

Original Paper

Camel, Donkey and Horse Cheese Making with a High Transformation Yield by Natural Thickeners and Lactic Coagulation

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Abstract

Making cheese from donkey and mares milk is considered unfeasible, due to difficulties in coagulation and curd forming. Encyclopedia of Dairy Sciences 2nd edition (2011) reported that no cheese is made from equid milk. However in 2015 a pioneering study of Iannella have reported the first protocols for making fresh donkey and mares cheese, subsequently there have been only a few protocols by other researchers. Anyhow, the low cheese yield of these protocols together with a higher cost of production of the raw material currently limit practical application. Also the processing of camel milk into cheese is technically more difficult than milk from other domestic dairy animals which may relate to the poor rennetability of camel milk. Therefore, a research project was planned by Iannella which has developed a technological procedure to produce cheese from camel, donkey and mares milk with a high transformation yield by the use of locust bean gum, k- carrageenan and lactic coagulation in a dedicated process, thus with a mini mal adjustments in the manufacturing technology and equipment. The whey proteins of milk with this method are withheld and this improve the efficiency of making cheese and increase further yield of cheese however preserving body and texture similar of cheese prepared by conventional processes. In the near future this protocol or similar, they could represent a source of innovative cheese and the development definitive of a new commercial scale of cheese from donkey, mares or camel milk or from others minor milks at the same time improving food and animal biodiversity and therefore all the ensuing benefits.

Keywords

donkey cheese, mare cheese, camel cheese, k-carrageenan

1. Introduction

Cheese from donkey and mares milk was never produced by traditional way. Previous investigation on rennet-induced coagulation of equid milk in Encyclopedia of Dairy Sciences 2nd edition (Uniacke-Lowe T and Fox PF, 2011) reported that mares milk does not form a gel during renneting by bovine chymosin and donkey milk, forms a very weak gel, therefore with out curd formation and that no cheese is made from equid milk. However, Iannella has surprisingly found that pure camel chymosin is able to curd effectively donkey and mares milk in an appropriate technological process (Iannella, 2015a; Iannella, 2015b) which in particular provides differently from other processes the warming of equid milk in a bain-marie; because Iannella also found that strangely equid milk subjected to a prior heat treatment, pasteurization or thermization, did not give a clot. The result of this not common protocol was a curd with elastic properties and intense syneresis. This type of curd was also used to produce stretched curd cheese like mozzarella with only donkey milk by Iannella (2017).

Subsequently only few investigation on enzymatic-induced coagulation of donkey milk have given good results. Sampajo (2017) used with success cyprosin extract from *Cynara cardunculus* for coagulation of donkey milk pasteurized, in the presence of CaCl_2 . A similar result was achieved by Iannella (2018) through commercial vegetal coagulant named “Galium-Prodor®” (*Cynara cardunculus* and other plants) but in raw donkey milk warmed in a water bath and acidified with starter culture.

Although Iannella for quality reasons does not agree with the idea of adding other milks to equid milk it is worth mentioning that a series of coagulation trials of Faccia et al. (2018) were performed on donkey milk, alone or fortified with goat milk (85/15 and 70/30, v/v), using calf rennet (75% chymosin, 25% pepsin) under different technological conditions. Donkey milk gave rise to sufficiently firm curd only at “extreme” technological conditions. D’Alessandro et al. (2019a) also adding with success cow milk in a ratio of 70/30 (v/v; donkey/cow milk). In another protocol, the addition of microbial transglutaminase together with rennet was proposed as a fortification agent for donkey milk cheese making (D’Alessandro et al., 2019b). Finally microbial rennet from *Rhizomucor miehei*, in raw donkey milk heated and maintained at the desired temperatures (46 °C) in a water bath, give soft curd (Faccia et al., 2020a, 2020b).

Unfortunately the authors do not explain the reason for heating the raw donkey milk in a bain-marie, probably as found by Iannella in 2015 and 2018 heating with traditional methods impair the enzymatic coagulation.

Anyway, the curd obtained in donkey milk, not fortified with other milks, is weak and is only suitable for making fresh cheese (D’Alessandro et al., 2020; Faccia et al., 2020a).

Although good results have been achieved using these techniques in terms of coagulation, according Iannella from a strictly enzymatic point of view, the efficacy of bovine chymosin in the previous methods cannot yet be affirmed in curdling donkey milk because the authors have used rennet which also contains pepsin.

