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To cite this article: F. Addeo, V. Alloisio, L. Chianese & V. Alloisio (2007) Tradition and innovation in the water buffalo dairy products, Italian Journal of Animal Science, 6:sup2, 51-57, DOI: 10.4081/ijas.2007.s2.51

To link to this article: https://doi.org/10.4081/ijas.2007.s2.51
Tradition and innovation in the water buffalo dairy products

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ABSTRACT: The first part of a two-part synthesis paper focuses on current research on optimization of Mozzarella cheese manufacture. Starting from technology of traditional cheese points susceptible of modification are identified for achieving greater yield and improving quality. In addition ingredients are identified which can enhance quality and performance characteristic of cheese also in term of economy. The second part considers the development of new cheese varieties whose technology has been developed in bovine milk. Water buffalo cheese reproducing bovine counterpart varieties like Gorgonzola, Grana, Asiago, Taleggio has been developed and are commercially available. Yogurt, and kefir, have also developed to enrich the panel of water buffalo dairy products.

Key words: Water buffalo, Mozzarella cheese, Technology, Novel cheese varieties, Novel fermented drinks.

INTRODUCTION - Water Buffalo Mozzarella cheese has the Protected Denomination of Origin (PDO) status under European Union regulations. It implies that any cheese-making practice must comply with the rules established by the National Standard for cheese production, including the milk origin and the approximate area within which the regulations permit it to be transformed to PDO cheese. For this cheese no milk standardization is allowed, pasteurization is optional; culturing is made by adding starter resulting from spontaneously acidified whey of the last day within the cheese plant; curd is cut, and cooked. Due to variable composition of milk and starter, yield and quality of cheese are highly variable. The manufacturing parameters affect the composition, and functional properties of Mozzarella cheese, including enzyme activity and starter culture composition and lactic acid bacteria survival which determine the cheese texture. Every process step and ingredient use needs to be optimized within each cheese factory in order to produce high-quality functionalized cheese. However, a certain number of indications such whey pH and milling pH have general validity as they affect cheese composition, proteolysis and functional properties.

Renneting and enzyme function in curd and cheese. The primary function of the coagulant is to obtain a curd that is successively stretched into cheese. Retention of chymosin is affected by curd pH. Curd cutting at higher pH reduces chymosin level retention and then casein proteolysis of the curd. Small amounts of the residual bovine rennet enzymes in the curd are carried out in the cheese. This residual coagulant is responsible for proteolysis
altering cheese texture during refrigerated storage brine. Mozzarella texture is important because of its influence on shredding, melting properties, and water release in Pizza cooking. The objective is therefore to reduce the coagulant activity in the curd and produce inactivation of chymosin/pepsin attaining temperatures sufficiently high during cooking and stretching. In general, these enzymes are more heat resistant to denaturation at lower pH. Therefore, a means to reduce the level of enzyme(s) entrapped in the curd is the control of the whey pH and milling pH. Cutting the curd in the pH range 6.4-6.1 has demonstrated higher levels of proteolysis for more acid whey. Therefore, a slow acid development decreases the rate of whey expulsion increasing the moisture of the curd and cheese yield. The acidification rate affords a progressive lowering of pH value so that curd can be stretched at various final pH. A 0.2 pH difference doesn't affect chemical composition or functional properties of water Mozzarella cheese. In contrast differences in mill pH value were effective in determining pH changes during cheese storage under brine. To limit proteolysis in acid curd is also suggested to use calf rennet with high levels of chymosin and very low pepsin content. As the pH of curd reduces, the pepsin activity increases. Pepsin is very proteolytic preferentially cleaving at aromatic amino acid residues (Keil, 1992). Specific cleavage of peptide bonds by pepsin occurs at optimum pH while this specificity is lost far from this value such as that of the cheese. Chymosin has very limited proteolytic effect on casein at any pH value. Therefore, the interest of cheese makers is towards use of rennet at high titer of chymosin and negligible pepsin. It is emphasized that casein proteolysis has negative effects on curd stretching and Mozzarella cheese properties.

