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Donkey cheese made through pure camel chymosin

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Cheese from donkey milk was never produced by traditional way. Research suggests that asinine milk by bovine chymosin form a very weak gel compared to the gel formed from bovine milk and with out curd formation. Encyclopedia of Dairy Sciences 2nd edition reported that no cheese is made from donkey milk, due to its particular caseinic composition that makes inefficient the traditional rennet coagulation. However, the author has recently discovered that pure camel chymosin is able to clot effectively the casein micelles of donkey milk. This investigation is aimed at experimental manufacturing of donkey cheese through pure camel chymosin. A total of five experiments were included in the present investigation. Before making cheese, the raw donkey milk samples were analyzed for their physico-chemical composition in order to better evaluate the successive properties of fresh donkey cheese. Cheese making using pure camel chymosin gave a yield of about 3.32% of fresh donkey cheese, however relative to the content of protein and fat of donkey milk. The fresh cheese after 12 h in molds, had a good content in total solids (35.65%). The low pH in cheese, after 30 min from the production (pH_{at} 30 min 5.34), shows the efficient activity of starter cultures in asses' milk, and allows a good protection against pathogens agents. This application represents an informative step for further trials and could be useful for industrial scale cheese processing of donkey milk. More research is needed to study the mechanism of enzymatic coagulation in equid species and if donkey cheese can be addressed, such donkey milk, as alternative food for people suffering from food allergies.

Key words: Donkey milk, camel chymosin, donkey cheese, milk clotting enzyme.

INTRODUCTION

The use of asinine milk by humans for alimentary and cosmetic purposes has been popular since ancient times, but it was not until the Renaissance that the first real scientific consideration was given to this exceptional milk, more recently it has been used successfully as a substitute for human milk in Western Europe (Vincenzetti et al., 2008), and as an alternative food for infants with food allergies (Salimei and Fantuz, 2012; Polidori and Vincenzetti, 2013) and to upregulate the immune response of healthy elderly humans (Salimei and Fantuz, 2012). Moreover, donkey milk whey proteins showed *in vitro* antiproliferative and anti-tumor activity (Mao et al., 2009). These properties have allowed its diffusion as fresh milk, milk powder and even in cosmetic products.

However cheese from donkey milk was never produced by traditional way and, in scientific literature, alternative

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production methods are not reported.

Cheese production and applications of enzymes

Cheese production represents one of the earliest biotechnological applications of enzymes (Szecsi, 1992). The active ingredients in this process were identified as the proteolytic enzymes pepsin and chymosin, previously referred to as 'rennet' (Foltmann, 1966).

The dairy industry characterizes rennet enzymes using two properties. The first is the milk-clotting activity (C) expressed in International Milk-Clotting Units (IMCU). It is determined by a standard method (International Dairy Federation, 2007) that describes the ability to aggregate milk by cleaving the Phe_{105}-Met_{106} bond or nearby bonds of κ-casein. The second property is the general proteolytic activity (P), which is the ability to cleave any bond in casein (Kappeler et al., 2006). The ratio between the two properties, the C/P ratio, captures the essential quality of a milk-clotting enzyme. The higher the value of this ratio the better the rennet, and in this regard chymosin is superior to all other known rennet enzymes (Foltmann, 1992). The C/P ratio of bovine chymosin towards bovine milk is higher than those of the chymosins from lamb, pig, cat and seal (Foltmann, 1970).

In bovine milk with rennet, chymosin hydrolyses the Phe_{105}-Met_{106} bond of κ-casein. As a result, the micelles lose steric stabilization and become susceptible to aggregation, particularly in the presence of Ca^{2+} (Walstra, 1990; Walstra et al., 2006).

Equine milk with calf chymosin is susceptible to hydrolysis at the Phe_{97}-Ile_{98} bond of equine κ- casein (Egito et al., 2001), but its hydrolysis is considerably slower than that of bovine κ-casein (Kotts *et* Jenness, 1976) and with out gel formation (Uniacke-Lowe *and* Fox, 2011).

