FACTORS THAT AFFECT THE THERMAL STABILITY OF BOVINE MILK AND THE USE OF ALCOHOL TEST IN THE MILK INDUSTRY – A REVIEW

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SUMMARY: The dairy industry requires good quality milk with an adequate yield that does not affect the industrial process. Therefore, to produce safe food for consumers aiming to increase the shelf life of the product, the before mentioned industry makes use of heat treatment (pasteurization or UHT – Ultra-high Temperature processing). Milk must have adequate quality and high-temperature resistance; otherwise, thermal stability problems may occur. The alcohol or alizarin test is used in dairy farms to identify milk samples that present over the normal acidity and to measure their stability before being transported to the consumer market. Thus, the objective of this review was to search, in the literature, for evidence regarding the factors that interfere in the heat stability of milk and the use of the alcohol test. False-positive results are usual in samples that make use of that test, showing cases of unstable non-acid milk and putting in jeopardy the reliability of the results.

Keywords: Casein; Chemical composition; Stability; Milk quality.

FATORES QUE AFETAM A ESTABILIDADE TÉRMICA DO LEITE BOVINO E A UTILIZAÇÃO DO TESTE DO ÁLCOOL NA INDÚSTRIA DE LÁCTEOS – UMA REVISÃO

Resumo: A indústria de lácteos requer leite de boa qualidade, com rendimento adequado e que não afete o processo industrial. Portanto, para a produção de alimentos seguros ao consumidor, visando aumentar o prazo de validade do produto, a indústria faz utilização de tratamento térmico (pasteurização ou UHT- ultra-high temperature). O leite precisa apresentar adequada qualidade e resistência a altas temperaturas, caso contrário problemas de estabilidade térmica podem ocorrer. O teste do álcool ou alizarol é utilizado na propriedade leiteira para identificar amostras de leite com a acidez acima do normal e mensurar a estabilidade do produto antes do transporte para a indústria. Desta maneira, objetivou-se com esta revisão buscar na literatura evidências sobre os fatores que interferem na estabilidade térmica do leite e na utilização do teste do álcool. Resultados falso-positivos são comuns em amostras que utilizam este teste, evidenciando casos de leite instável não ácido e colocando em dúvida a confiabilidade dos resultados.

Palavras-Chave: caseína, composição química, estabilidade, qualidade do leite

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Introduction

Brazil is ranked fifth in global milk production, with 35.1 billion liters produced in 2018, and the intake of milk, on an industrial scale, was 24.3 billion liters in the same year. The *per capita* consumption of milk in Brazil is of 60 liters/inhabitant/year, while the consumption of dairy products totals 173 liters/inhabitant/year (EMBRAPA, 2018). To meet this demand, the dairy industry requires excellent quality raw materials, with proper yield, besides not affecting the industrial process (HANUŠ *et al.*, 2018). Therefore, for the production of consumer-safe foods, the dairy processing industry must be aware of milk components, level of contamination and stability (SILVA *et al.*, 2012).

Bovine milk is composed of lipids, carbohydrates, mineral proteins, and vitamins, and it is considered a nutritionally complete food for human consumption (CAPPOZZO *et al.*, 2015; CHAVAN *et al.*, 2016; O'SULLIVAN; COTTER, 2017). The presence of water in the milk, combined with the high pH nutritional compounds, are an environment conducive to microbial development, requiring the use of heat treatment (pasteurization or UHT – Ultra-high Temperature processing) to ensure food safety and increase the shelf life of the product (CLAEYS *et al.*, 2013; MCAULEY *et al.*, 2016). Milk must have adequate quality and high-temperature resistance, without coagulating, when subjected to these types of heat treatment; otherwise, issues of thermal stability may occur (LE *et al.*, 2015). Some factors that are related to milk composition may affect thermal stability, such as milk proteins (mainly casein), lactose, acidity, urea and saline composition (calcium, citrates, and phosphates) (MURPHY *et al*., 2016). Other weighting factors regarding milk composition are cow age, lactation period, the incidence of mastitis and the animals' diet (CLAEYS *et al.*, 2014).

In Brazil, the legislation requires technical regulation for products of animal origin, such as raw milk, in which the alcohol or alizarol stability test (alcoholic solution with alizarin pH indicator) is performed on the dairy farm to identify high bacterial contamination in milk samples, being mandatory to determine the acidity and the stability of the product before shipping it to the consumer market, regulated by normative instructions IN – 76 and IN – 77 (FAO, 1996; BRASIL, 2018).

According to Barros (2001), the alcohol test carried out in dairy farms determines the acceptance or rejection (in this case, the milk is discarded) of the product by the industry at the time of its collection. The rejected milk presents protein precipitation by the test due to the reduction of pH via lactose fermentation and lactic acid production because of the presence of microorganisms in the milk. However, the relationship between pH, lactic acid and the microorganisms load may be influenced by the presence of psychrotrophic agents in the milk, which do not metabolize lactose into lactic acid, bringing about high bacterial counts that are not

always attributed to acidity (SAMARŽIJA *et al*., 2012). The reduced stability of milk in the presence of alcohol testing leads to problems in equipment at the manufacturing step. Coagulation during pasteurization or UHT process results in obstruction and blockages in the process, as well as problems in the manufacture of creamy liqueurs, such as fat-plug. Besides, with emulsion, the instability of shelf life is reduced (RADFORD *et al*., 2004). Another not least issue that interferes with those alcohol testing results is the unstable non-acid milk syndrome (UNAM), which is unstable in alcohol testing without acidity of microbiological origin (TSIOULPAS, 2007; FAGNANI, 2016).

In countries such as Brazil, Uruguay, Cuba, Argentina, India, and South Africa, alcohol testing is still used by the dairy industry, in which this UNAM phenomenon is frequently detected. In other countries such as Australia, Canada, United States, New Zealand, and some European countries, alcohol testing has already been abolished as a safe and efficient practice in detecting stability problems because, in these countries, the milk quality is high. And it is different from Brazil regarding the degree of technification and knowledge of those technologies by farmers and even by the dairy industry (BATTAGLINI *et al.*, 2013; MARTINS *et al.*, 2015).

Unstable non-acid milk presents physicochemical changes in milk quality due to multifactorial causes, which are associated with metabolic, physiological and nutritional disorders, as well as determining factors for milk synthesis. The loss of casein stability present in the alcohol test is one of the biggest changes, resulting in positive precipitation without high acidity (above 18 ºD) (MARQUES *et al.*, 2007; ZANELA *et al.*, 2009). UNAM is a problem that requires attention, as it leads to the condemnation and disposal of milk in the farm, giving rise to economic losses to the farmer, as well as to the industry, which no longer collects a larger volume of milk for processing.

Noting the increasing importance given to the quality of the raw material by the dairy industry, this review aimed to find, in the literature, evidences on the factors that interfere in the thermal stability of bovine milk and the use of alcohol testing in the dairy industry.