**Starter culture identity.** The variable composition of the home made natural starter does not allow speculating on neither the lactic acid bacteria identity nor the dominant flora. For bovine Mozzarella typical thermophilic starter cultures include lactobacilli and streptococci. The relative proportion of rod to cocci is attentively controlled because of its potential effect on proteolytic activity. The symbiotic effect of the starter cultures results in the early acid lactic production by cocci followed by rods in the final stages fermentation. Lactobacilli are largely proteolytic bacteria supplying assimilable nitrogen to weakly proteolytic streptococci by degrading mainly casein. Uncontrolled composition of starter cultures in buffalo Mozzarella cheese changes the acid production and net time required to attain ripened curd for stretching. Dominance of lactobacilli during cheese making affords a greater casein proteolysis thus affecting rheological parameters of curd and cheese. To standardize the bacteria composition of natural starter, incubation at predetermined temperature or using temperature gradient reproducing whey cooling at room temperature has been assayed. Some fermenting vats with controlled temperature or temperature gradient are being used. Reproducible results have been obtained and standard quality of cheese improved. Novel solutions are required for standardizing natural starter cultures.

**Mozzarella for Pizza.** With the increasing use of water buffalo Mozzarella cheese it become important the quantity of residual sugars of Mozzarella cheese. Residual galactose in cheese is responsible for undesirable browning of cheese during oven cooking Pizza. Starter cultures containing lactic acid bacteria strains able to equally convert glucose and galactose to lactic acid are needed. This has been realized by addition to the starter of lactobacilli or pediococci strains able to ferment galactose.
In cheese plants where buffalo Mozzarella is produced no clear temperature profile is distinguishable for curd ripening. There are cases where temperatures compatible for thermophilic bacteria growth are used; others favor mesophilic bacteria; finally, there are cases where growth of both types of lactic acid bacteria is favored along a single cheese making. This depends on the extremely variable temperature of curd ripening during the day and along the seasons. Some of these drawbacks may be overcome by planning the use of thermostatic vats for curd ripening which are nowadays lacking in the majority of cheese plants. Therefore, even in the hot season, mesophilic lactic bacteria are favored which can result in a delayed time for curd ripening.

**The role of calcium.** Storage in brine results in a progressive softening of Mozzarella cheese. Calcium level of cheese is important as it imparts texture and functionality to Mozzarella cheese. Higher the calcium content, higher is the cheese firmness. During curd ripening a progressive pH decrease makes soluble some colloidal calcium phosphate associated with the casein micelles which is released in the whey. The calcium retained by the curd must represent about 25% as compared to the original content. Higher calcium content decreases shredding aptitude of the curd which melts with difficulty. Other observations indicate a softer curd when the calcium content is lowered. Finally, no rapid test is available for controlling, like as the pH measure, the evolution of casein bound calcium. Current assays are focused on the complementary soluble whey calcium evaluation. The calcium content of the cheese is determinant either for an appropriate storage in brine or for use in Pizza topping. Studies of the melting properties of Mozzarella cheese have indicated that micellar calcium decreases under the action to the tomato sauce acidity. Therefore, the chemical migration of calcium from cheese to tomato sauce reduce must be kept into account when cheese is destined to melt as string adjuncts.

![Cheese stretcher-cooker](image)

**Figure 1.** Cheese stretcher-cooker used in the experiments for determined best cheese yield and controlled humidity of cheese.

**Curd stretching apparatus.** Engineering systems analysis of *pasta filata* process during Mozzarella cheese manufacture has demonstrated that: (1) Mechanical and thermal treatments during *pasta filata* process have a significant influence on the composition and properties of the cheese. (2) Empirical relationships has been developed between system variables (SME, specific mechanical energy and TE, exit temperature of melt) and cheese properties; these relations suggests that optimization of the operation to increase the cheese yield is possible (Yu and Gunasekaran, 2005). The cheese moisture content decreased as SME increased. A quadratic relation linked cheese moisture content and TE, FDM (fat-in-dry-matter) and SME and FDM and TE. Moisture (%) = b x SME + c; Moisture (%) = a x TE² + b x TE + c; FDM (%) = a x SME² + b x SME + c; FDM (%) = a x TE² + b x TE + c. These relationships suggest that optimization of the chee-
se yield is possible by adjusting the *pasta filata* process operating parameters. In particular, temperature and screw speed need proper adjustments to avoid microstructure breakdown in the cheese. High-speed screws cause curd splitting until temperature increases sufficiently to value necessary to melt the curd which then becomes more and more prone to deformation under the shear force of the turning screws. Increasing screw rate produces a continuous decrease of cheese humidity with a firmer texture. This has important repercussions on the functional properties of Mozzarella cheese. It seems that the screw speed might also influence retention of fat in cheese and oil-off on Pizza cheese.