Preliminary research, suggests that asinine milk under the action of bovine chymosin, forming a very weak gel, G' of 10–15 Pa, after 60 min at 30°C, compared to 180–200 Pa for bovine milk coagulum under similar conditions (Uniacke-Lowe and Fox, 2011) and with out curd formation. This result is due by the particular caseinic composition of donkey milk, because in asses' milk the k-casein, the key protein in the clotting process, is present only in traces (Amadoro et al., 2011). Differently, k-casein represents about the 13% of total caseins in cow's milk (Malacarne et al., 2002); small quantities in horse (Malacarne et al.,2002); and 5% in camel (Jardali et Ramet, 1991).Therefore, it is not surprising that the majority of pastoral systems have produced at least one type of cheese, no traditional methods exist for making cheese from donkey milk; in Encyclopedia of Dairy Sciences 2nd (Fuquay and Fox, 2011) was reported that no cheese is made from donkey milk.

However, until today, only through lactic fermentation or thermo-acid coagulation could produce a dairy product from donkey milk (i.e. yogurt cheese, ricotta, cottage cheese without rennet), but these products have very different organoleptic, nutritional and rheological characteristics from a cheese obtained with the use of coagulating enzymes, from rennet.

A decisive step in the possibility to use donkey milk in the dairy sector, for cheese production, has been made recently thanks to the surprising finding of the italian food technologist, Iannella (2014), which has discovered that pure camel chymosin, enzyme found in camel rennet, is able to clot effectively donkey milk (Iannella,2014).
For this reason pure camel chymosin now used to curdle camel milk (Bruntse, 2011), can also be used for donkey cheese production (Chr Hansen Co. and Iannella, 2015).

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 donkey milk. Without wishing to be bound to any theory, it is contemplated that curd is formed when raw donkey milk is treated with pure camel chymosin (Iannella, 2014) with a proper dairy technology, because Iannella found that donkey's milk subjected to a prior heat treatment, pasteurization or thermization, did not give a clot (Iannella, 2014).

Taking into account the complexity of the natural casein substrate, Iannella affirms that the improved milkclotting activity of camel chymosin can be attributed to variations on the surface charge, at the binding sites, that facilitate the association between camel chymosin and the casein micelles.

The promising industrial applications of camel chymosin were first reported by Kappeler et al. (2006), who have shown that camel chymosin synthetized by *Aspergillus niger* have different characteristics than bovine chymosin. Camel chymosin exhibits a 70% higher clotting activity for bovine milk and has only 20% of the unspecific protease activity for bovine chymosin. This results in a sevenfold higher ratio of clotting to general proteolytic activity. Kinetic analysis showed that half-saturation is achieved with less than 50% of the substrate required for bovine chymosin and turnover rates are lower (Kappeler et al. 2006). Camel chymosin, moreover, contains two additional positive patches that favor interactions with the substrate. The improved electrostatic interactions arising from the variations on the surface charges and the greater malleability both in domain movements and substrate binding of camel chymosin contributed to better milk-clotting activity in bovine milk (Langholm Jensen et al., 2013). In addition, an increase flexibility of the relative orientation of the two domains in the camel enzyme contributes to improved substrate binding by camel chymosin in casein micelle (Langholm Jensen et al. 2013).

While raw camel milk cannot be clotted with bovine chymosin (FAO, 2001; Ramet, 2001), a high clotting activity was found with camel chymosin (Kappeler et al., 2006). However the ability of pure camel chymosin to clot donkey milk (Iannella, 2014) was a surprising found because as mentioned k-casein is present in trace amounts in casein micelles of asses' milk (Amadoro et al., 2011). Taking into account the complexity of the natural casein substrate, Iannella hypothesizes that the improved milk clotting activity of camel chymosin can be attributed to variations on the surface charge, at the binding sites, that facilitate the association between camel chymosin and the casein micelles. Iannella supposes that the key proteins in the efficient coagulation process, by pure camel chymosin, of donkey milk are especially β-caseins.

The best breeding Asinine management practices, which has improved the quantity and quality of milk produced, combined with the growing interest in donkey's milk due to its nutraceutical properties, might spawn the interest of producing cheese with donkey milk.

However, the processing of donkey milk into cheese is technically more difficult than milk from other domestic dairy animals.

This is mainly due to its low total solids and casein content. This investigation is aimed to develop cheese making technology from donkey milk through pure camel chymosin, and could be useful for industrial scale cheese processing of donkey milk.

MATERIALS AND METHODS

Sample collection and physicochemical properties of donkey milk

Fresh whole donkey milk was obtained from Montebaducco pharma© (1990), specialized in livestock and production of donkey milk; District in province of Reggio Emilia (Italy).