Milk quality and its composition in Brazil

Bovine milk is characterized as an emulsion of fat globules in suspension of casein micelles in the aqueous phase, with the presence of lactose molecules, whey proteins and solubilized minerals. It is basically composed of water (87.3 %), total solids (12.7 %), total proteins (3.3 % to 3.5 %), fat (3.5 % to 3.8 %), lactose (4.9 %) and minerals and vitamins (0.8 %) (CHANDRAKANT; SINGH, 2018). Some variations may occur, influenced by the animal breed (Table 1) (SORMOLI, 2013). Milk quality refers to characteristics that influence the nutritional

value, yield, and safety of dairy products that are produced, being determined by composition, hygiene and safety aspects with nutritional, sensory and technological quality (MONARDES, 2004). For Marques, Fischer, and Zanela (2010), milk quality must present chemical, microbiological and organoleptic composition and a number of somatic cells that meet international quality requirements, being free of any kind of adulterants, disinfectants, and antibiotics.

Table 1 – Chemical composition of milk from different bovine breeds.

Breed	Fat (%)	Protein (%)	Lactose (%)	Dry Matter (%)
Holstein	3.5	3.1	4.9	12.2
Jersey	5.5	3.9	4.0	15.0
Zebu	4.9	3.9	5.1	14.7
Brown Swiss	4.0	3.6	5.0	13.3

Adapted from Órdoñez *et al*. (2005); Gonzalez (2001).

According to reports by Tsenkova *et al*. (2001), the somatic cell count (SCC) acting on the secretion of milk indicates aspects of mammary gland health, being widely used as an indicator of subclinical mastitis and as a measure that determines the quality of the analyzed milk. Another important issue referred to SCC is that when its levels in the milk are high, there is a reduction in casein, fat and lactose concentrations in milk, increased enzymatic activity and reduced quality and yield of dairy products (REIS *et al.*, 2013).

As for the nutritional factors of the animal, they cause problems regarding the composition of the milk, affecting its quality, because there is a need of paying extreme attention to the type of food provided, its availability, way of conservation and, above all, adjusting the diet according to the needs of the animals. Genetic factors (species and breed), environmental factors (stress, season, management), intrinsic factors of the animal (age, stage of lactation, number of lactations), extrinsic factors (health and bacterial contamination) are also responsible for the milk quality, all in which the search for stability within the production system must be constant (ZANELA *et al*., 2014).

Milk quality standards are controlled in Brazil by the Ministry of Agriculture, Livestock and Supply (MAPA), in which, in 2019, the normative instructions IN-76 and IN-77 came into force. IN-76 deals with the product's characteristics and quality in the industry, while IN-77 defines criteria for obtaining milk with quality, safe for the consumer, which ranges from the organization of the farm, its facilities, and equipment, until the training and qualification of those who are responsible for daily tasks, the systematic control of mastitis, brucellosis and

tuberculosis (BRASIL, 2018). The minimum standards established for the industrial receiving of milk regarding its chemical composition are 3.0 % of fat, 2.9 % of protein and 8.4 % of defatted dry extract. The maximum number of somatic cell count (SCC) is 500,000 cells/mL^{-1} of milk, and the total bacterial count (TBC) is 300,000 CFU/mL^{-1} of milk. Regarding the physical characterization, milk must have a titratable acidity between 14 and 18 ºD and must be stable in an alcoholic solution with at least 72 °GL of ethanol (BRASIL, 2018).

Heat Stability of Milk (HSM)

Milk stability is considered as the fundamental time for visual coagulation to occur at a given pH and temperature, and it is directly related to the ability of milk to resist coagulation at certain temperatures (LEITNER *et al.*, 2016). The heat stability of milk is multifactorial, being punctually linked to its protein structure, more specifically in the casein fraction, which is largely responsible for the physical structure of milk (HANUŠ *et al.*, 2018).

Thermal Treatment

The dairy industry in Brazil has, regarding heat treatment, a need for application, but its intensity should be measured not to cause damage to the quality of processed food. UHT (Ultra High Temperature) milk, known as long-life milk, is homogenized for 2 to 4 seconds at a temperature between 130 ºC and 150 ºC by a continuous flowing thermal process, and then it is immediately cooled to below 32 ºC and packaged under aseptic conditions in sterile and hermetically sealed packaging (BRASIL, 1997). The heat treatment of milk is extremely intense and destroys opportunistic contaminating microbiotas, the pathogenic and sporulated ones, proving to the consumer to be a safe final product (HUPPERTZ, 2016). According to Fox's (1981) studies, milk, when subjected to heating, can be classified into five groups due to changes that precede coagulation: acid production, calcium phosphate precipitation, casein modifications, darkening reaction (Maillard) and interaction of sulfhydryl groups, including whey proteins or urea.

O'Connell & Fox (2016) concluded, in their studies, that the effect of the temperature influences milk constituents, causing significant variations in both protein and salt balance. Warming and denaturation of soluble proteins are observed effects that give rise to the β-lactoglobulin/κ-casein complex, resulting in physicochemical disorders of milk (HANUŠ et al., 2018). In saline equilibrium, calcium phosphate precipitation occurs in association with the micellar phase and the reduction of soluble inorganic calcium and phosphorus contents. This loss

of salt balance due to warming is only moderately recovered due to the solubilization of colloidal calcium phosphate fractions (CHAVAN, SEHRAWAT, MISHRA, & BHATT, 2016).

Intrinsic factors that affect the heat stability of bovine milk

Casein

Milk stability is due to the organization of caseins in micelles. Casein is one of the milk proteins and is synthesized in the epithelial cells of mammary gland and secreted in the form of micelle, and can be called phosphoproteins containing serine-linked phosphate radicals (P-se), present in several regions of the polypeptide chains, presenting more hydrophilic or more hydrophobic zones (amphipathic activity), being more susceptible to proteolysis (CHANDRAKANT; SINGH, 2018). The micelle represents 95% of the casein form in milk and are the main components responsible for maintaining the heat stability of milk (MURPHY *et al.*, 2016).

Somewhat, all fractions of casein seem to be involved with the term stability (caseins α, β, κ and γ), especially K-casein, which, being on the surface of the micelle, interacts with other whey proteins and also with calcium, which promotes an electrostatic change, favoring the aggregation of casein molecules and facilitating their precipitation (O'CONNELL; FOX, 2016). To prevent precipitation of casein micelles, κ-casein plays an important role in stabilizing the micelle in the presence of calcium (CHANDRAKANT; SINGH, 2018). When water is added to milk or soluble calcium is decreased, casein stability increases because its micelle retains water equivalent to three times its weight, while maintaining stability (HUPPERTZ, 2016).

Another factor that impairs casein stability is the development of proteolytic psychrotrophic bacteria, which act at low temperatures (0 ºC to 15 °C), producing thermostable enzymes that eventually act on K-casein and destabilize the milk stored in the cooler (FAGNANI, BELOTI; BATTAGLINI, 2014). According to Gaucher, Mollé, Gagnaire, & Gaucheron (2008), the action of psychrotrophic protease enzymes occurs differently among fractions of milk proteins, with K-casein being the most susceptible to the action of these enzymes, whereas proteins of serum are resistant to protease attacks, as well as pH imbalances. Table 2, which is at the end of this research, shows the summary of some studies on casein behavior concerning milk thermal stability.