**Novel cheese varieties.** Water buffalo milk produced within the PDO area is almost entirely destined to Mozzarella production. However, during winter when the consumption of the fresh unripened *pasta filata* cheese dramatically decreases assays have been done to obtain novel varieties of cheeses from water buffalo milk. Indeed a series of assays in cheese plants have been documented in the past. Camembert-, Gouda-, and Stracchino-like cheese have been produced without no appreciable success within the time. In particular, the protein profiling of the Camembert cheese made with water buffalo milk has been also documented (Addeo *et al.* 1982). One of the main problems encountered in the technology of buffalo cheese is the difficulty of converting milk into natural ripened cheese even after

![Figure 2.](image_url)  
**Figure 2.** Hard pressed cheese with Grana-like texture. Ingredients: bovine and water buffalo milk; rennet; salt; lysozyme; lactic acid bacteria; E171; (a) Registered mark; (b) weight ~32kg; cylindrical form 38-45 cm diameter and 22-25 cm height; (c) clock-shaped form of ~16kg; cylindrical form 38-45cm diameter and 11-12 cm height; vacuum packaged cheese: slice (d) 4kg; (e) 1kg; (f) 1kg wrapped in oxygen barrier plastic package; (g) 250gr weight Take-away slice in EthylPolyStyrene package; Length of ripening: 14 months minimum; Composition and nutritional facts (100g cheese serving): 382,5 Kcal (1590,7 KJoule); Humidity 31.81g; Protein 29.96g; Carbohydrates 2.56g; Fat 28.05g (saturated 20.70g); NaCl 1.69g; Calcium 978mg; P 550mg; cholesterol: 149 mg; pH 5.72.
Fermented milk and whey beverages

Yogurt. Water buffalo milk yogurt is a creamy delicacy high in calcium and free from artificial thickeners due to higher casein content (~1.3-1.35fold) with respect to bovine. It is higher in calcium, higher in protein, and lower in cholesterol and also rich in Vitamin E. Made from fresh natural buffalo milk with traditional yogurt culture has been recently produced with addition of probiotic cultures acquiring both unique taste and biological properties. The application of probiotics to yogurt becoming bio-yogurt strengthens the importance of the survival of probiotic microorganisms in acid fermented milks as probiotic carrier. Two basic procedures have been applied for making probiotic bacteria containing

Figure 3. Three varieties of buffalo cheese. (a) Soft cheese. 2 months ripening. Ingredients: bovine and water buffalo milk; rennet; salt; lactic acid bacteria starter. Composition and nutritional facts (100g cheese serving): 357.2 Kcal (1480.3 KJoule); Humidity 43.74 g; Protein 21.46g; Carbohydrates 0.02g; Fat 30.14g (saturated 23.83g); NaCl 2.51g; Calcium 394 mg; P 500mg; Cholesterol: 41 mg; pH 5.64; (b) Blue cheese. 2 months ripening. Ingredients: bovine and water buffalo milk; rennet; salt; starter (L. bulgaricus and S. termophilus); Penicillium roqueforti spores. (b) weight ~ 12kg; cylindrical form 25-30 cm diameter and 15-17cm height; packaging in EthylPolyStyrene plastic foil; shelf-life 45days at +4/+6°C; Composition and nutritional facts (100g cheese serving): 357.2 Kcal (1480.3 KJoule); Humidity 43.74 g; Protein 21.46g; Carbohydrates 0.02g; Fat 30.14g (saturated 23.83g); NaCl 2.51g; Calcium 394 mg; P 500mg; Cholesterol: 41 mg; pH 5.64; (c) 1.5kg slice. Composition and nutritional facts (100g cheese serving): 348.7 Kcal (1443.7 KJoule); Humidity 49.87g; Protein 18.53g; Carbohydrates 0.01g; Fat 30.50g (saturated 23.36 g); NaCl 1.85g; Calcium 131mg; P 190 mg; Cholesterol 52mg; pH 6.46. (c) Hard cooked pressed cheese. bovine and water buffalo milk; rennet; salt; lactic acid bacteria starter. Plastic coat E171; Composition and nutritional facts (100g cheese serving): 354.2 Kcal (1468.8 KJoule); Humidity 44.32g; Protein 22.82g; Carbohydrates 0.12g; Fat 29.16g (saturated 21.93g); NaCl 1.25g; Calcium 201mg; P 310mg; Cholesterol 42mg; pH 5.76.
yogurt. Milk is added of particular strains of lactic acid bacteria and/or bifidobacteria which are selected in view of the beneficial action which they may manifest on the health of the consumer. Probiotic bacteria such as *Lactobacillus acidophilus* and *Bifidobacterium* spp. grow slowly in milk because of lack of proteolytic activity (Klaver *et al.*, 1993). Therefore, fermentation would be carried out at a controlled temperature for 1-2 days, up to reaching a final pH value of 3.8-4.5 and a total concentration of lactic acid bacteria of at least $10^9$ c.f.u./g. It is important to prevent the pH from reaching lower values because they could affect the vitality of the acid-sensitive lactic acid bacteria thus reducing the level of viable cells in the product ready for consumption.

