Efeito do Armazenamento na Qualidade de Leite UHT

Effect of Storage on Quality of UHT Milk

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Resumo

O tratamento térmico do leite garante a segurança microbiológica e aumenta a vida de prateleira. No entanto, provoca alterações nas propriedades nutricionais, sensoriais e/ou tecnológicas. O objetivo deste estudo foi avaliar a qualidade do leite UHT (três marcas, dois lotes de cada) armazenados por 150 dias, em relação aos parâmetros físico-químicos, contagem microbiológica, sedimentação, gelificação e (hidroximetilfurfural) HMF livre e total. As alterações foram observadas nos seguintes padrões de qualidade: densidade, índice crioscópico, extrato seco e gordura. Todas as amostras cumpriram as normas para a contagem de aeróbios mesófilos. Sedimentação foi observada em todas as marcas, aumentando depois de 150 dias, variando de baixo (1,00 g) a moderada (1,01-2,99 g). A gelificação foi observada em duas marcas na parte inferior da embalagem nos dias 120 e 150. O conteúdo de HMF livre e total aumentou em todas as marcas, durante os 150 dias de armazenamento, indicando perdas nutricionais no produto. O HMF livre e total variou 117,63-124,63 mmol / L e 124,20-131,64 mmol / L, respectivamente, após 30 dias de armazenamento. Após 150 dias, os teores máximos foram 157,02 mmol / L e 170,59 mmol / L para HMF livre e total. Os resultados indicaram problemas físico-químicos e nutricionais nas marcas avaliadas.

Palavras-chave: Gelificação. Índice de Hidroxymetilfurfural. Legislação. Sedimentação, Tratamento Térmico.

Abstract

Heat treatment of milk ensures microbial safety and increases its shelf life. However, it causes changes in the nutritional, sensory and/or technology properties of milk. The aim of this study was to evaluate the quality of UHT milk (three brands, two lots each) stored for 150 days, regarding the physicochemical parameters, microbiological count, sedimentation, gelation and free and total (hidroxymetilfurfural) HMF. Changes were observed in the following quality standards: density, cryoscopic index, dry extract and fat. All samples met the standards for aerobic mesophilic counts. Sedimentation was observed in all brands, increasing after 150 days, ranging from low (1.00 g) to moderate (1.01 to 2.99 g). Gelation was observed in two brands at the bottom of the packaging at 120 and 150 days. The free and total HMF contents increased in all brands during the 150 days of storage, indicating nutritional losses in the product. The free and total HMF ranged from 117.63 to 124.63 µmol/ L and from 124.20 to 131.64 µmol/ L, respectively, after 30 days of storage. After 150 days, the maximum contents were 157.02 µmol/ L and 170.59 µmol/ L for free and total HMF. The results indicated physicochemical and nutritional problems in the brands evaluated.

Keywords: *Gelation. Heat Treatment. Hydroxymethylfurfural Index. Legislation, Sedimentation.*

1 Introduction

According to Ordinance No. 370 of September 4, 1997 of the Ministério da Agricultura, Pecuária e Abastecimento (BRASIL, 1997) which defined the Technical Regulation of Identity and Quality of UHT milk, UHT (Ultra High Temperature) milk is the homogenized milk submitted for 2 to 4 seconds at temperatures between 130 °C and 150 °C by using a continuous-flow thermal process, followed by immediate cooling to a temperature below 32 °C and packed under aseptic conditions in sterile and hermetically sealed containers. Although the heat treatment of milk ensures microbial safety and increases its shelf life, it also causes changes to the nutritional, technological and sensory properties, including gelation and sedimentation (CLAEYS *et al.*, 2003). In addition, the heat-induced Maillard reaction may occur, having the hydroxymethylfurfural (HMF) as intermediate or end product, formed by the degradation of hexoses. For this reason, HMF is used to assess heat treatment of milk (GUERRA-HERNANDEZ *et al*., 2002).

Different problems associated to the quality of UHT milk are reported in Brazil, both concerning physicochemical and microbiological standards (BERSOT *et al.*, 2010; COELHO *et al*., 2001; LONGHI *et al.*, 2012; MARTINS *et al.*, 2005; MARTINS *et al.*, 2008), or technological problems related to gelation and sedimentation, which often happen due to the poor quality of the raw material (TOPÇU *et al.*, 2006; VESCONSI *et al*., 2012).

Psychrotrophics counted in raw milk is a determining factor of milk quality, since these microorganisms produce heat resistant proteolytic and lipolytic enzymes (SANTANA *et al.*, 2001). These enzymes can cause degradation of milk constituents or generate compounds able to modify its physicochemical properties (MARTINS *et al.*, 2008).