The animals were of indigenous breed (*Romagnola*). The milk samples were collected from 40 donkey between 2° to 4° month of lactation.

Individual milks samples, not refrigerated, were mixed in one batch to perform donkey cheese manufacturing in the laboratory. The tests were started within 2 h from collection occurred just after the morning milking**.**

Coagulant

Pure camel chymosin, named FAR-M® (2011) and product from Chr Hansen©, Denmark, was used; product in highly-stable powder. Dosage used in all tests was 4 g /50 L of donkey milk.

Coadjuvant

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.

Skimmed milk samples were analyzed for pH values by using pH meter (Hanna Instruments HI221 pH/mV/ORP), percentage of fat, total protein and lactose, was determined by automatic milk analyzer device (Lactoscan MCC which uses ultrasonic technology) calibrated for donkey milk.

Donkey cheese manufacturing

A total of five trials of cheese-making from donkey milk were conducted, 1 test per day for 5 consecutive days, to prepare fresh donkey cheese.

Fifteen liters of donkey milk, kept at room temperature, were taken in a stainless steel container and heated to 37°C in a water bath at 43°C. Then the milk was inoculated with thermophilic starter cultures *(L. delbrueckii ssp. bulgaricus* and *S. thermophilus*), after 1 h 30 min was added pure camel chymosin, FAR-M® (0.4 g /5 L of donkey milk).

The milk was incubated for 5 h, at room controlled temperature at 37°C. After the curd formation, most of the whey was removed, and the curd was cut and then the remaining whey was drained off.

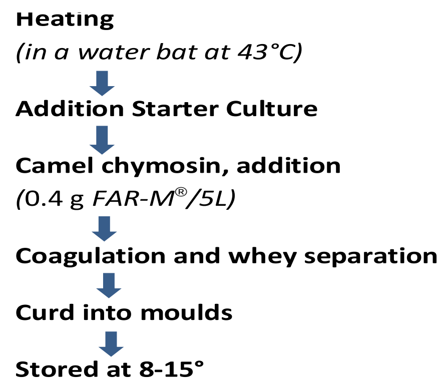

Figure 1. Flow diagram for the manufacture of fresh cheese from donkey milk. "Nativity equid donkey cheese making method , by Giuseppe Iannella©.

The curd was moulded and pressed at room temperature. Cheese was kept in the mould, weighed, sampled and stored at 15°C for further evaluation.

Physico-chemical evaluation of fresh donkey cheese

Cheese yield

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

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

The total solids

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

Statistical analysis

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

RESULTS AND DISCUSSION

The flow steps for the manufacturing of fresh donkey cheese are summarized in Figure 1.

Starter cultures are responsible for the production of lactic acid, which improved curd firmness and inhibited the growth of undesirable bacteria in the curd. In addition the pure camel chymosin in previous studies has shown an optimum pH at 5.1 (Kappeler et al., 2006). The longest time of coagulation (5 h) is due to different caseins in donkey milk, which may related to the poor rennet aptitude of donkey milk. The gross composition of donkey milk differs considerably from milk of the principal dairy species. In comparison with bovine milk, donkey milk contains less fat, protein, inorganic salts but more lactose, with a concentration close to that of human milk (Table 1).

Table 1. Average percentage composition (g/100g) found in donkey's milk, compared with other milk (Salimei et al. 2004; and Guo et al. 2007).

Parameter	Donkey	Cow	Uman
pH	7.0-7.2	6.6-6.8	7.0-7.5
Proteins	1.5-1.8	3.1-3.8	0.9-1.7
Fat	0.3-1.8	3.5-3.9	3.5-4.0
Lactose	5.8-7.4	4.4-4.9	6.3-7.0
Ashes	0.3-0.5	0.7-0.8	0.2-0.3
Solid content	8.8-11.7	12.5-13.0	11.7-12.9
Casein %	45	78	28

Table 2. Average chemical composition (Mean±SD) of donkey milk used for cheese preparation (five replications).

Parameter	Value in donkey milk
pH	7.06±0.06
Fat (%)	1.11±0.34
Protein (%)	1.61±0.12
Lactose (%)	6.08±0.28

Table 3. Physico-chemical parametres (Mean±SD) of Fresh donkey cheese.

