evaluated using Mars iQ Air Haake (Vreden, Germany), molecular advance rheometer system fitted with a Couette measuring geometry with a diameter of 25 mm. The shear rate varied from 0.00185 to 116 s^{-1} (Hassan, Ipsen, Janzen, & Qvist, 2003) and shear stress was registered at increasing shear rate. Continuous shear was applied with a delay time of 5 s between measurements at a given shear rate. The changes in viscosity during the coagulation process were measured by a SV-10 vibroviscosimeter (A&D Company, Tokyo, Japan) as reported by D'Alessandro et al. (2019). In brief, a small portion of milk was inserted into the plastic tray included within the instrument and the viscosity was measured at time 0 and after 30 and 60 min from the addition of rennet.

The chemical analyses performed on the cheese samples were total protein (Kjeldahl method) and moisture (IDF, 4:1986) and Urea-PAGE as reported by Andrews and Alichanidis (1983); moreover, the yield and the yield on dry matter were calculated. Finally, the body of the cheeses was empirically assessed by touch and cutting with a knife.

2.4. Statistical analysis

All samples were analysed in triplicate and results were subjected to the analysis of variance (ANOVA) and post-hoc Tukey's HSD (honestly significant difference) test to verify differences between means by Xlstat (Addinsoft, France). The differences were considered significant at 95% probability level ($p < 0.05$).

3. Results and discussion

3.1. Milk samples

Table 1 shows the gross compositions of the control milk (C), control milk fortified with 10% bovine skim milk (C-Fortified), ultrafiltered milk (UF) and ultrafiltered milk fortified with 10% bovine skim milk (UF-Fortified). The pH value of C was around 7.30, in agreement with the literature (Bhairav, 2020; Iannella, 2015), and after fortification with bovine skim milk it decreased to 7.10 because of the lower pH of this type of milk. The pH value in the UF samples also decreased, reasonably due to the treatment applied, as ultrafiltration was performed on raw milk at room temperature for a few hours; as a result, the growth of indigenous bacteria led to a pH reduction. The protein content in the control milk was lower than the average reported in the literature (1.4 versus 1.65%; Faccia et al., 2018, 2019; Massouras, Triantaphyllopoulos, & Theodossiou, 2017); as expected, fortification with bovine skim milk increased the content to 1.63%. The ultrafiltration treatment caused an almost double increase (2.72%), with the final content not very far from that of cow milk; the fat content also increased, passing from 0.35 to 0.7%.

The effect of ultrafiltration on milk viscosity (η) measured using a rotational rheometer, before and after rennet addition, is reported in Fig. 2. Before rennet addition (Fig. 2A), the difference between C and UF milk was clearly visible by the average viscosity values registered during the last stage of the analysis, when the stability was reached. Considering that the ultrafiltration treatment had almost doubled the protein concentration, the difference was less than expected (1.6 versus 1.4 mPa s for UF and C, respectively; $p < 0.05$). This result should depend on the fact that, differently from protein and fat, the dry matter content did not double, since the UF process does not allow the retention of lactose, which is the main macro-constituent of donkey milk. In fact, UF membranes are not able to reject lactose that, instead, is retained by nanofiltration

Table 1
Main parameters of milk used for the cheesemaking trials.[a]

Milk sample	pH	Protein (%)	Fat (%)
C	7.30 ± 0.02[a]	1.4 ± 0.08[c]	0.35 ± 0.05[b]
C-Fortified	7.10 ± 0.03[b]	1.63 ± 0.1[b]	0.35 ± 0.05[b]
UF	7.08 ± 0.01[b]	2.72 ± 0.07[a]	0.71 ± 0.05[a]
UF-Fortified	7.06 ± 0.03[b]	2.82 ± 0.11[a]	0.72 ± 0.05[a]

[a] Abbreviations are: C, donkey milk; C-Fortified, donkey milk + 10% bovine skim milk; UF, ultrafiltered donkey milk; UF-Fortified, ultrafiltered donkey milk + 10% bovine skim milk. Data are the mean ± sd; different superscript letters in a column indicate significant differences ($p < 0.05$).