According to the Brazilian Association of Long Life Milk, the long-life milk represents 78% of liquid milk consumed in the country (MILK POINT, 2013).

Due to the high consumption of UHT milk in Brazil and the problems caused by heat treatment and poor microbiological quality of milk in natura, this study aimed to evaluate the quality of UHT milk during 150 days of storage by determining its physicochemical and microbiological properties, sedimentation, gelation and hydroxymethylfurfural index (HMF).

2 Material and Methods

Three different brands (M1, M2, M3 – two lots each) of UHT milk were evaluated for five different periods (30, 60, 90, 120 and 150 days after manufacturing), totalling 30 samples. The analyses were carried out from April to August 2011. The physicochemical and microbiological analyses were performed in triplicate and duplicate, respectively. A single analysis of gelation, sedimentation and HMF index (free and total) was performed for each lot in each period.

For physicochemical analyses, the UHT milk samples were characterized by fat content by Gerber method; titratable acidity by Dornic; non-fat milk solids calculated by the Fleishmann formula; density at 15°C (g m L^{-1}), and freezing point using an electronic digital cryoscope (ITR®, Esteio, Brasil). All determinations were carried out in triplicate and in accordance with the Association of Official Analytical Chemists methodology (ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1995).

In the microbiological analyses, samples were incubated at 35-37 °C for seven days prior to microbiological analyses, according to the procedures described by International Dairy Federation (HOUGHTBY *et al.*, 1992). After this period, the samples were plated onto Plate Count Agar (Himedia, Mumbai, India) and subsequently incubated at 35 °C for 48 hours. The results were expressed in colony-forming units per milliliter of milk (CFU m L^{-1}). Microbiological analyses were carried out in duplicate.

Gelation was determined as described by Bizari *et al.* (2003). All packages (bricks) containing the milk sample were opened and the contents carefully poured out. Next, non-trained panelists observed whether any sediment had settled on the bottom of the packaging. Each panelist was asked to quantify sedimentation by assigning a rating on a scale ranging from zero to 5, in which 0 indicated absence of sediments and 5 complete sedimentation of the content.

The sediment formation was determined as described by Ramsey and Swartzel (1984). After 24 hours of drying at ambient temperature, the UHT milk cartons were weighed (P1), and then washed to remove the excess sediment. A new weighing was performed after 24 hours (P2). The sediment content (in grams) was calculated by the difference between P1 and P2, and classified as low, when sedimentation was up to 1.00 g, moderate (1.01 g 2.99 g) and high (above 3.00 g) (VESCONSI *et al.*, 2012).

The quantification of Free and Total HMF was performed by spectrophotometer (Cintra 5, GBC Equipamentos Científicos, São Paulo, Brazil), with readings at 443 nm using thiobarbituric acid as substrate for Total HMF. Free and Total HMF contents were calculated using a calibration curve of 5-hydroxymethylfurfural P.A. (Merck, Darmstadt, Germany) (PEREIRA *et al.*, 2001).

The results were evaluated by analysis of variance (ANOVA) and Tukey's test ($p<0.05$) using the Software Program Statistica v. 8.0 (STATSOFT, 2000).

3 Results and Discussion

Brazilian law establishes as quality parameters of UHT whole milk minimum 3% fat, acidity between 14 and 18°D, and minimum nonfat milk solids of 8.2% (BRASIL, 1997). The legislation does not establish physicochemical standards for density and cryoscopic index of UHT milk, but these parameters are important for milk quality. Thus, the parameters established for pasteurized milk were used to discuss the results, which are density from 1028 to 1034 g m L^{-1}, and cryoscopic index from -0.530 °H to -0.550 °H (BRASIL, 2011). Optimal pH values should range from 6.6 to 6.8 at 20 °C (WALSTRA *et al., 1999*).

Brazilian law (BRASIL, 1997) does not establish shelf life for UHT milk, which is determined by industry. The only requirement related to validity is that the product should meet all physicochemical and microbiological requirements until the end of its shelf life. Previously, most brands had established a shelf life of 180 days. Currently, this period is established in 120 to 150 days, which suggests problems in milk quality during prolonged storage.

The results of the physicochemical analyses during 150 days of storage are shown in Table 1.