Table 2 – Summary of studies of casein behavior in relation to milk thermal stability.[a]

Experimental approach	Results	Reference
Lactation period		
Two 6 weeks' periods. Period 1 cows were in mid lactation. Period 2 cows were in late lactation.	Milk samples in mid lactation were more thermally stable than in late lactation. Milk produced during late lactation has a higher plasmin activity, promoting hydrolysis of casein, which may contribute to reduced thermal stability.	O'Connell *et al*. (2017)
Plasmin		
It was added 6 mgL^{-1} of plasmin to examined its effect on the heat stability of raw, pre-heated, serum protein-free or concentrated skim milk.	Plasmin activity markedly affected the heat stability–pH profile of skim milk and serum protein-free milk, apparently by altering the properties of the casein micelles.	Crudden *et al*. (2005)
Samples were incubated at 37°C for 0, 3, 7 and 16 days, after which samples were analysed for plasmin activity, plasminogen-derived activity and proteolysis.	Plasmin-induced casein proteolysis was observed and the degree of hydrolysis was primarily correlated with plasmin activity, which results in gradual loss of colloidal stability of the micelles.	Gazi *et al*. (2013)
Seasonal variation		
Milk samples were collected monthly from a mixed-herd of spring- and autumn-calving cows during a year.	Season did not influence the heat stability characteristics of the milk, however ethanol stability of autumn milk or winter milk at pH 7.0 was lower than that of spring or summer milk.	Lin *et al*. (2017)
Transglutaminase		
Heat-induced coagulation of unconcentrated reconstituted skim milk was determined after incubation with transglutaminase.	Treatment with transglutaminase reduced heat stability of milk at pH <6.6.	Huppertz (2013)
pH		
Fresh bulk raw milk was acidified with HCl at pH 6.4 (T2), 6.1 (T3), 5.8 (T4) and 5.5 (T5), viewing analyzed in terms of casein molecules. Control milk suspension (T1) was prepared in the same conditions.	The obtained suspensions were progressively demineralized and partial dissociations of casein micelles were observed. Suspensions T1 and T2 were more stable than T3 which have an intermediate stability, and T4 and T5 which were the most unstable. These decreases of stability were related to the presence of small casein particles and the quantitative reduction of casein micelles.	*Silva et* al. (2013)
Contamination		
Fresh bulk milk was microfiltrated and then divided into two parts: one served as the control milk and the second was incubated for 2 hours at 4°C in the presence of the psychrotrophic bacteria *Pseudomonas fluorescens.*	P. fluorescens induced instability in the corresponding UHT milk during storage. This instability was detected by the presence of a sediment, a low value in the phosphate test and the formation of aggregates. These macroscopic instabilities were related to decreases in the negative charge and hydration of casein micelles.	Gaucher *et al*. (2011)
The heat-stable protease Ser2 is secreted by the species *Serrati liquefaciens*, a psychrotrophic bacteria frequently found in raw milk. The enzyme was purified and added to microfiltered raw milk before UHT treatment.	A visual destabilization appeared after 8 days of storage with the presence of sediment. This confirmed that the presence of the protease Ser2 in raw milk can be one of the main causes of UHT milk destabilization.	Baglinière *et al*. (2017)

[a] Abbreviations are: HCl, hydrochloric acid; UHT, ultra-high temperature.

pH and Acidity of Milk

Bovine milk has natural acidity formed from its acids and chemical composition, being considered normal levels of titratable acidity between 14 and 18 ºD, and pH between 6.6 and 6.8. (BRASIL, 2011). Some factors have direct involvement in the acidity of bovine milk, such as lactation period, inflammatory and infectious processes of the mammary gland, endogenous and exogenous enzymatic action, with minimal and distinct changes in the heat stability of milk (BARBOSA et al., 2008). The maximum point considered for milk stability is close to a pH of 6.7 due to the aggregation of whey proteins (albumin and globulin) with the micellar surface, but when the pH exceeds 6.9, there is a dissociation of whey proteins and also K-casein, reducing milk stability (SINGH, 2004; O'CONNELL; FOX, 2016). Milk heating processes interfere with its pH, given that lactose is broken down into organic acids, leading to a pH reduction and resulting in displacement of the maximum milk stability range (CHANDRAKANT; SINGH, 2018).

Casein is highly sensitive to acidity, as when milk becomes acidic, its positive ions neutralize the negative charge of casein, thus decreasing the repulsive force between molecules, favoring the association and making them less heat-stable (FOX; BRODKORB, 2008). By the time its isoelectric point is reached (pH 4.6), in other words, when total neutralization of negative casein charges occurs, the micelle flocculates and unite retaining fluid (serum). At the same moment, the contraction of the casein micelle begins, expelling the serum, causing dehydration and calcium output from the micelle (O'CONNELL; FOX, 2016). An acidic pH (below 6.5) reduces the ionic strength that maintains the micellar structure, shifting calcium from the colloidal phase to the soluble one, causing an increase in ionic calcium (BELOTI, 2015).

Ionic Calcium

Ionic calcium has a high degree of importance, since it is considered the main factor of interference in milk stability, acting both in heat and ethanol stabilities, and it is distributed in three phases: colloidal, soluble and ionic (O'CONNELL; FOX, 2016). Approximately 70 % of the calcium is in the colloidal phase and in the form of calcium phosphate bounded to casein micelles. The remaining 30 % is in the form of solution, distributed among bonds with citrates and phosphates or as free ions (FAGNANI *et al*., 2014). The presence of salt in milk reduces the ability of casein to maintain its physical structure, and the mineral salt saline balance between both phases (colloidal and soluble) must be preserved. However, when an imbalance occurs, there will always have compensation, resulting in an increase in ionic calcium, which goes from the soluble to the colloidal phase, causing milk instability (HOLT, 2004).

Ion calcium levels are affected by various aspects, such as temperature, pH, milk storage time, while concerning animal factors, are affected by lactation time (cows in early and late

lactation have higher levels of ionic calcium), the time between the collection of samples and measurement of calcium (levels may increase up to eight hours after collection) (BARBOSA *et al*., 2008). Changes in pH directly influence calcium mobilization from one phase to another. Reductions in pH cause calcium displacement from the colloidal phase to the soluble one, increasing ionic calcium (RENHE et al., 2018). Increased levels of ionic calcium in milk tend to increase ionic strength by binding ions to protein-charged groups, leading to reduced electrostatic repulsion that favors protein agglutination and reduces the stability of casein, stabilizing salts to maintain the integrity of the micelle (CHANDRAKANT; SINGH, 2018). When this type of electrostatic repulsion occurs, it leads to a micellar breakdown, which is a result of increased micelle hydrophobicity. When the level of calcium is reduced, there is an increase of negative charges of micelles, intensifying the repulsion between them, making precipitation difficult (BELOTI, 2015). This can be explained by the sensitivity of casein to calcium, as phosphoserine residues agglomerate and are negatively charged and bind to calcium, affecting the stability of micelle (CHANDRAKANT; SINGH, 2018).