It is worth remembering that Chymosin are the preferred enzyme in the cheese making process, since

specificity for K-casein is high, general proteolytic activity, especially with regard to milk proteins, is low, and optimal activity is achieved at mildly acidic conditions (Iannella, 2015a, 2015b). Other aspartic proteases, such as pepsin and microbial peptidases, exhibit a broader proteolytic activity and are less suitable for cheese production, since bitter, hydrophobic peptides, formed by undesirable proteolytic action, impair the sensory value of the cheese (Iannella, 2015b). Therefore, if the effectiveness of any chymosin is not clearly demonstrated, not all rennets can be used to curdle equid milk because there would be a clot given by the pepsin or other peptidases.

However Iannella (2015b) had speculated that the improved milk clotting activity of camel chymosin, compared to bovine chymosin, in equid milk can be attributed to variations on the surface charge, at the binding sites, that facilitate the association between camel chymosin and equid casein and to the improved flexibility of camel chymosin in the ability to accommodate the substrate. Iannella (2015b) also states that the heating of equid milk is a critical point in the process of equid cheese production, for this reason must take place in a “soft way”, e.g., in a water bath and hypothesizes that the absence of clot in equid milk heat-treated (thermised, pasteurized or heated with the traditional method on the fire) is due to changes in casein binding sites. This unpredictable result is typical only of equid milk and probably also the coagulability of raw camel milk by rennet according to Iannella can be improved by using this heating method.

Unfortunately, computational methods for proteins have not yet reached a state that enables the modelling of the interactions of an entire casein micelle with chymosin, in particular the pure camel chymosin with casein micelles of equid milk. However, without wishing to be bound to any theory empirically is contemplated that curd firmness is formed when raw equid milk is coagulated with pure camel chymosin (Iannella, 2015b).

Even though the results obtained were encouraging, the main problem in all these protocols remains the scarce cheese yield that together a higher cost of production of these milks currently limit practical application which could not be overcome substantially by increasing the milk productivity of the animals being strongly linked to genetics.

The processing of camel milk into cheese is also technically more difficult than milk from other domestic dairy animals (Qaader et al., 2015). This is mainly due to its low total solids content, unique composition and casein properties. Camel milk contain low milk solids and its cheese processing ability is poor (Khan et al., 2004). Farah and Ruegg (1989) reported that, because of differences in availability of K-casein, camel milk has more large casein micelles than does cow milk, which may relate to the poor rennetability of camel milk. Also due to major variations between the primary K-casein structures of the two species (Kappeler et al., 1998). Normally, production of cheese from camel milk requires the use of rennet preparations containing a higher amount of pepsin or of microbial coagulant. This results in cheese with an impaired taste not accepted by local consumers (Farah, 1996). In any case a high clotting activity was found with camel chymosin (Kappeler et al., 2006) but animal derived preparations of camel chymosin are not feasible since camels are rarely slaughtered at infancy, therefore Kappeler et al. (2006) decided to

produce the enzyme by fermentation of a genetically modified *Aspergillus niger* strain. Hansen© (Denmark) delivered recently new coagulant agent named “Chy-Max M” containing transgenic camel chymosine (Sorensen et al., 2011). In any case the camel milk products developed thus far are still limited (Dziuba et al., 2014; Berhe et al., 2017).

Recently though, various factors have led to renewed interest in the use of alternative for making cheese. Such factors include the high price and limited availability of ruminant stomachs, dietary regimes such as lacto-vegetarianism, religious restrictions (i.e., Kosher and halal) or the ban on recombinant rennet in some countries.

Therefore, a research project was planned by Iannella to find a new way to produce cheese from camel, donkey and mares milk overcoming the obstacle of the difficult enzymatic coagulation, therefore without rennet and under simple local conditions, possibly with a high transformation yield and a minimal adjustments in the manufacturing technology and equipment.

In the most conventional cheese making processes, the whey proteins of milk, although highly nutritious, are removed from cheese curd in the whey drainage step. So another goal of Iannella project was to find a method which holding the whey protein to improve the efficiency of making cheese and to increase further the yield of cheese, however preserving body, texture and taste similar of cheese prepared by conventional processes.

To achieve this goal, Iannella (2020) has foreseen the use of thickening substances in particular by their characteristics kappa-carrageenan and locust bean gum in a dedicated process.