Table 1: Physicochemical parameters of 3 brands of UHT milk analyzed in five different periods (30, 60, 90, 120 and 150 days of storage) from April to August 2011

Brand	Physicochemical parameters						
	Storage (days)	**Density ($g\ mL^{-1}$)**	**Cryoscopic** índex (ºH)	**NFMS* (%)**	**Fat (%)**	**pH**	**Titratableacidity (ºD)**
M1	**30**	1028.12^{b}	-0,538 ^{b}	8.02 ^{ab}	3.35 ^{a}	6.53 ^{b}	15.5 ^{b}
	60	1028.40^{b}	-0,547 ^{a}	7.84^{b}	3.35 ^{a}	6.64 ^{b}	14.5^{c}
	90	1026.93^{c}	-0,545 ^{ab}	7.61 ^{c}	3.20 ^{b}	6.82 ^{a}	14.0^{c}
	120	1028.60 ^{b}	-0,547 ^{a}	8.04^{ab}	3.32 ^{ab}	6.82 ^{a}	14.3^{c}
	150	1029.40 ^{a}	-0,543 ^{a}	8.23^{a}	3.02^{c}	6.60 ^{b}	17.0 ^{a}
M2	**30**	1028.05^{cd}	-0,541 ^{a}	8.11 ^{a}	3.00 ^{a}	6.68^{c}	15.3 ^{b}
	60	1028.80 ^{ab}	-0,549 ^{a}	8.11 ^{a}	3.00 ^{a}	6.79 ^{b}	15.0 ^{b}
	90	1027.60 ^{d}	-0,548 ^{a}	7.66 ^{a}	3.02 ^{a}	6.78 ^{b}	15.0^{b}
	120	1028.40 ^{bc}	-0,549 ^{a}	7.80 ^{a}	2.83 ^{ab}	6.86 ^{a}	15.0 ^{b}
	150	1029.40 ^{a}	-0,550 ^{a}	7.68 ^{a}	2.63 ^{b}	6.63^{c}	18.0 ^{a}
M3	**30**	1028.25 ^{b}	-0.549 ^{a}	7.19 ^{a}	3.35 ^{a}	6.73^{cd}	14.8 ^{b}
	60	1027.83 ^{b}	-0.557 ^{a}	7.43 ^{a}	3.35 ^{a}	6.79 ^{bc}	14.3 ^{b}
	90	1026.60^{c}	-0.553 ^{a}	7.40^{a}	3.04 ^{ab}	6.86 ^{ab}	14.3 ^{b}
	120	1028.30 ^{ab}	-0.557 ^{a}	7.91^{a}	2.92 ^{ab}	6.91 ^{a}	15.0 ^{b}
	150	1029.40 ^{a}	-0.552 ^{a}	7.90^{a}	2.45 ^{b}	6.68 ^{d}	17.0 ^{a}

* Nonfat milk solids.

^{a,b,c}:For each brand and parameter, values with equal superscript letters in the same column do not differ statistically (p <.05).

Source: Research data.

Reference values: density from 1028 to 1034g ml^{-1}; cryoscopic index from -0.530 ºH to -0.550 ºH; 8.2% minimum NFMS, 3% minimum fat, titratable acidity from 14 to 18 ºD; pH from 6.6 to 6.8 at 20 °C.

Regarding to aerobic mesophilic counts, all brands had attended to the minimum quality requirements throughout the period of the analysis (150 days), with peak populations of 100 CFU m L^{-1}.

All samples showed significant increase in density on days 30 and 150 ($p<0.05$). Water addition and skimming process reduce the milk density (TRONCO, 2003). On day 90, the density of the samples M1, M2 and M3 was below the recommended value. However, in later periods (120 and 150 days) it recovered the normal values, which can be due to the gelation and sedimentation processes that may increase viscosity (LONGHI *et al*., 2012) and decrease fat content, which was also observed in the present study (M1 only after 150 days).

Among the components responsible for lowering the cryoscopic index (CI) are some minerals, certain soluble proteins, lactose and chloride, the last two being the main factors that cause this change. In contrast, the water addition converges for values close to zero (TRONCO, 2003). In this study, after 60 days of storage the brand M3 presented CI values below the minimum reference (-0.550 °H), ranging from -0.552 to -0.557 °H, with no significant difference ($p>0.05$) during storage.

The nonfat milk solids (NFMS) correspond to all milk components except fat and water (TRONCO, 2003). In this study, although NFMS varied for all brands, only M1 had statistically significant results in the storage period (from day 30 to 120) ($p<0.05$). M3 showed values below the minimum required by law (8.2%) over 150 days of storage. Increasing water content can cause dilution of the NFMS, thus reducing its concentration in milk (MARTINS *et al*., 2008). However, no water was added to the samples of the present study, since no increase was observed in the cryoscopic index. The equilibrium in the cryoscopic index can be due to the addition of sodium citrate, preservative added legally to UHT milk (BRASIL, 1997) that lowers its freezing point (TRONCO, 2003).