Stabilizing Salts (Citrates and Phosphates)

The mobility of milk salts in their soluble and colloidal phases establish direct equilibrium relationships with the stability of bovine milk, mainly due to the increase of calcium in the soluble phase and the decrease of phosphate and citrate activity (HUPPERTZ, 2016). According to Fox, (1981) about 94 % of citrate is found in the soluble phase, 85 % of which is associated with calcium and magnesium, but only 6 % is represented in the colloidal form, which contributes to the union of casein micelles, being synthesized inside the epithelial cells of the mammary alveolus (SILVA, 2012).

Warming milk at certain temperatures results in the loss of stability of casein micelles due to the interference on the saline balance of milk so that colloidal calcium and phosphate (before bounding casein) will separate from it and precipitate into the tricalcium phosphate form (BELOTI, 2015). Therefore, the dairy industry that uses UHT treatment in Brazil adds citrates and phosphates to milk, since they can increase the stability of casein, reducing its tendency to aggregation, coagulation, and precipitation (O'CONNELL; FOX, 2016). Animal feed is directly correlated with citrate levels, as forages are its main sources. Low bulk diets determine low citrate yields (SILVA, 2004). Phosphate establishes a reduction in ionic calcium in milk, thus ensuring increased stability, but its effect is short-lived compared to citrate, as it remains longer in the soluble phase (MELETHARAYIL *et al*., 2018).

Phosphates are divided into two groups in casein micelle – casein-bound covalent organic phosphate as part of the side chains of phosphoserine residues; and inorganic phosphate, in the form of calcium phosphate crystals that are displaced from the micelle by acidification and whose interaction has a more ionic characteristic than the covalent one (MCMAHON, 2010). The colloidal calcium phosphate bonds with the α, β and κ-CN fractions, which are responsible for micelle stability and are called crosslinking bonds, which manifest themselves as an agent that neutralizes residues of phosphorine, positively charging it, binding to negative sites by micellar calcium phosphate (GAUCHERON, 2005). This same author states that colloidal calcium bonds are broken when the milk is in an acid medium, being irreversible even if the pH is stabilized. All inorganic phosphate is solubilized at pH 5.2, with the destruction of inorganic micellar calcium phosphate, that is, calcium that is bound to inorganic phosphate and phosphoserine. At a pH of 3.5 calcium is fully solubilized (BELOTI, 2015). If there is no phosphate to remove soluble calcium, even at high pH, milk shows reduced stability against alcohol testing (FAGNANI *et al*., 2016).

Seroproteins in Milk

Certain factors that interfere with the way milk becomes unstable to heat treatment are linked to kappa-casein (k-CN) and soluble beta-lactoglobulin (β-Lg), as seroproteins end up denatured when heated and react with k-casein causing instability (SINGH, 2004; ANEMA & LI, 2003). The issue is because beta-lactoglobulin (β-Lg) is a thermolabile seroprotein, from its arrangement in sulfhydryl groups combined with its calcium sequestration capacity at a certain pH. Dissociation of β-Lg begins at temperatures between 30 ºC and 55 ºC, reaching its peak at 80 ºC and pH 6.5 or 60 ºC and pH 8.0, causing denaturation, which is one of the reasons for sedimentation problems in UHT milk equipment faced by the dairy industry in Brazil, requiring the use of stabilizers in the process (BELOTI, 2015).

Urea Nitrogen in Milk

High milk urea levels increase milk urea stability due to reduced acidity or transformation of urea into cyanate, which tends to react in the presence of protein increasing negative micelle charges, thus providing higher repulsion force, increasing thermal stability of milk (MARTINS *et al*., 2015).

N-urea levels in milk are directly linked to the feed that the animals are given to, always referred to the protein composition of the diet, in which a sample with a value above 3.2 % of protein should not exceed 18 mg/dL^{-1} of urea nitrogen. Higher levels indicate an excess of

degradable protein in the diet or a soluble carbohydrate deficit, even resulting in low lactose levels in the milk (PEREZ Jr., 2001; MARQUES *et al*., 2010).

As for the cheese industry, this increase in milk urea can become a problem, since it tends to reduce the clotting time, and the protein responsible for cheese mass is replaced by N-urea (BELOTI, 2015).

Lactose of Milk

Being the main carbohydrate in milk, lactose determines its volume by representing 50 % of milk osmotic pressure, associated with sodium, chlorine and potassium ions in the mammary gland. Lactose drags about ten times its weight in water, and an increase in lactose concentration above 50 % of normal results in instability of type A milk at pH 6.4 to 6.7 and carries the minimum stability point to more alkaline pH values (SINGH, 2004). The addition of lactose to milk negatively interferes with its stability due to the increased rate of pH reduction by the transformation of lactose into lactic acid when milk is outside the minimum stability pH range (6.6 to 6.8), where the decrease in stability is also due to the increase in ionic calcium concentration, taking into account the temperature conditions in which this milk is stored (CHAVEZ *et al*., 2004).

Bovine Milk Temperature

Some aspects of milk stability are related to the temperature at which milk is subjected. Fagnani, Battaglini, Beloti, Urbano, & Bronzol (2016) pointed out the reduction of k-casein fraction stability with ionic calcium content due to the cooling time of the milk sample. According to the same authors, cooling causes changes and disorganization in saline balance (P and Ca) between micelles and soluble phase, increasing serum calcium, phosphorus and casein concentrations, and pH increases rapidly (0.3 – 0.4 pH unit between 38 and 6 °C). Micellar casein at 20 ºC represents 93 to 95 % of the total milk casein, but if refrigerated at 2 ºC becomes 80 to 85 %, causing an imbalance between caseins and directing the soluble phase, reducing the average diameter of the micelles (HUPPERTZ, 2016). Milk preheating alters milk stability in the pH range from 6.4 to 6.8 due to changes in the system's ionic balance by temperature-induced calcium phosphate precipitation (FAGNANI *et al*., 2014).

These events directly affect the dairy industry, as they result in changes in technological properties, such as increased coagulation time, changes in curd consistency and reduced yield of cheese production up to 10 %. A possible solution to restore its properties is the use of pre-maturation, adding calcium chloride ($CaCl_2$) mixture for initial pH adjustment (BELOTI, 2015).

Plasmin

Bovine milk contains the complete plasmid system: plasmin, plasminogen, plasmin activators (PAs), and plasmin and PAs inhibitors (KELLY; MCSWEENEY, 2003). Plasminogen is activated in plasmin by PAs, which are divided into two main classes, the urokinase (u-PA), associated with somatic cells, and the tissues (t-PA), associated with casein micelles (WHITE *et al.*, 1995). Unlike other structures of the plasmid system, plasmin and PAs inhibitors occur in the serum phase of milk (WEBER; NIELSEN, 1991).

Plasmin is a technologically important structure in bovine milk, as it reduces the quality of dairy products. α_{s1}-casein is susceptible to plasmin proteolysis, while α_{s2}-casein and β-casein are used as substrates. Plasmin activity drives effects on milk and dairy products, such as shorter rennet coagulation time, reduced curd firmness, lower cheese yield, UHT milk gelation over time, development of bitter milk flavors and degradation of casein-based products during storage (MARA *et al.*, 1998; CRUDDEN, 2005).

