Artigo

ASSESSMENT OF PHYSICOCHEMICAL AND MICROBIOLOGICAL QUALITY OF UHT WHOLE MILK AND PROTEOLYSIS DETERMINATION DURING STORAGE

Avaliação da qualidade físico-química e microbiológica de leite UHT integral e determinação da proteólise durante armazenamento

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ABSTRACT

The aim of this work was aimed to evaluate physicochemical and microbiological quality of UHT whole milk, market in Londrina-PR, Brazil, as well as estimate gelation and proteolysis during storage period of 150 days at room temperature. Physicochemical analyses included determination of fat content; titratable acidity; ethanol stability; total solids; solids-not-fat; pH, density and freezing point. For proteolysis estimative, total nitrogen, non-casein nitrogen and non-protein nitrogen were quantified. Gelation estimation was carried out as well as mesophilic aerobes enumeration. Results obtained in this work demonstrate increase of gelation and proteolysis during storage and samples in disagreement with physicochemical and microbiological standards. The problems found in UHT milk quality standardization associated with increasing proteolysis and gelation during storage could lead to product rejection by the consumer.

Keywords: UHT milk; ultra-high-temperature milk; gelation; storage.

RESUMO

O objetivo deste trabalho foi avaliar a qualidade físico-química e microbiológica de amostras de leite UHT integral, comercializados em Londrina, PR, Brasil, assim como a estimativa de geleificação e proteólise

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durante o período de armazenamento de 150 dias a temperatura ambiente. Análises físico-químicas incluíram determinação da porcentagem de gordura, acidez titulável, estabilidade ao álcool, sólidos totais, sólidos não gordurosos, pH, densidade e ponto de congelamento. Para estimativa da proteólise, nitrogênio total, nitrogênio não-caseico e nitrogênio não-protéico foram quantificados. Também foi realizada estimativa de geleificação contagem de micro-organismos aeróbios mesófilos. Os resultados obtidos neste trabalho demonstram aumento da geleificação e proteólise durante armazenamento e amostras em desacordo com padrões físico-químicos e microbiológicos. Os problemas encontrados na padronização do leite UHT associados ao aumento da proteólise e geleificação durante o armazenamento podem levar à rejeição do produto pelo consumidor.

Palavras-chave: leite longa vida; leite UAT; geleificação; estocagem.

1 INTRODUCTION

Due to its convenient storage at room temperature for periods that can be as long as 4 months, UHT consumption has increased over the past years in Brazil. According to data from Brazilian Association of Long Shelf Life Milk, its market share that was of 20,2% in 1994 reached 74,6% in 2009 (ABVL, 2011).

UHT treatment of milk is used with the objective of reducing significantly the microbiota in order to make it microbiologically stable for storage at room temperatures, when aseptically packed. However, some alterations can occur during storage of UHT milk, limiting its shelf life, mainly increase of viscosity, gelation and sedimentation. Among the factors involved in gelation are the type and severity of heat treatment, temperature of storage, fat percentage and proteolysis induced by both endogenous enzymes and heat-stable enzymes produced by psychotropic microorganisms (HILL, 1988; DATTA; DEETH, 2001).

Since the amount of psychotropic microorganisms in raw milk is determinant for milk quality due to production of these heat-stable lipolytic and proteolytic enzymes (SANTANA et al., 2001), long storage periods of refrigerated milk previous to heat treatment allow increase of heat-stable enzymes (DATTA; DEETH, 2001).

Brazilian legislation (BRASIL, 1997) does not establish expiration date for UHT milk, which is determined by each industry individually. However, within its shelf life, the product must comply with all physicochemical and microbiologic requirements determined by law. Formerly, most of the available brands in market established a shelf life of 180 days. Currently this period has been reduced to 120 days, what may indicate quality problems with UHT milk during storage period.

Several authors in Brazil report problems related with UHT milk quality regarding disagreement with physicochemical and microbiological standards (COELHO et al., 2001; MARTINS et al.; 2005; MARTINS et al., 2008; BERSOT et al., 2010; DOMARESKI et al., 2010).

Problems with UHT milk quality, the effect of raw milk over physicochemical characteristics of UHT milk and also proteolysis occurrence during storage were reported by researches in different countries such as France, Turkey, Spain, Australia, Sudan and Pakistan Pakistan (AULDIST et al.1996; GARCIA- RISCO et al., 1999; TOPÇU et al., 2006; TEKINSEN et al., 2007; GAUCHER et al., 2008; HASSAN et al., 2009).