Carrageenan is a natural carbohydrate (polysaccharide) obtained from edible red seaweeds. However, despite the utilization of carrageenan in a wide range of dairy products, there is little information about the use in cheese-making (Błaszak et al., 2020). It is known that the Carrageenan is used in the dairy industry due to its ability to interact with casein (Piculell, 2006). The underlying mechanism is electrostatic interaction between positively charged milk proteins and negatively charged polysaccharides. The correct concentration of carrageenan, pH and temperature treatment can improve cheese yield and whey protein recovery, as well as improved cheese structure, which is desirable for cheese producers (Dybing & Smith, 1998; Błaszak et al., 2018). Carrageenan, mainly kappa-carrageenan, is known for coagulating whey proteins. It is also a good replacement for emulsifying salts, to stabilize cheese fat (Dybing & Smith, 1998). Locust bean gum is used as an additive in food industry due to its thickening and stabilizing property. Locust bean gum or carob gum is a galactomannan obtained from seed endosperm of carob tree, i.e., *Ceratonia siliqua*. It gives viscosity when added to different food products and ultimately improves the textural and other functional properties through water phase management (Barak et al., 2014). Locust bean gum also shows a useful synergistic increase in gel strength on blending with other gums such as xanthan gum, k-carrageenan, etc. (Barak & Mudgil, 2014). To solve the problem of phase separation, carrageenan is used in combination with locust bean gum which leads to phase stabilization dairy systems via casein and carrageenan electrostatic interaction (Schorsch et al., 2000).

In conclusion, the aim of this work was to provide a new method of Iannella to making donkey, mare and camel cheese by processes that incorporate ionic gums, kappa-carrageenan and locust bean gum, into milk prior to lactic fermentation to reinforce milk gels and coagulum structure, thus increasing product firmness and yield of cheese, however preserving body, texture and taste similar of cheese prepared by conventional processes.

2. Materials and Method

2.1 Materials

Fresh whole donkey milk was obtained from Italian Livestock. Milk was immediately cooled to 5 ± 1 °C and maintained cold until use.

Camel's milk was found in powder from the Emirate company "Camelicious" Emirates Industry for Camel Milk & Products. Camel milk powder was rehydrated according to the manufacturer's instructions and immediately cooled to 5 ± 1 °C and maintained cold until use.

Mare's milk was found in powder from the German company Zollmann Stutenmilch GmbH. Mare's milk powder was rehydrated according to the manufacturer's instructions and immediately cooled to 5 ± 1 °C and maintained cold until use.

Commercial thermophilic starter culture for fresh cheese (Freeze-dried DVS of *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*) was used for acidification of milk. Dosage used as indicated on the package.

Locust bean gum in powder from the company Special Ingredients Italia. Final dosage: 3 grams per liter of milk. Dissolved according to the manufacturer's instructions.

Kappa-carrageenan in powder from the company Special Ingredients Italia. Final dosage: 1,5 grams per liter of milk. Dissolved according to the manufacturer's instructions.

2.2 Method

For each type of milk (from donkey, mare, camel) three cheese-making tests were carried out

In each trial two litres of milk was taken in a stainless steel container and heated to 40 °C.

Later, the ionic gums are added in milk; in any case after dissolving them separately in water (2% w/w).

After milk was heated to 85 °C for 7 minutes to promote the denaturation of whey proteins and the formation of complexes with caseins and at the same time activate k-carrageenan.

The temperature of milk was brought down to 40 °C. The starter culture was then added. The milk was incubated for about 4 h at 40 °C. When the pH had reached the value of 4,5 the gel was cut and the most whey was drained off in cheesecloth. The curd was moulded and pressed for 30 min at 8 °C.

Milk (from camel, donkey, horse)
Addition of ionic gum (tot locust bean gum 3 g/L of milk; tot k- carrageenan 1,5 g/L of milk)
Heating to 85 °C for 7 min
Cooling at 40 °C and addition of starter cultures
Optional addition (i.e., salt, culture for ripening, rennet, enzyme)
Coagulation up to pH 4.5
Cutting of the gel and drainage
Moulding
Storage at 8 °C
Seasoning

Figure 1. Technological Procedure Proposed by Iannella for Camel, Mares or Donkey Milk Cheesemaking by Ionic Gum, k-carrageenan and Locust Bean Gum and Lactic Coagulation

2.3 Physico-chemical Evaluation of Fresh Donkey Cheese

Cheese yield (after 12 h in mould) was calculated using an analytical balance, and was expressed as a percentage. Cheese yield % = weight of cheese (g)/weight of the milk sample (g) x100.

pH were measured using pH meter (Hanna Instruments HI221 pH/mV/ORP), after 1 h that cheese was in the modules.

The total solids of fresh donkey cheese, after 12 h in modules, were analyzed following standard procedures (AOAC, 1995).