Extrinsic factors that affect the heat stability of bovine milk

Lactation Period

The lactation period of the animal has a direct influence on the heat stability of milk due to changes in its constituents since during lactation the potassium and lactose proportions are constantly decreasing, peaking at the beginning and decreasing until the end of lactation. direct binding to the cow's nutritional factor. For chlorides and sodium, levels start to low and rise, peaking at the end of lactation (O'CONNELL; FOX, 2016). As a consequence of these occurrences, there is a protein concentration in the milk, increasing the formation of the β-Lactoglobulin/κ-casein complex, and calcium and phosphorus follow the same pattern, in which ionic calcium has an increase in the beginning and end of lactation, interfering in the stability of milk (BARROS, 2002).

Herd Nutrition

Another factor directly linked to milk stability is the feeding that lactating animals receive, in which the compensatory capacity of the animal's organism should reduce the effects of nutritional imbalances on milk stability. Feed restriction of animals from alcohol test is negatively seen in numerous studies in the literature (GABBI *et al*., 2016) (will be approached in item 4.2.3), and a small number of studies that elucidate the relationship between nutrition and the heat stability of milk (ZANELA *et al*., 2014). According to Murphy, Martin, Barbano, & Wiedmann, (2016), low serum glucose levels would be the triggering factor of milk instability,

which is confirmed by the reduced lactose levels. Another factor is that diets that lead to lower intake of citric acid precursors will determine a lower level of produced citrate, which strongly interferes with milk stability (ZANELA *et al*., 2009).

Bovine Breed

High-production Holstein cows are more likely to show changes referred to the heat stability of milk (PONCE; HERNANDES, 2001). This breed has a lower frequency of allele B for k-casein when compared to Jersey cows. According to the studies of Robitaille, Britten, & Petitclerc, (2001) with the expression of B alleles greater than alleles A has, there is a positive imprint to ensure greater milk stability against alcohol testing because it needs higher concentrations of ethanol to produce any type of instability in milk proteins.

Regarding breeds with crossbreed *Bos taurus taurus* vs *Bos taurus indicus*, in a study carried out by Botaro *et al*. (2009), the milk produced by Holstein cows was more stable to the alcohol test when compared to Girolando cows, besides presenting higher presence of B allele than the crossbred milk.

Mastitis, Somatic Cell Count (SCC) and Total Bacterial Count (TBC)

Animal health is directly related to the thermal stability of milk. Animals with mastitis elevate the passage of sodium chloride directly from blood to milk, causing disorganization of the salt balance. Mastitic milk is three times more likely to be unstable when compared to healthy cow milk, and the increase in milk pH is responsible for this occurrence, due to the greater permeability of the mammary epithelium to small particles and ions, as mastitis modifies the vascular permeability of milk-secreting cells, (Na, Cl, P and K) (FAGNANI *et al*., 2014). Mastitis, when established in animals, tends to elevate undesirable characteristics of milk, such as proteolytic enzymes, salts, and rancidity and reduces desirable protein, fat and lactose levels, as well as cheese production and heat stability (OLIVEIRA *et al.*, 2013). The reduction in lactose concentration caused by mastitis due to breast tissue damage decreases the capacity of synthesis by the glandular epithelium, affecting the volume of milk produced (LEITNER *et al.*, 2016).

Milk with high somatic cell index (SCC) has high enzymatic activity, with increased proteolysis and lipolysis in the udder, both before milking and after the animal is milked. Somatic cell lysosomes are provided with proteolytic enzymes, such as cathepsin D, which from K-casein can produce caseinmacropeptide, which in high concentration ends up causing milk coagulation (HUPPERTZ, 2016).

High levels of total bacterial count (TBC) interfere in the composition of bovine milk, causing an increase in the concentration and passage of blood proteins and a reduction in lactose (BUENO, 2008). When milk TBC increases, there is also an increase in the action of proteolytic enzymes, which will modify the structure of casein micelles and reduce the repulsion capacity between them, generating heat stability disorders (SILVA, 2004). When we talk about levels of SCC and TBC, we need to emphasize that they will not always be correlated, because the microorganisms have a great reproductive capacity, which may be a reflection of the mastitis scenarios or poor quality of the water used in the hygiene of the utensils. Or even failure of the milk cooling system (RAMIRES *et al*., 2009; WINK; THALER NETO, 2009).

Alcohol/Alizarol Test in Dairy Industry

In the late nineteenth century, one of the main problems in the dairy industry was the uncooled milk that turned sour. The researchers of that time sought to solve this problem through a detection method of this fermented milk. A German researcher called Martinn, concluded in his experiments, in 1890, that by mixing equal parts of milk and alcohol, he could find acidity of microbiological origin. In the following years, the researcher Wilhelm Morres, in 1910, added to the test a color indicator, the alizarin dye (able to infer milk pH by colorimetry). Thus, the first milk quality test was created, providing safety to the industry, since if the tested milk formed lumps (coagulation) it would be sour and could not be industrialized (DEETH; LEWIS, 2017). The test of alcohol or alizarol was of great value to the world dairy industry because it made it possible to prevent acidic milk, such as colostrum or mastic milk from being processed, thereby reducing quality problems and coagulation in the heating plates of the dairy pasteurizer. Consequently, 1930 brought with it the best test to measure the thermal resistance of milk, because, by logic, if it coagulated in alizarol the same would happen in the pasteurizer. The following year, the reliability of the alcohol test was cast in doubt by Ramsdell, who concluded that no researcher could prove that this test was reliable in determining whether or not milk could withstand heating. Almost two decades later, Davis and White (1956) found no relationship between thermal stability and ethanol stability, suggesting that the thermal stability of milk is not only related to bacterial acidity, but other factors interfere in protein stability.

Nowadays, the first test that milk is subjected to after being milked and properly cooled is still the alcohol or alizarol test. Its application is done in the dairy farm, being a decisive instrument for quality milk approval and subsequent charging for dairy (ZANELA; RIBEIRO, 2018). This test evaluates the stability of milk proteins, which, when subjected to the dehydration caused by alcohol, is possible to estimate the resistance of milk to heat treatment in the industry, since milk with low hygienic quality during its production may present reduction of pH by

lactose fermentation in lactic acid, thus resulting in increased protein instability (OMOARUKHE *et al*., 2010). One reason why this test is still widely used in some Latin American countries, in Africa, and the Far East by the dairy industry is because of its low cost coupled with the speed and simplicity of execution in regions with large number of low technological dairy farms (KASSA *et al.*, 2013; HORNE, 2015; RATHNAYAKE *et al.*, 2016).

Alcohol test is regulated in Brazil by the Industrial and Sanitary Inspection of Animal Products (Riispoa) since 1952. In 2002, the country introduced the National Milk Quality Improvement Program, supported by Normative Instruction No. 51 (BRASIL, 2002) of the Ministry of Agriculture, Livestock and Supply (MAPA). In 2011, the NI-51 was replaced by the NI-62 (BRASIL, 2011). Nowadays, the national legislation in force is NI-76 and NI-77, which establishes technical regulations of milk production, quality, collection and transport to the industry, where specifically NI-76 has parameters from production to reception in the industry, and NI-77 is responsible for the criteria for approving it into the industry until it is shipped (BRASIL, 2018). According to NI-76, moments before the milk is loaded on the farm, it must be shaken before the alcohol test (with a minimum concentration of 72 °GL) and must be stable to the test (negative result). However, graduations of 76; 78 and up to 82 °GL of alcohol have been used by various industries, hoping to select better quality milk.