Hassan et al.(2009) analyzing UHT milk in Pakistanconcluded from the whole study, that there were an increase in sedimentation value, fat separation, titratable acidity during storage, while decrease was found in pH and protein percent during storage of 12 weeks. Tekinsen et al. (2007) observed in one milk trend, from five analyzed in Turkey, thatthe difference in pH values before and after the incubation during the UHT process was higher than 0.5 and the number of the total aerobic mesophilic microorganism was 101-1000 CFU/mL in 6.67% samples. According the authors, Turkish Standard Institution indicates in UHT milk that, in sterilization controls, the difference in pH values and after the incubation (at 30ºC for 15 days) should be lower than 0.5 and Food Codex suggests that the number of total alive bacteria should be 10 or less in 0.1 mL after the same incubation.

Garcia-Risco et al. (1999) concluded that extensive casein degradation and severe changes in the electrophoretic pattern of the proteins present in the serum fractions were observed during storage of direct UHT milks at 25°C. The authors founded a high proteolytic activity against k-casein, that could be responsible for the hydrolysis of serum-liberated k-casein or could have enhanced the liberation of beta lactoglobulin and para-k-casein complexes through proteolysis of micellar k-casein.

Therefore this work aimed to evaluate physicochemical and microbiological quality of UHT whole milk, market in Londrina-PR, Brazil, as well as estimate gelation and proteolysis during storage period of 150 days at room temperature.

2 MATERIAL AND METHODS

Sampling

Three brands of UHT whole milk were purchased the local market of Londrina city of Paraná state, Brazil. All analyzed brands of milk from this study were produced by establishments with authorization from Brazilian's Federal Inspection Service for both

production and commercialization of its products, in agreement with regulations set by Ministry of Agriculture, Livestock and Supply (Ministério da Agricultura, Pecuária e Abastecimento - MAPA).

To each brand two different processing batches or production lots were evaluated, three cartons of milk were used for each lot one for physicochemical analyses, one for microbiological examinations, and the last for estimating gelation. Samples were analyzed at five different time periods, after 30, 90, 60, 120, and 150 days of production, toting up 30 samples (two lots of each of the three brands evaluated over five different periods). The chosen periods of time included the expiration date (120 days) plus 30 days after expiration (150 days).

Physicochemical assessment

Physicochemical analyses were performed in triplicate. Analyses included determination of fat content by the Gerber method; titratable acidity by the Dornic method; ethanol stability using 68% v/v, 72% v/v and 78% v/v alcohol solutions; total solids (TS) and solids-not-fat (SNF) by indirect Akermann disk method; pH determination by potentiometric method, density at 15 °C ($g.mL^{-1}$) and freezing point using an ITR® (MK 540) electronic digital cryoscope (BRASIL, 2006). Proteolysis estimation was conducted through determination of total nitrogen, non-casein nitrogen and non-protein nitrogen by Kjeldahl method (AOAC, 1995).

Microbiological assessment

For microbiological analyses, UHT milk cartons were previously incubated at 36 ± 1ºC for 7 days. Next, samples that did not presented visual alteration of package were submitted to mesophilic aerobe enumeration. Decimal dilutions were performed in saline peptone solution 0,1%. Dilutions were then plated in Plate Count Agar (PCA) and incubated at 32 ºC ± 1 ºC for 48 hours (APHA, 1992).

Gelation estimate

Gelation estimative was determined in accordance with Bizariet al. (2003). All packages (bricks) containing milk were opened and the contents carefully poured out. Next, non-trained panelists observed whether or not any sediment had settled on the bottom of the packaging. Each panel member was asked to quantify sedimentation by assigning a rating on a scale ranging from 0 to 5, with 0 indicating absence of sediments,1sediment formation only at the bottom of the packing, 2 approximately 25% of sediment from the bottom of the packing, 3 sediment committing about 50% of the content, 4 sediment committing about 70% of the content and 5 complete sedimentation of the content.Presence of sediment is related to proteases action, which can be originated from bacteria or may be naturally present in milk, as plasmin (HILL, 1988).

Physicochemical analyses were evaluated through variance analysis (ANOVA) and Tukey's test ($p<0.05$), with the objective to detect differences between averages values. To evaluate proteolysis were used linear regression and correlation analysis, using the Statistica software program (STATSOFT, 2000).

3 RESULTS AND DISCUSSION

During the 150 days of storage pH and titratable acidity of the three UHT whole milk brands were maintained within legal standards, respectively 6,6 to 6,8 and 0,14 to 0,18 g of lactic acid.$100mL^{-1}$(BRASIL, 1997), except for one sample that presented 0,19 g of lactic acid.$100mL^{-1}$at 150 days, however already after its expiration date. Significant reduction ($p<0,05$) in pH and consequent increase in acidity ($p<0,05$) were perceived during storage of 150 days. The average pH during storage were 6,67, while average acidity were 0,165 g of lactic acid.$100mL^{-1}$.