Test results are expressed as Mean ± Standard deviation (SD) of five test using Excel 2010 as a statistical program.

3. Results and Discussion

The manufacturing procedure and the characteristics of the cheeses are summarized in Figure 1 and Table 1, respectively.

Three trials separate were conducted to study the manufacturing of fresh soft cheese from camel, donkey and mares milk. For each cheese 3 tests were repeated.

The ionic gums, k carrageenan (1,5g/L of milk) and locust bean gum (3 g/L of milk), are added before fermentation, in order to boost the formation of the gel. However theoretically others ionic gums like iota and λ-carrageenan, xanthan, guar gum, agar, gum arabic, alginates, etc. can be conveniently added to find the best conditions between gel structure / syneresis of the cheese/ water retention. Certainly some structural properties will change in the cheese but not the basis of the method and its effectiveness.

Lactic starter cultures, *L. delbrueckii* ssp. *bulgaricus* and *S. thermophiles*, were used for cheese manufacturing. However any lactic acid-producing bacteria used in conventional cheesemaking can be used in the process of the current protocol.

Lactic cultures are primarily responsible for the production of lactic acid, which improve milk

coagulation and suppresses the growth of undesirable bacteria in the coagulum and produce the flavour compounds that contribute to the aroma of fresh cheese. The fermentation step is carried out until the pH of the milk is reduced to preferably about 4.5 but a range 4.3 to 5.0 is possible.

In any case, various ingredients or culture for ripening can be added after adding starter cultures, also rennet or other coagulating enzymes can be optionally added but in this case for their secondary effect in cheese ripening.

Cheese was difficult to make from camel, donkey and mares milk under conventional conditions, but empirically in this protocol is contemplated that success was achieved when pH of milk was lowered at about 4,5 by starter cultures and k-carrageenan and locust bean gum was added and activated prior to the fermentation. The author states that the fresh cheeses produced in this way have firmness, texture and body of conventional fresh cheese.

The Structure and physical properties of many types of cheese, mozzarella and parmigiano being two examples, are imparted by coagulation of the milk proteins using rennet or other coagulating enzymes. The enzyme treatment of milk modifies kappa casein and produces a coagulum in the presence of calcium ions. Tan un benefit of this method in that the action of coagulating enzymes to modify a major portion of the kappa casein causing aggregation or coagulation is not required. It is an object of this method to reduce or eliminate the enzyme coagulation step in making cheese thus saving time and overcoming the obstacle of the difficult enzymatic coagulation of donkey, mares, camel milk and its low yield in cheese.

In the most conventional cheese making processes, the whey proteins of milk, although highly nutritious, are removed from cheese curd in the whey drainage step. Instead with this method according to the author it are withheld. The author speculated this result thanks to the observation of the drainage liquid that passes through cheesecloth which was colorless and also through the high yield in cheese obtained (16-18%) and its good content in total solid (Table 1).

The author states that cheeses obtained with this method can be seasoned with all other cheeses

Table 1. The Results of New Cheese Making Process

Parameter	Camel cheese	Donkey cheese	Mares cheese
pH at 1 h	4,48 ±0.21	4,55 ±0.41	4,52 ±0.55
Total solids at 12 h	32,45 ±3.51	31,34 ±4.25	31,85 ±3.33
Yield at 12 h	18.42 ±5.51	16.34 ±4.45	17.48 ±4.76

4. Conclusion

This empirical protocol provide the processes (Figure 1) for preparing cheese without rennet allows to overcome the obstacle of the difficult enzymatic coagulation of mare, donkey and camel milk. The processes involve adding a relatively small but effective amount of k carrageenan and locust bean gum

before lactic fermentation to reinforce milk gels and coagulum structure. The whey proteins of milk with this method are withheld thus increasing product firmness, yield of cheese, however preserving body and texture similar of cheese prepared by conventional processes. Anyhow, this procedure requires only a minimal adjustment in the manufacturing technology and equipment and can be used also under simple local conditions. Obviously they still remain to be explored the chemical-nutritional and microbiological information.

Thanks to the excellent results obtained in the near future this protocol or similar, could represent a source of innovative cheese and the development definitive of a new specific production chains / industrial scale of cheese from donkey, mares, camel milk or from others minor milks (i.e., from yak, mithun, musk ox, bactrian camel, llama, alpaca, reindeer and moose) which have unfortunately been overlooked by many researchers.

This will also help to improve the socioeconomic condition of the farmer at the same time improving food and animal biodiversity and therefore all the ensuing benefits.

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