To reduce the fermentation time, yogurt bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*) are added to probiotic products. Thus a complex starter composition consisting of the traditional species *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* and probiotic species *Lactobacillus acidophilus* and *Bifidobacterium* is employed to make probiotic yoghurt. Probiotic bacteria make bio-yogurt amongst the ‘functional foods’ for its beneficial properties in preventing and treating acute diarrhoea and gastroenteritis. Prior to the fermentation step or at the end of the fermentation phase it is possible add various substances for the purpose of obtaining a desired taste, flavour, consistency or any other defined function. Amongst these substances “prebiotics” defined as “nondigestible food ingredients that may beneficially affect the host by selectively stimulating the growth and/or the activity of a limited number of bacteria in the colon” are frequently added. Thus prebiotics escape digestion in the upper gastrointestinal tract and are metabolized by a limited number of the microorganisms comprising the colonic microflora. Prebiotics are principally oligosaccharides (fructo-oligosaccharides, inulins, isomalto-oligosaccharides, lactitol, lactoscerose, lactulose, pyrodextrins, soy oligosaccharides, transgalacto-oligosaccharides, xyl-o-oligosaccharides) mainly stimulating bifidobacteria growth, for which reason they are referred to as bifidogenic factors. Therefore bio-yogurt could contain a variety of compounds exhibiting biological activities, among which a number of bioactive casein peptides exhibiting opioid agonistic and antagonistic activities, immunomodulation, anti-thrombotic, ACE-inhibitor, and anti-microbial activities. A fermented milk beverage of buffalo based on cheese whey and soymilk mixture has been also elaborated. A mixed substrate was prepared by selective combination, which contained buffalo cheese whey 35%, soymilk 30% and cow milk 35%. The substrate mixture was fermented by a mixed culture of *Lactobacillus casei* shirota and *Bifidobacterium adolescentis* at 37°C for 8 h keeping a 1 : 1.5 proportion between the lactic and bifidobacteria within a 5% (v/v) inoculum size. The fermented beverage was lightly extra-flavoured with vanilla essence and subjected to chemical, microbiological and sensory evaluations during storage for 28 days at 4°C. Except a slight variation in the acidity, no other properties changed even after 28 days. There were viable cells of the bacterial strains within the recommended limits (6.8 × 108 cells for *L. casei* and 2.3 × 107 cells for *Bifidobacterium*. No negative changes were found in the sensory characteristics of the beverage allowing its good acceptability in all during the storage period (Macedo *et al.* 1999).

**Kefir.** Kefir is a fermented milk product that originates in Asia. The kefir produced from sheep and bovine milk possesses various biological properties. Instead of kefir grains a complex starter made by lactic acid bacteria (lactobacilli, lactococci, leuconostocs), acetic acid
bacteria and yeast mixture has been recently formulated in various ratios by starter manufacturer. Production of ethanol and carbon dioxide by lactose fermenting yeast (mainly *Kluyveromyces lactis*, *Kluyveromyces marxianus*), as well as non lactose fermenting yeasts (*Saccharomyces cerevisiae*) has been observed along 1-year assay. The major end products of the fermentation were identified as lactic acid, acetaldehyde, acetoin, diacetyl, ethanol and CO₂ in buffalo milk. Fermentation was stopped at pH about 4.8 by refrigeration while alcohol ranged from 0.5% to 2%; fat content about 8% with a dominant acid taste slightly yeasty. Compared to typical bovine, the buffalo kefir was sharp, acid and yeasty flavour like the bovine counterpart. Due to well balanced composition of kefir starter the fermented beverage was made in a reproducible manner. From the constant content of alcohol, acetic acid content and sensory attributes during the 1 month storage it was concluded that beverage was stable.

**CONCLUSION** - These considerations allow concluding that the complex multi-step process of Mozzarella making must stimulate cheese makers to promote applied researches to implement controls of parameters decidedly influencing cheese functionality and yield. This kind of research requires day-to-day experiments with slight modifications of parameters and checking for the positive or negative changes produced in the cheese. This could afford a better understanding how the processing changes influence cheese yield and its functionality and indicating precise guidelines for cheese making. On the other hand, other products can be obtained from water buffalo milk or cheese whey such as whey proteins, single protein fractions, whey protein hydrolysates, fat, butter from pure species, fractionated fat, and other cheese varieties.

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