Reduction of pH during storage can be caused by formation of acids in Maillard reactions, by dephosphorylation of casein, degradation of lactose and also by proteolysis (AL-SAADI; DEETH, 2008; GAUCHER et al., 2008).

As for the remain physicochemical parameters evaluated, as fat content, density, freezing point (FP), total solids (TS) and solids not-fat (SNF) non significant alteration ($p >0,05$) was observed within storage period. However, irregularities were detected in all of these parameters analyzed, demonstrating disagreement with minimum requirements established by legislation (BRASIL, 1997).

Fat content below standard value of 3% (BRASIL, 1997) was detected in 25 (83,3%) of 30 evaluated samples. Average fat content was 2,42%, with minimum of 1,5% and maximum of 3,15%. Concerning solids percentage, average values were 8,39% and 10,90%, respectively to SNF and TS. Legislation establishes minimum of 8,2% to SNF (BRASIL, 1997) consequently, 20 (66,67%) samples were in disagreement with standards. Though a legal standard to TS is not determined, if minimum value of 11,5% is considered - fixed for raw milk (BRASIL, 2002) - 26 (86,67%) samples would be irregular.

Despite the fact that freezing point and density determination are ofmajor importance for quality evaluation, Brazilian legislation does not determine standards for these parameters in UHT milk. If pasteurized milk standards were applied (BRASIL, 2002), 4 (13,3%) out of 30 samples would be below minimum density (1,028 $g.mL^{-1}$) and above maximum FP (-0,530°H). Average density obtained was 1,029 $g.mL^{-1}$, with minimum of 1,020 $g.mL^{-1}$and maximum of 1,031 $g.mL^{-1}$.

Given the low fat content detected it would be expected that density and SNF reached higher values, what was not perceived in the obtained results. Along with that, FP results that presented 13,4% (4) above -0,530°H. These alterations may be associated with fraudulent water addition, which may be originated in the raw material or yet from failure during UHT heat treatment by direct steam injection, which may lead to increase of water percentage (MARTINS et al., 2008).

On the other hand 50% of samples presented FP below -550°H. The components responsible for lowering FP are lactose, some minerals, soluble proteins and dissolved gases (oxygen, nitrogen and carbon dioxide). Sodium citrate, a casein stabilizer, that has addition permitted in UHT milk up to 0,1% (BRASIL, 1997) may as well deepen the freezing point of milk.

Except for one sample with 150 days of storage, UHT whole milk samples were stable to different alcohol tested percentages (up to 78% v/v). It is kwon that stability of protein fraction of milk decreases with acidity raise, which was true for this sample that presented 19 g of lactic acid.100mL^{-1} at the same period.

In the three UHT milk brands analyzed during 150 days of storage, were observed a progressive increase of primary and secondary proteolysis which can be demonstraded by increased percentages of non-casein nitrogen (NCN) (r^2=91.68%)and non-protein nitrogen (NPN)(r^2=97.81%) (Fig. 1).This results are in accordance with findings reported by Gaucher et al. (2008) where the UHT process effect was observed through decreases in non-casein nitrogen content and in micellar hydration and by an increase in casein micelle size for UHT milks.

When where compared a correlation between NCN, NPN and gelation process, a strong positive correlation (r =0,83) between NCN and NPN where founded, and moderate positive correlation it was obtained between NNP x gelation (r =0,54) and NPN x gelation (r =0,68).

Regarding gelation, an increase was noticed for the three brands evaluated. Gelation initiated at 60 days of storage for 66,67% of samples. After 150 days 66,67% reached degree 2 of the scale (BIZARI et al., 2003), which corresponds to sediment or gelation of approximately 25% of package content. Longhi et al. (2012) using the same technique to evaluate UHT gelation, noticed after 90 days in the five brands, sediment formation only at the bottom of the pack.