The alcohol test consists of mixing equal parts of milk and alcohol in the salut acidimeter (Figure 1). It is a way to evaluate the formation of clots or lumps in the milk, since the added ethanol causes changes in the K-casein layer, reducing micellar charge and calcium phosphate precipitation, occurring coagulation (STUMPF *et al.*, 2013). In general, with this addition of alcohol to milk, there is a reduction of the dielectric constant of the medium, providing interaction between charges on the most superficial part of casein, decreasing the negative micellar charges and their repulsion force, promoting coagulation, which means that alcohol neutralizes the negative charges of milk proteins.

Figure 1. Acidimiter of salut (ethanol pistol) used to perform the test.

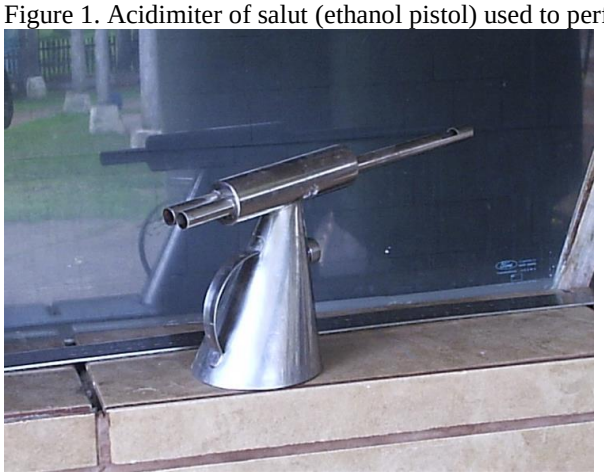


Photo: ZANELA; RIBEIRO (2018).

Milk is considered stable when there is no precipitation (Figure 2), but when the result is positive to the alcohol test, the carrier rejects it, not collecting it, causing damage to both the farm and the industry that has reduced its volume milk intake.

Figure 2 Stable milk on the left, unstable milk on the right with clots.

Photo: ZANELA; RIBEIRO (2018).

The alcohol test with alizarol (Figure 3) has the presence of the alizarin coloring, which reacts by changing color according to the pH in milk, allowing simultaneous observation of casein flocculation and color turning due to pH change. It consists of mixing in a test tube 2 mL of milk, 2 mL of 0.2 % alizarin coloring and 0.1 % of alcohol (TRONCO, 2013). When the pH is too acidic or basic, the change in color is easily noticed, otherwise, when it is in the normal pH range (6.6 to 6.8), the change in color is small, causing doubts and uncertainty in the evaluation of the results (ZANELA; RIBEIRO, 2018).

Figure 3 Ethanol test with alizarin.

Photo: SANTOS; SILVA (2013).

The pH of the alcoholic solution used in the alcohol test may bring false-positive results if this parameter is not adjusted to near neutrality. Vizzotto *et al*. (2012) evaluated alcoholic

solutions with ethanol concentration ranging from 68 to 92 °GL without pH adjusted and alcoholic solutions with pH adjusted (close to neutrality: 6.9 to 7.1) at the same concentrations. The authors observed that extremes (both low and high pH) increased the frequency of positive samples over the adjusted solution. Another key issue in this test is the need to dilute alcohol to the correct concentration (volume/volume) and maintain this concentration until it is used, as a slight elevation of 1-2 °GL can produce quite different results. This fact explains why the results using 72 °GL alcohol is different from those using 74 °GL in the alcohol test (TRONCO, 2013).

When milk is considered normal without coagulation (pH 6.6 around neutrality), it acquired a reddish brick color (titratable acidity 14 to 18 ºD). Milk samples with pH 5.5 and 6.0 are highly acidic (above 21 ºD) and are yellowish with coagulation. However, milk samples with high pH, 7.0 to 7.5 (alkaline milk) are violet and purple. This correlation of pH and visually made colors can be misjudged between analyzes, damaging the dairy sector.

Relation Between Heat and Ethanol Stability of Milk

Industry in this test uses the alcohol concentration proportional to the rigor, with which it is desired to subject the milk to heat treatment in the processing industry, so coagulation tends to occur due to high acidity or saline imbalance when promoting the destabilization of micelles by alcohol. According to Tronco (2013), the higher the alcohol concentration, the better the product's thermostability and the better the milk conservation conditions. However, according to the studies by Machado, Fischer, Stumpf and Stivanin (2017), this correlation of increased alcohol content and increased heat resistance is not true. This correlation was widely questioned by authors seeking to assess whether the alcohol test could measure heat resistance of milk, but they have always come to low correlations, making this prediction uncertain (CHEN *et al*., 2014; HORNE, 2016; DEETH; LEWIS, 2017). During the heating process, a series of chemical changes occur, generating basic differences between the thermal stability of milk and the stability of alcohol, where seroproteins act on the stability of milk, forming the β-lactoglobulin/κ-casein complex, while pH interferes over calcium composition in different phases of the alcohol test (CHRAMOSTOVÁ *et al.*, 2016). Horne and Muir (1990), when comparing the heat stability of milk compared to the alcohol test, found similarities between the methods in the pH adjustment and responded, at least qualitatively, in the same way to the increase or decrease of available calcium. Negri, Chavez, Taverna, Roberts, and Speranza (2001), when evaluating alcohol-stable milk samples at 78°GL, submitted to heat stability test and presented longer coagulation time than alcohol-unstable milk samples at 72 °GL.

Alcohol test, however, has shown positive results in milk with acceptable pH and acidity values (pH of 6.6 to 6.8 and/or titratable acidity of 14 to 18 °D) and SCC and TBC within the appropriate values (COSTABEL *et al.*, 2009). According to Oliveira *et al.* (2011), 40 % to 50 % of milk samples presenting acidity within the acceptable values, precipitate in the alcohol test, being the farm penalized. Studies conducted by Molina, González, Brito, Carrillo, and Pinto, (2001) and Silva *et al.* (2012) demonstrated that the progressive increase in alcohol content increased the number of positive samples of milk to the alcohol test. Evaluating these events, the way the alcohol test is used by the industry should be reevaluated, as it ends up penalizing the farm with false-positive results.

These results are due to a confounding factor called Unstable Non-Acid Milk (UNAM), which is unstable in alizarol (lumps) but not acid in the Dornic acidity test, which measures the amount of lactic acid from bacteria. One way for the carrier to eliminate the false positive doubt is to boil the milk on the farm if it is not coagulating it is probably UNAM, which can be interpreted in the industry platform with the Dornic acidity result (14 and 18 °D). These facts call into question the reliability of alcohol test and draw our attention to the fact that perhaps it is time to abolish this method as a good raw material classifier for the dairy industry, following examples from European and North American countries.