Parameter	Value in donkey cheese
pH $_{10min}$	5.34±0.08
Yield $_{at\ 12\ h}$	3.32±0.31
Total solids (%) at 12 h	35.65 ± 4.35

Moreover, the fat content, according to Salimei et al. (2004) and Guo et al. (2007) shows marked variability indicating that it could be affected by breed, breeding area forage, milking technique, interval between milkings and mainly stage of lactation as also reported by Fox (2003), with a negative trend throughout lactation.

Mean composition of donkey milk used for manufacturing cheese samples is shown in Table 2. The protein contents was in the range of the normal values reported in the literature (Salimei et al., 2004; Guo et al., 2007) whereas fat content, which is a parameter more variable in ass milk, was higher than reported (Table 1).

Physicochemical evaluation of fresh cheese made from donkey milk

In all the samples, the coagulum obtained with donkey milk, after 5 h from the addition of pure camel chymosin (FAR-M®), was a precipitate in the form of clot, with elastic properties.The pure camel chymosin utilized in this investigation, FAR-M® is able to allow the production of donkey's cheese with doses of 0.4 g / 5 L. Table 3 shows some physico-chemical parameters of fresh donkey cheese, after 10 min in the mold for pH and after 12 h for yield and solids content.

The curd (3.32%) with a good content in solid substance (35.65%) was obtained when cheese was prepared following the previous procedure. In a previous work Iannella (2014) has observed that only raw donkey's milk, therefore not heat treated nor pasteurized, is able to clot; for this reason the donkey's milk cannot be heat treated in this process.

The negative result in donkey milk heat-treated is due to the change of proteins conformation in response to this process; this result in donkey milk is very important and this prevents the subsequent enzymatic reaction.

Conclusion and recommendation

Manufacture of fresh cheese is feasible. The average cheese yield obtained from donkey milk (3.32%) however, was lower than that reported for cow milks, depending to the contents of protein and fat of this milk (Tables 1 and 2). The result is promising and shows possibilities of improving the quality of such cheese in the future.

In the process, the use of starter cultures is necessary because they are responsible for the production of lactic acid, which improve curd firmness and suppresses the growth of undesirable bacteria in the curd, obtained from raw milk. In addition the pure camel chymosin have optimum pH at 5.1 (Kappeler et al., 2006), therefore also for this reason the pH of the milk must be lowered. These data indicate that cheese making without use of starter cultures should be discouraged.

However, more research is needed to study the mechanism of enzymatic coagulation of donkey milk, with pure camel chymosin and to improve cheese yield.

Further investigation is warranted to determine if there are differences in the coagulation properties of asinine and equine milk and if donkey cheese can be to people sensitive to milk protein allergies

Conflict of interests

The author did not declare any conflict of interest.

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REFERENCES

Amadoro C, Colavita G, Salimei E (2011) *Il latte*, 35

AOAC (1995). Official Methods of Analysis, 17thed. Washington, DC: Association of Official Analytical Chemists.

Broe ML, Van Den Brink J, Kirsten Harboe M (2011) inventors; Chr. Hansen A/S, Inc., assignee. Coagulation of milk. United States patent US 20110008492 A1. 2011 January 13

Bruntse A (2011). Camel cheese Manual PDF. www.infonet-biovision.org

Chr. Hansen Co, Iannella G (2015). From Camel to donkey cheese – FAR-M® creates new business opportunities. http://www.chr-hansen.com/news-media/singlenews/from-camel-to-donkey-cheese-far-mR-m-creates-new-business-opportunities.html, 30-03-2015.

Egito AS, Girardet J-M., Miclo L, Mollé D, Humbert G, Gaillard J-L(2001). Susceptibility of equine κ- and β-caseins to hydrolysis by chymosin. Int. Dairy J. 11, 885-893

FAO (2001). The Technology of Making Cheese from Camel Milk (Camelus dromedarius). Animal Production and Health Paper, Vol. 113.Rome: Food and Agriculture Organization of the United Nations.

FAR-M® (2011). http://www.chr-hansen.com/products/product-areas/enzymes/our-products/coagulants/far-mr.html

Foltmann B. (1966). A review on prorennin and rennin. C. R. Trav. Lab. Carlsberg 35:143-231.

Foltmann B. (1970). Methods Enzymol. 19: 421–436.

Foltmann B. (1992). Chymosin: a short review on foetal and neonatal gastric proteases. Scand. J. Clin. Lab. Invest. 52:65-79.