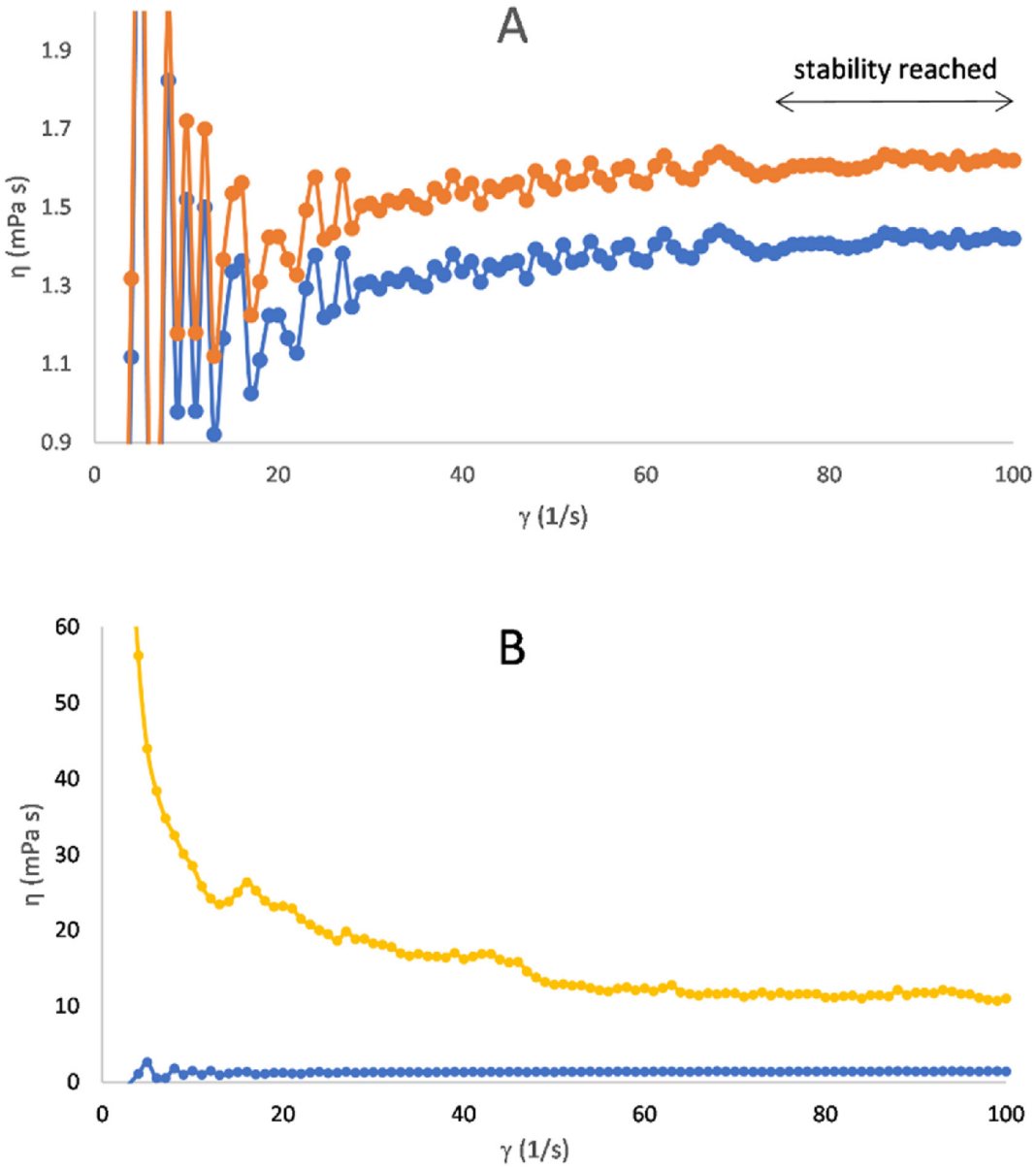

Fig. 2. Viscosity curves [expressed as η(mPa s)/γ (1 s) measured by the rotational rheometer] of (A) control milk (●) and ultrafiltered milk (●) and (B) control milk sample with calf rennet added (●) and ultrafiltered milk sample calf rennet added (●).

(Cuartas-Uribe et al., 2009; Faccia, Natrella, Loperfido, Gambacorta, & Cicco, 2021).

After rennet addition (Fig. 2B), the differences became even more evident. In fact, viscosity of UF milk immediately increased reaching a mean value of 11.60 mPa s at the equilibrium, about 7-fold higher than C milk that, in turn, gave rise to the same curve reported in Fig. 2A, since coagulation did not take place over time. As observed in Fig. 2 the curve of UF milk tended to decrease dramatically with time: it depended on the fact that rotation progressively disrupted the coagulum. For this reason, the measurements during cheesemaking were carried out by the vibro-viscosimeter.