In addition to heat treatment, gelation and the intensity of milk primary proteolysis can be caused by activity of endogenous milk enzymes, originated from somatic cells and heat-stable enzyme produced by psychotropic microorganisms (TOPÇU et al., 2006). Secondary proteolysis includes the degradation of proteins and large peptides to smaller peptides and amino acids by the activities of intracellular and extracellular enzymes from bacteria (KALIT et al., 2005). Raw milk produced in Brazil presents

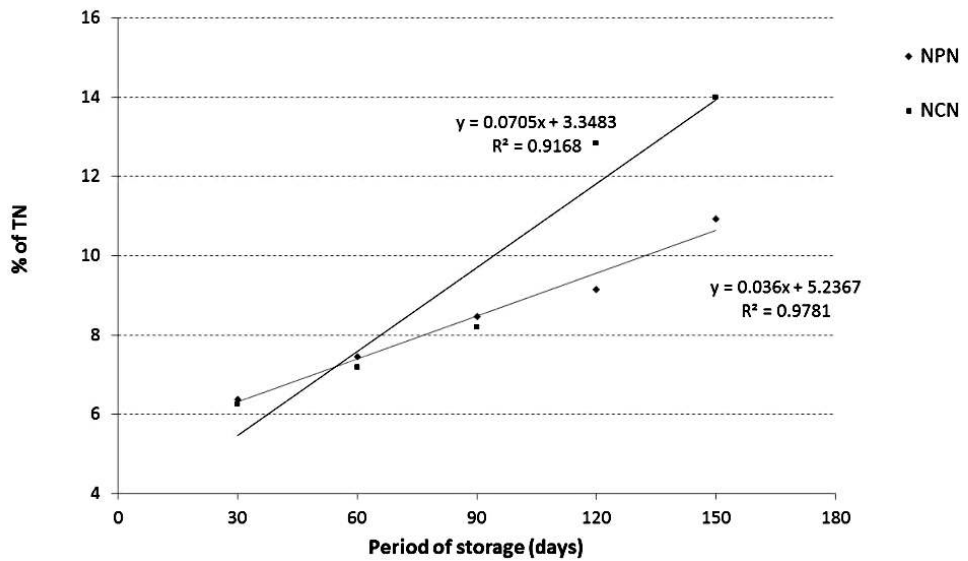

Figura 1 – Changes on non-casein nitrogen (NCN) and non-protein nitrogen (NPN) percentages obtained for three UHT whole milk brands commercialized in Brazil, during 150 days of storage at room temperature.

microbiological quality predominately unsatisfactory and high counts of psychotropic microorganisms in raw milk are reported in several regions of the country, reaching values that vary from 7 x 10^5up to 1,4 x 10^9CFU.mL^{-1} (SANTANA et al., 2001; FAGAN et al., 2005; MATTOS et al.,2010; SILVA et al., 2011).

Proteolysis of UHT milk during storage at room temperature is a major factor limiting the shelf life through changes in its flavor and texture (DATTA et al., 2002). The changes ultimately reduce the quality and limit the shelf life of UHT milk via development off flavors, fat separation and sedimentation, liberation of volatile fatty acids such as butyric acid and oxidation of free or unsaturated fatty acids (DATTA et al., 2002).

Two of the three evaluated brands did not conform to microbiological standards, presenting counts higher than 100 CFU.mL^{-1} of mesophilic aerobes, after 120 days of storage, thus within their shelf life. The third brand only presented counts above limits after expiration date, at 150 days. Average counts were of 9,69 x 10 CFU.mL^{-1}, with minimum of <1 and maximum of 8,25 x 10^2 CFU.mL^{-1}. Counts of mesophilic aerobes above limits in UHT milk was also reported by other authors in Brazil (COELHO et al., 2001; MARTINS et al., 200; BERSOT et al., 2010).

Mesophilic aerobes detected in UHT samples are possibly heat-resistant spore forming microorganisms, mainly from *Bacillus* genera that can survive UHT treatment (HUEMER et al., 1998). *Bacillus sporothermodurans* has been isolated worldwide, but mainly in European countries as an usual contaminant of milk by the UHT system. Even being nonpathogenic, the presence in UHT milk is undesirable because it can contribute for standards limiting mesophlic aerobic count, that is of 100 CFU/mL (BRASIL, 1997).Inadequate storage conditions and processing or post-treatment contamination are other possible explications for the detection of these microorganisms.

Results obtained in this study demonstrate that despite important, increase of gelation and proteolysis during storage are not the only quality problems in this product. Problems detected in the evaluated samples related to standardization can be originated from the treatment itself or from low quality raw material employed in production.

4 CONCLUSIONS

Were observed alterations resulting from storage period of 150 days were observed only to pH, titratable acidity, viscosity, proteolysis and gelation.

UHT whole milk samples evaluated in this study are in disagreement with established standards. Irregularities in fat content, total solids and solids not-fat were observed for all three UHT evaluated brands, in addition to low density and freezing point above established by legislation.

Only one of the brands met microbiological standard for mesophilic aerobes prior to expiration date.

The problems found in this study with UHT milk standardization associated with increasing proteolysis and gelation during storage should be thoroughly controlled by industries, since it could lead to consumer's rejection.

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