Unstable Non-Acid Milk (UNAM) Facing Alcohol Test

Unstable Non-Acid Milk (UNAM) is termed as a change in milk quality resulting from multifactorial imbalances within the production system (ZANELA; RIBEIRO, 2018). Loss of casein stability on alcohol test is one of the main variations with false-positive results, with no evidence of high acidity (above 18 °D), followed by physicochemical changes in milk, such as saline imbalance and proportion of divalent cations. (CHAVEZ *et al*., 2004). Because they are unaware of this UNAM factor, many farmers end up discarding this positive milk to the alcohol test, as they associate it with acid milk, causing damage to both the farm and the industry, which loses in raw material uptake. The alcohol concentration used at the time of testing is one of the key points for triggering UNAM. IN-76 states that milk should be stable to alcohol/alizarol at a minimum concentration of 72 °GL (BRASIL, 2018). However, the dairy industries have increased the level of this concentration, using up to 82 % alcohol content, causing a reduction in the normal levels of acidity of milk, that is, exerting more radically its dehydrating action. Thus, a milk that passed the alcohol test with an alcohol content of 72 °GL could not withstand an alcohol concentration of 74 or 78 °GL while maintaining the same titratable acidity of 18 ºD.

The earliest reports of UNAM around the world were cited in the literature in countries such as Japan (YOSHIDA, 1980), Italy (PECORARI *et al.*, 1984), Iran (SOBHANI *et al.*, 1998),

Cuba (PONCE, 2001), Uruguay (BARROS *et al.*, 1999), Argentina (NEGRI *et al.*, 2001), Brazil (DONATELE *et al.*, 2003; MARQUES *et al*, 2007; ZANELA *et al*, 2009; ROMA Jr, 2007; LOPES, 2008, OLIVEIRA *et al*., 2013; WERNCKE *et al.*, 2016). In Brazil, studies making use of diagnosis of UNAM began to be carried out at EMBRAPA in 2002, where in the Southern region of the country, Zanela *et al*. (2006) evaluated occurrences of UNAM, and 3,353 samples of milk were analyzed for alcohol test (76 °GL) and titratable acidity. According to the results, the vast majority (72.2 %) of the samples were alcohol-test positive (with protein instability) and presented normal titratable acidity, characterizing that the milk instability observed in the study did not originate from the presence of lactic acid. Over the next two years, the same researcher analyzed 2,396 milk samples in a mesoregion of the Southern country and found out that 55.2 % of them had UNAM. In the state of Rio de Janeiro, Donatele *et al*. (2003) found out that 59.6 % of the dairy cows analyzed produced 72 °GL Alizarol test positive milk, with no known factor determining acidification. It was observed that 13.6 % of the positive samples had acidity between 14 and 18 ºD, that is, within the normal standard established by the Brazilian legislation.

Roma Jr. *et al.* (2007) conducted a study analyzing 2,981 samples from the states of Minas Gerais, Rio de Janeiro and São Paulo, from October 2005 to September 2006. The authors found that 7.4 % were classified as UNAM presenting coagulation in the test with 78 °GL of ethanol. The highest incidence period was in early Autumn (March), with a fall from early Spring (September). This pattern was related to the low quality of forages between the periods mentioned. Battaglini *et al*. (2016) analyzed 322 samples of raw milk, with 72 °GL alcohol test, and 92 (28.6 %) were classified as normal milk, 138 (42,8 %) as UNAM and 92 (28.6 %) as acid milk. This occurrence was similar to that found by Zanela *et al.* (2009), with occurrence ranging between 30 and 40 %. In studies conducted by Fischer *et al.* (2012), in which unstable milk samples were evaluated at different alcohol concentrations, the result pointed out that the higher the concentration used, the higher the number of cases of UNAM.

UNAM Losses to Dairy Industry

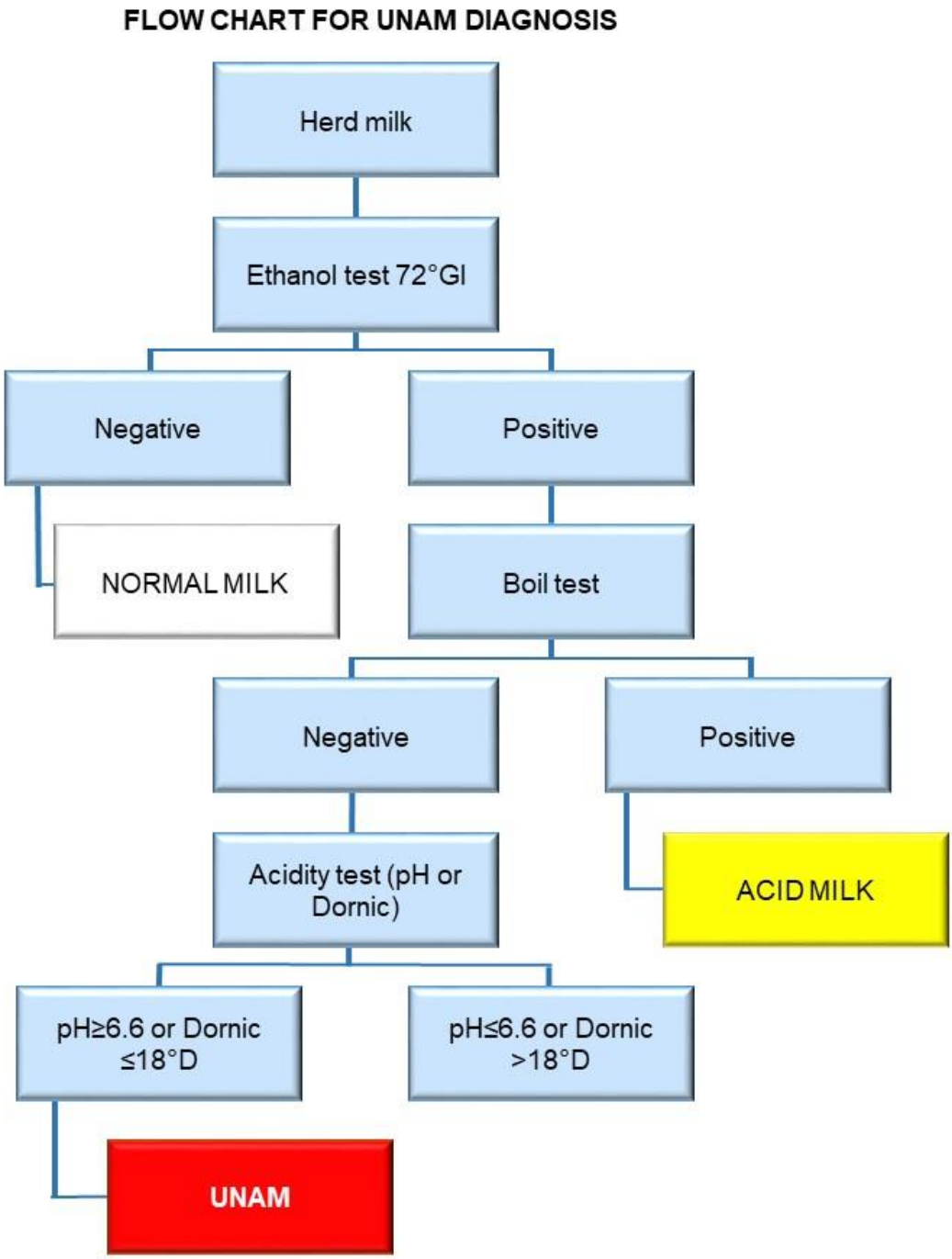
There is a close relationship between the composition of milk and its industrial yield, which is attributed to the casein fraction. Santos and Fonseca (2007) reported that a decrease of 0.5 % in total solids or 0.1% in protein may mean a loss of 5 tons of milk powder or 1 ton of cheese, respectively, for each million liters of processed milk. UNAM causes much damage to the dairy industry, as casein becomes unstable as temperatures rise during thermal processing, which can promote milk coagulation and protein deposition on equipment. This can lead to a higher number of disruptions to cleaning, a problem that does not make processing impossible