3.2. Cheesemaking

The cheesemaking process was successfully concluded only for the ultrafiltered samples. In fact, C and C-Fortified did not coagulate at all, whereas C6 and CS6 only formed localised weak clots. The results of the viscosimeter measurements on UF samples is shown in Fig. 3. As viscosity is strongly influenced by temperature, this parameter was carefully monitored during coagulation and it was found that the trend was the same for all samples. As expected, the initial temperature was 40 °C, which was the temperature at which rennet was added, and after 60 min, it dropped to 21 °C. In general, viscosity always registered an uptrend over time, but the increase varied among samples, resulting in different levels of firmness of the coagulum. The initial values were similar to those reported above with the rotational rheometer, and ranged from 11.65 to 11.9 mPa s. After 30 min, all samples reached a viscosity of 25−27 mPa s, except for UF that only reached a value of 11.78 mPa s; a different behaviour was observed in the successive phase. In fact, viscosity at 60 min was 27.1 mPa s for UF-Fortified (double than the initial value), whereas the two acidified samples almost quintupled the initial value (55.6 mPa s for UF6 and 60 mPa s for UFS6); UF sample showed the lowest increasing rate (from 11.78 after 30 min to 17.07 mPa s after 60 min).

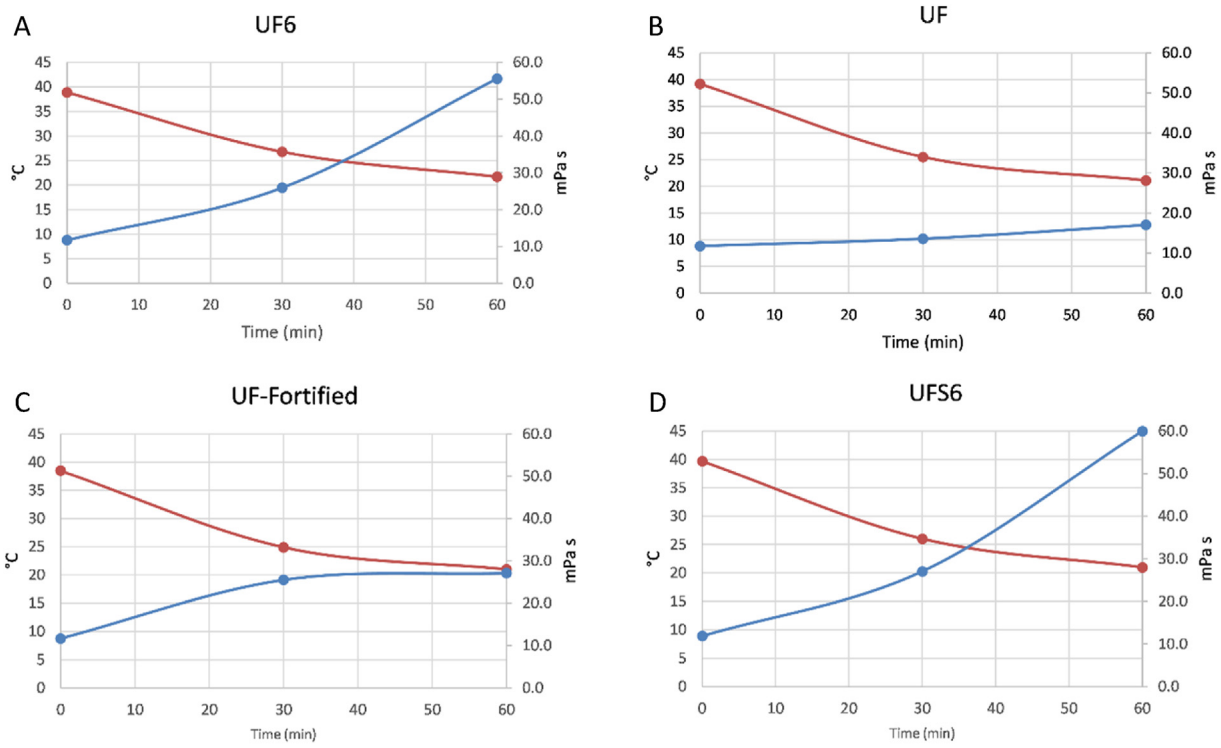

Fig. 3. Viscosity (●, mPa s) and temperature (●, °C) values measured by vibroviscosimeter each 30 min from the addition of the calf rennet to milk until 60 min of curd resting: A, ultrafiltered donkey milk acidified to pH 6.3 with lactic acid (UF6); B, ultrafiltered donkey milk (UF); C, ultrafiltered donkey milk + 10% bovine skim milk (UF-Fortified); D, ultrafiltered donkey milk acidified to pH 6.3 by starter fermentation (UFS6).