Fox PF (2003). The major constituents of milk. In Dairy Processing (ed. CRC press), Washington, DC. pp. 7-19.

Fuquay JW, Fox PF (2011). Equid Milk. Encyclopedia of Dairy Sciences, 2nd edn., Vol. 3, Academic Press, San Diego, CA, USA, pp. 518-529

Guo HY, Pang K, Zhang, XY, Zhao L, Chen SW, Dong ML, Ren, FZ (2007). Composition, Physiochemical properties, nitrose fraction distribution, and amino acid profile of donkey milk. J. Dairy Sci. 90:1635-1643.

Iannella G (2014). Coagulation of donkey milk through pure camel chymosin– FAR-M®. Article recorded by a notary, attesting certain date at 14/07/2014. Available from the author.

International Dairy Federation (2007). Determination of Total Milk-clotting Activity of Bovine Rennets. ISO 11815:2007. Geneva: International Organization for Standardization.

Jardali Z, Ramet JP (1991). Composition et taille des micelles du lait de dromedaire. Le lait 65

Kappeler SR, van den Brink HJ, Rahbek-Nielsen H, Farah Z, Puhan Z, Hansen EB, Johansen E (2006). Characterization of recombinant camel chymosin reveals superior properties for the coagulation of bovine and camel milk. Biochem. Biophys. Res. Commun. 342:647-654.

Kotts C. et Jenness R (1976). Isolation of κ-casein-like proteins from milk of various species. J. Dairy Sci. 59, 816-822.

Langholm Jensen J, Molgaard A, Navarro Poulsen J-C, Harboe MK, Simonsen JB, Lorentzen, AM, Hjerno K, van den Brink KB, Qvist KB, Larsen S (2013). Camel and bovine chymosin: The relationship between their structures and cheese-making properties. Acta Crystallogr. D Biol. Crystallogr. 2013; 69:901-913.

Malacarne M, Martuzzi F, Summer A, Mariani P (2002). Protein and fat composition of mare's milk: some nutritional remarks with reference to human and cow's milk. Int. Dairy J. 12:869-877.

Mao X, Gu J, Xu YSS, Zhang X, Yang H, Ren F (2009). Anti-proliferative and anti-tumor effect of active components in donkey milk on A549 human lung cells. Int. Dairy J. 19:703-708

Montebaducco pharma© (1990). Asinine farm, specialized in production of donkey milk. Quattro Castella – Reggio Emilia – Italy. http://www.montebaduccopharma.it

Polidori P, Vincenzetti S. Use of Donkey Milk in Children with Cow's Milk Protein Allergy. Foods. 2013; 2(2):151-159.

Ramet JP (2001). The technology of making cheese from camel milk (Camelus dromedary). Animal. Production and Health Paper. No. 113. Rome, Italy: F.A.O.

Salimei E, Fantuz F (2012). Equid milk for human consumption Int. Dairy J. 24:130-142

Salimei E, Fantuz F, Coppola R, Chiofalo B, Polidori P, Varisco G (2004a). Composition and characteristics of ass's milk. Anim. Res. 53:67-78.

Szecsi PB (1992). The aspartic proteases. Scand. J. Clin. Lab. Invest. 52, 5-22.

Sorensen J, Palmer DS, Qvist KB, Schiott B (2011). Initial stage of cheese production: A molecular modeling study of bovine and camel chymosin complexed with peptides from the chymosin-sensitive region of kappa-casein. J. Agric. Food Chem. 59:5636-5647.

Uniacke-Lowe T (2011). Studies on equine milk and comparative studies on equine and bovine milk systems. PhD Thesis, University College Cork

Uniacke-Lowe T; Fox PF (2011). Equid Milk. University College Cork, Cork, Ireland 2011 Elsevier Ltd, 523

Vincenzetti S, Polidori P, Mariani P, Cammertoni N, ET AL (2008) Donkey's milk protein fractions characterization. Food Chem. 106:640-649.

Walstra P (1990). On the stability of casein micelles. J. Dairy Sci. 73:1965-1979.

Walstra P, Geurts TJ, Noomen A, Jellema A, van Boekel MAJS (2006). Dairy Technology:Principles of Milk Properties and Processes. CRC/Taylor Francis. New York.

Vincenzetti S, Polidori P, Mariani P, Cammertoni N, Fantuz F, Vita A (2008). Donkey's milk protein fractions characterization. Food Chem, 2008, 106, 640-649.