but generates difficulties and increased costs (SILVA *et al.*, 2012). Also, this casein instability is associated with UHT milk gelatinization and it is one of the major problems affecting this product. UHT milk is one of the most consumed dairy products, given its practicality of preservation and use and also its long commercial life (CLAEYS *et al.*, 2013; MCAULEY *et al.*, 2016). According to Fischer *et al.* (2012), the use of UNAM milk in industry causes a reduction in dairy processing yields, as this type of milk may have lower levels of lactose, protein and in some situations fat. However, Zanela, Fischer and Ribeiro (2006) conducted an experiment in which milk from Jersey cows with a positive and negative alcohol content of 76 °GL that was subjected to the process of making the beaten yogurt, where no changes in fermentation time, pH and viscosity were observed of whipped yoghurt made with UNAM. Costabel (2009) used 72 and 80 °GL alcohol-unstable milk in the industrial processing of cheese and observed that the percentage of protein retention in the clot was higher in the samples with 72 °GL alcohol instability. Those unstable to 80 °GL alcohol had a higher percentage of fat retention and total solids. However, this did not result in changes in industrial yield and no significant differences were observed between cheese processing with 72 and 80 °GL alcohol test positive and negative samples. Today, the use of UNAM is still viewed with suspicion by the industry and no more appropriate action is defined when this type of milk is diagnosed, except for disposal on the farm.

How to Diagnose UNAM

When the alcohol test on the farm shows a positive result for a milk without acidity, the carrier can boil this milk. If it precipitates, it is really a case of sour milk, but if it remains stable at boiling, the acidity test (pH or Dornic) should be applied. If the pH result is between 6.6 and 6.8 or Dornic is between 14 and 18ºD, it is UNAM. Zanela (2009) exemplified these results in a UNAM diagnostic flowchart (Figure 4).

Figure 4 Flow chart of UNAM diagnosis.



Source: Zanela (2009).

Causes of UNAM

UNAM is reported as a multifactorial problem, and its causes are not yet fully understood. Cases usually occur due to a nutritional imbalance that the animal undergoes and due to the influence of seasonality, with the highest frequency of cases of UNAM during autumn (FISCHER, 2012). There is a large variation in incidence between times of the year, occurring mainly during the pasture in-betweens (at the end of the Summer pasture cycle, associated with the lack of Winter pastures). Other causes, such as diets with mineral imbalance, acid-base changes in animals, sudden changes in diet (BARROS, 2001), energy deficiency (PONCE; HERNÁNDEZ, 2001), malnutrition (ZANELA, 2004) and advanced stage of lactation may also promote instability. There are indications that high fiber silages and excess protein concentrates, factors that can alter the calcium-magnesium balance, may also lead to positive alcohol test reactions (GABBI *et al.*, 2016; WERNCKE *et al.*, 2016). Digestive and/or metabolic changes were related to decreased milk stability, possibly due to metabolic acidosis induced by ruminal acidosis (PONCE; HERNANDES, 2001) or the addition of anionic salts to the diet during lactation to induce metabolic acidosis (MARQUES *et al*., 2011), where the reduction of stability was related to the reduction of pH and the increase of ionic calcium.

Heat stress is also one of the causes of UNAM, as studies by Faria *et al.* (2017) Holstein cows were subjected to high temperatures and without access to shade for five days and were perceived as a significant reduction in milk stability on alcohol test, with values reached 70.83 °GL. The authors suggest a reduction in stability as a result of metabolic acidosis, as claimed by Marques *et al.* (2011), in compensatory response to respiratory alkalosis triggered by increased respiratory rates.

Dietary restriction with consequent malnutrition or nutritional imbalance stands out for reducing milk stability in the alcohol test. The restriction, caused by the reduction of 40 to 50 % in the amount of food, reduced milk production and increased the frequency of UNAM occurrence (ZANELA *et al*., 2006). Barbosa *et al.* (2012) found out that milk from cows subjected to dietary restriction showed more instability to the alcohol test. In these experiments, the pictures of UNAM were quickly established, about 2 days after the change in the animals' diet. Fruscalso *et al.* (2013) concluded that low availability of dry matter in pasture or restriction of grazing time are also considered forms of food restriction. The 50 % reduction in pasture supply (Tifton 85) decreased the minimum alcohol concentration required to destabilize the samples from 75.8 to 69 °GL. According to Stumpf *et al*. (2013), the relationship between dietary restriction and reduced stability could be related to increased permeability of tight junctions between mammary epithelial cells. Consequently, there is a greater influx of sodium and eventually paracellular chlorides, which would increase ionic strength or promote saline

imbalance and, consequently, reduce the net negative charge between casein micelles, increasing their chances of coagulation. In contrast, some experiments were performed in an attempt to correct the UNAM problem, such as the studies by Marques *et al.* (2010), who, using diets corrected for energy and protein signaled improvement in results with lower incidence of the problem. Oliveira (2015) adjusted the diet of animals fed on sorghum silage, elephant grass, and commercial concentrate, using the same nutrients, but to reach 100 % of the requirements according to the NRC (2001), and found that the adjustment increased milk stability. In general, a balanced diet while maintaining the nutritional requirements of animals improves milk stability when facing the alcohol testing, but the total recovery of stability may take from one to three weeks, as each animal organism responds differently.

Conclusions and Future Perspectives

According to what has been presented, it can be concluded that the biggest challenge faced by the dairy industry in Brazil today is to obtain quality milk to maintain its heat stability. This prevents the precipitation of casein micelles in the equipment during the industrial process, which would result in clogging and blockage, and allows this raw material to obtain excellent yield in dairy products.

Another relevant factor in the Brazilian dairy sector is its undeniable social and economic importance. However, unlike European and North American countries, Brazil still has microbiological quality problems in milk, needing to apply the alcohol test as a guarantee of safe milk uptake. Changes in milk stability in alcohol test are described in different regions of the world and in Brazil as a serious and multifactorial problem and for causes not yet well understood, but which cause great damage to all links in the milk production chain, since UNAM even without high acidity is rejected and undervalued by the dairy industry.

Alcohol test is still considered a good alternative because it is fast, practical and inexpensive, but what is expected from quality regulatory bodies such as MAPA is that for the coming years this test will be abolished as a method for classifying raw material quality in the dairy industry. For such an event to occur, milk produced in the country can no longer present microbiological problems or the test must be reviewed and replaced, to offer greater reliability in the results and avoid losses for both dairy farmers and industry.

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