The results obtained on the non-ultrafiltered samples allow a series of considerations to be made. First, the failure in coagulation of C and C-Fortified samples confirmed the non-feasibility of using donkey milk for cheesemaking without suitable modifications of the process, even after the addition of bovine casein (at least at the level considered in the experimentation, future trials should consider other fortification ratio). Second, the poor coagulation of the two pre-acidified milk samples were in contrast with the outcomes of previous researches (Faccia et al., 2018, 2019), in which lowering pH to 6.3 allowed rennet coagulation and the formation of the coagulum that, although very soft, had sufficient firmness to be slowly converted into a curd suitable for moulding. In that case, it was speculated that pre-acidification reduced the surface charge of the casein micelles, with consequent reduction of thickness of the solvation shell that prevents inter-micellar interactions at natural pH (i.e., above 7) (Jaubert, Durier, Kobilinsky, & Martin, 1999).

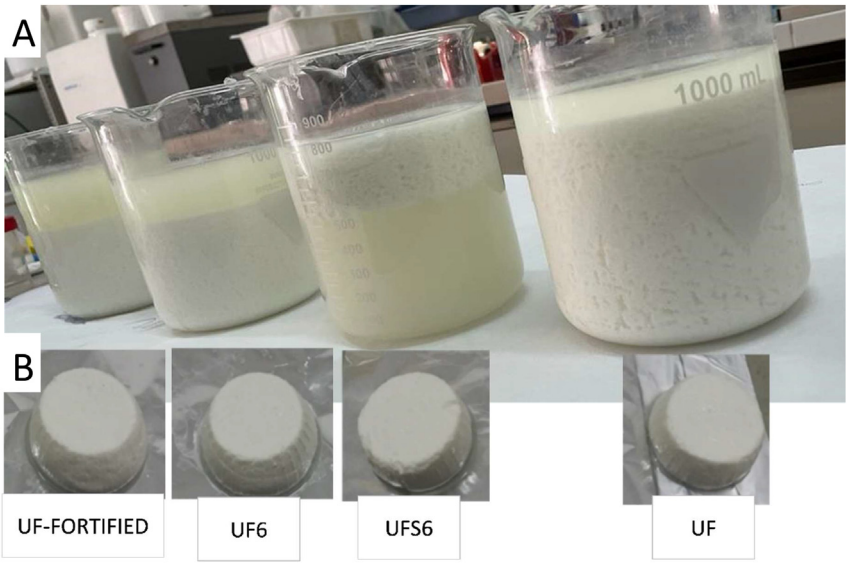

Fig. 4. Appearance of (A) the curds after curd cutting during whey separation and (B) the corresponding moulded cheeses. Starting milk samples were: UF-Fortified, ultrafiltered donkey milk + 10% bovine skim milk; UF6, ultrafiltered donkey milk acidified to pH 6.3 with lactic acid; UFS6, ultrafiltered donkey milk acidified to pH 6.3 by starter fermentation; UF, ultrafiltered donkey milk.

Table 2
Donkey cheese composition after 24 h refrigerated storage.[a]

Sample	Protein (%)	Moisture (%)	Yield (%)	DMY (%)
UF	17.05 ± 0.45[c]	73.53 ± 0.24[a]	9.60 ± 0.04[a]	2.54 ± 0.11[b]
UF-Fortified	19.18 ± 0.41[a]	71.58 ± 0.55[b]	7.7 ± 0.03[b]	2.48 ± 0.09[b]
UF6	19.01 ± 0.21[b]	70.25 ± 0.48[b]	8.05 ± 0.05[b]	2.38 ± 0.15[b]
UFS6	16.54 ± 0.33[c]	73.89 ± 0.74[a]	10.97 ± 0.06[a]	2.86 ± 0.13[a]

[a] Abbreviations are: UF, ultrafiltered donkey milk; UF-Fortified, ultrafiltered donkey milk + 10% bovine skim milk. UF6, ultrafiltered donkey milk acidified to pH 6.3 with lactic acid; UFS6, ultrafiltered donkey milk acidified to pH 6.3 by starter fermentation. Data are the mean ± sd; different superscript letters in a column indicate significant differences ($p < 0.05$).

However, failure to obtain the cheese in the present experimentation, despite of milk pre-acidification, could be due to the poor protein content of the milk in comparison with the previous researches (only 14.0 versus 17.3 and 22.1 g L^{-1}). It is likely that at low level of concentration, the casein micelles are too distant to interact with each other with sufficient strength; consequently, the casein content should be the most important limiting factor of coagulation, and it should be very interesting to assess the "minimum casein threshold" needed to obtain a clot. This hypothesis was confirmed by the results obtained on the UF milk samples, which all rapidly coagulated upon rennet addition. The coagulation of the two samples with pH above 7 indicated that the high pH value does not represent an obstacle when a suitable protein content is present. Very interestingly, the time needed from addition of rennet to cheese moulding (20–25 min, on the average) was much lower than that reported in the literature. In fact, coagulation time was 34 and 159 min in the case of pre-acidification and combined use of rennet and transglutaminase, and 42 and 180 min in the case of pre-acidification and addition of rennet without transglutaminase (D'Alessandro et al., 2019, 2021; Faccia et al., 2018, 2019). Iannella (2015) reported a much longer time (5 h) in a protocol involving acidification and use of camel chymosin.

The curds obtained were soft but easy to be moulded, and behaved differently in terms of whey expulsion and hardening of the body during refrigerated storage (Fig. 4). The one obtained from UF milk was the most fragile and expelled only low amount of whey at the end of the process; on the other hand, it lost a huge amount of moisture during refrigerated storage, suggesting that the protein network in the cheese was too weak and porous to be able to retain water over time. UF6 and UF-Fortified behaved similarly in terms of whey expulsion both during the in-vat-phase and after refrigerated storage and produced cheeses with similar firmness. Differently, UFS6 curd was thick and firm, tended to spontaneously separate from the whey and to float on it. A possible explanation of floating is the presence of entrapped gas, deriving from fermentation by indigenous microorganism of the raw milk during the phase from for acidification to moulding (about 2.5 h), could explain this particular behaviour. As the other three curds did not behave in the same way, it is probable that EPS played a role by making the network firmer and less porous and allowing the gas to be entrapped.

The chemical characteristics of the cheeses after refrigerated storage overnight are shown in Table 2. Due to the concentration process, the cheese yields were much higher than those that have been reported in the literature, with the highest value observed for UFS6 (10.97%), followed by UF (9.60%). Of course, the reduction of the milk volume caused by ultrafiltration must be taken into

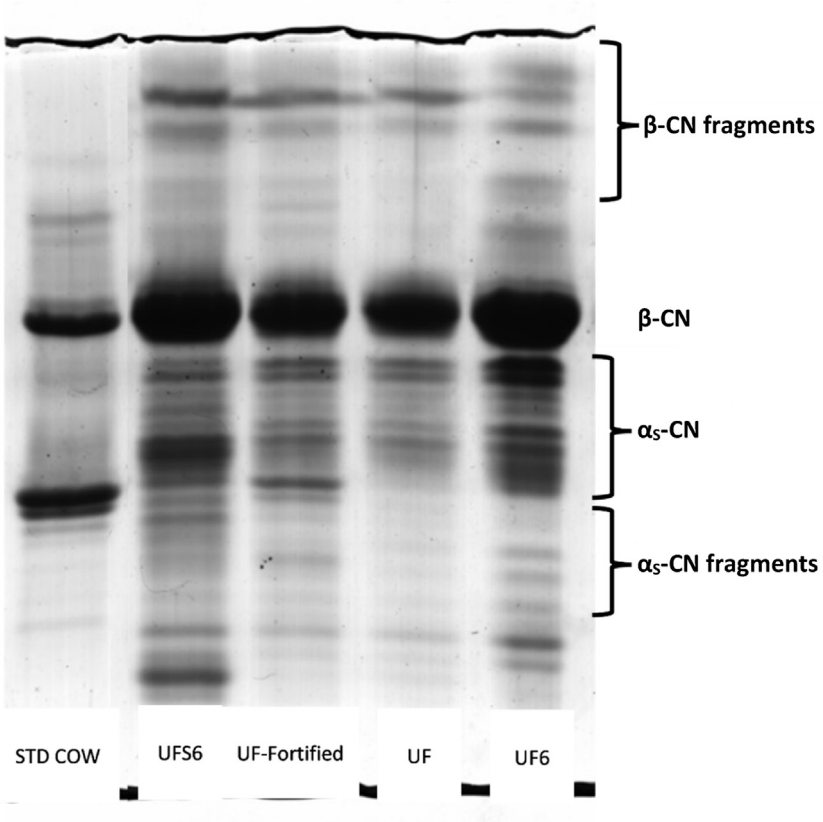

Fig. 5. Urea-PAGE of experimental cheese caseins: lane 1, bovine casein standard; lane 2, UFS6 cheese (made from ultrafiltered donkey milk acidified to pH 6.3 by starter fermentation); lane 3, UF-Fortified cheese (made from ultrafiltered donkey milk + 10% bovine skim milk); lane 4, UF cheese (made from ultrafiltered donkey milk); lane 5, UF6 cheese (made from ultrafiltered donkey milk acidified to pH 6.3 with lactic acid). Identification of the bands was done according to Chianese et al. (2010).

consideration for comparing the obtained yields with those reported in the literature for non-ultrafiltered milk (from 3 to 7%, depending on the richness in protein of the milk) (D'Alessandro et al., 2019, 2021; Faccia et al., 2018, 2019; Iannella, 2015). However, if we consider that the milk used obtained in the present experimentation had very low protein content, the yield improvement seems very promising, as can be inferred from the values of the yield on dry matter (DMY). These values, which give information about the extent of retention of the milk macroconstituents into the cheese, were higher than those reported in the above research. In particular, the value found for UFS6 cheese (2.86%) was the higher than the other three samples that, in turn, did not show significant differences among each other. It is likely that EPS, whose formation continued during the refrigerated storage, fortified the texture and at the same time retained the moisture (Perry, McMahon, & Oberg, 1997). Other authors reported similar results, highlighting the role of EPS-producing starters, as they act as texturiser increasing the viscosity and improving the hydration of the product (Liu et al., 2023; Shih, 2010).

Overall, the obtained results indicate that the low casein content, rather than the scarce presence of κ-casein, is the most important obstacle to rennet coagulation of donkey milk. Nevertheless, the role of κ-casein needs further investigation, since the present study had two main limitations: (i) fortification was not performed by directly adding pure κ-casein, but by adding bovine skim milk (that is, all caseins in micellar status) and (ii) the level of fortification tested was only one and was rather low (theoretically 0.37 g L^{-1}).

Finally, Fig. 5 shows the Urea-PAGE patterns of the cheeses; identification of the bands was done by comparison with the pattern reported by Chianese et al. (2010). The presence of cow milk in the UF-Fortified sample was clearly visible, and some differences in the number of bands were detected among the samples, since UF and UF-Fortified samples had a fewer number. From a quantitative point of view, the intensity of the casein bands appeared more intense in UFS6 and UF6. As these two samples had in common the pre-acidification treatment, it could be hypothesised that this technological operation allowed better recovery of caseins. Regarding the unidentified bands, the most remarkable difference regarded that at the bottom of the electropherogram, which was only present in UFS6 cheese. Its identification could be the purpose of a future investigation, but considering the high electrophoretic mobility it could be a product of casein degradation formed by a possible proteolytic activity of the starter.

4. Conclusion

The results of the investigation gave useful information for better understanding the low aptitude of donkey milk to rennet coagulation and for developing an effective cheesemaking protocol. Pre-concentration of the milk by ultrafiltration prior to rennet addition allowed to easily obtain a curd with sufficient firmness, even without pre-acidification. The most relevant conclusion that can be drawn from the study is that the low protein concentration is the main cause of the poor response of donkey milk to rennet, even though the high pH value also plays a role. Ultrafiltration and pre-acidification with EPS-producing starter seems to be the best way to prepare donkey milk cheese with a good firmness and satisfactory yield. Further research is needed to assess if, by suitable modifications to the cheesemaking protocol, the moisture content in the curd can be lowered to replicate the typical texture of a semi-hard cheese, easier to be marketed. Considering the high commercial value expected for this type of dairy product, and the non-excessive cost of a small-sized ultrafiltration plant, the production of donkey cheese at farm level seems to be feasible.

CRediT authorship contribution statement

Giuseppe Natrella: Methodology, Investigation, Visualization, Formal analysis, Data curation, Writing – original draft. Aristide Maggiolino: Investigation, Resources, Validation, Writing – review & editing. Pasquale De Palo: Investigation, Supervision, Resources, Writing – review & editing. Marina Mefleh: Formal analysis, Data curation, Writing – review & editing. Michele Faccia: Conceptualization, Methodology, Supervision, Visualization, Validation, Writing – review & editing.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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