All brands showed a significant decrease ($p<0.05$) of fat content during 150 days of storage. After 120 days, the brands M2 and M3 showed fat contents below the minimum required by law for whole milk (3.0%). The lowest values were found for M2 at 150 days of storage, with a significant difference ($p<0.05$) when compared with the values obtained on days 30, 60 and 90. The sample M3 showed a significant difference ($p<0.05$) in fat content when comparing the results found at day 150 with day 30 and 60. Several factors can affect the milk fat content including the diet of the animal, rapid ruminal degradation, stress (FONSECA; SANTOS, 2000), or fraudulent skimming (LONGHI *et al.*, 2012).

The mean pH values of the samples ranged from 6.53

(M1 after 30 days of storage) to 6.91 (M2 after 120 days). All brands showed pH values within the standards required by law for all storage periods. Concerning the titratable acidity, all samples were within the limits established by law. However, significant increases ($p<0.05$) were observed for M1, M2 and M3 from day 30 to 150.

The results found in this study differ from literature regarding aerobic mesophilic counts, since the samples presented populations of aerobic mesophiles above the standard values (BERSOT *et al.*, 2010; COELHO *et al.*, 2001; LONGHI *et al.*, 2012; MARTINS *et al.*, 2005).

The sedimentation and the Free and Total HMF contents of each brand refered to an average of 2 different lots. In this study, the sediments at the bottom of the packages increased over the 150 days for all brands. The brand M2 showed the highest sedimentation from 90 to 120 days of storage and gelation grade 1. However, after 150 days, the highest sedimentation degree was observed for brand M1 (1.81 g; moderate), without occurring gelation. The brand M3 presented the lowest sedimentation degree, ranging from low (0.04 g, after 30 days of storage) to moderate (1.01 g, 150 days of storage). For the brand M3, the gelation occurred only at the bottom of the package (grade 1). Vesconsi *et al*. (2012) found an increase in sedimentation of whole milk over 120 days of storage, which reached 10 g for the samples stored at 31°C, a value much higher than that observed in the present study.

Some researchers believe that the phenomenon of gelation and sedimentation are initially induced by the action of heat-resistant enzymes (proteases) naturally existing in milk or derived from bacteria, especially psychrotrophics. These enzymes have the ability to degrade casein and promote aggregation of the micelles (FONSECA; SANTOS, 2000; SHAH, 1994). Thus, gelation and sediment formation are also related to the poor microbiological quality of raw milk used in the UHT process (VESCONSI *et al*., 2012).

The gelation mechanisms include changes in milk protein, association and dissociation of calcium ions, formation of polymers from the Maillard reaction, formation and dissociation of κ-casein complex and whey proteins, and participation of natural enzymes (plasmin) and bacterial proteases produced by psychrotrophics present in milk (HILL, 1988; LEWIS, 1986).

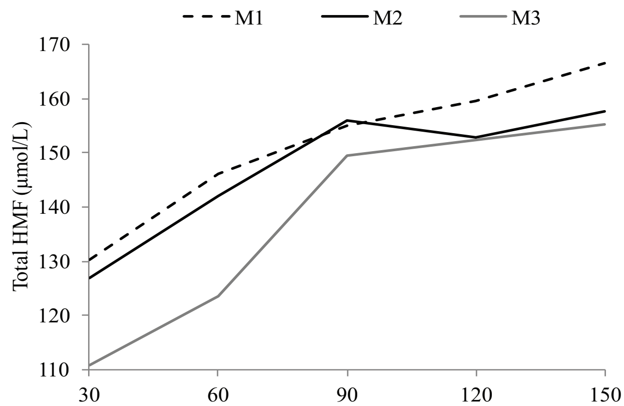
Sedimentation can be an effect of the progressive loss of protein stability in the milk subjected to heat treatment. When milk is heated, the stable colloidal particles are destabilized by changes affecting space and electrostatic interactions responsible for maintaining the integrity of the micelles, including lower pH, deposition of calcium phosphate onto micelles, dephosphorylation of the casein, and polymerization reactions (FOX; MCSWEENEY, 1998).

According to Vesconsi *et al*. (2012), sedimentation reduces the shelf life and may lead to rejection of UHT milk by the consumer, being regarded as one of the major problems identified by the industries that process UHT milk.

The Maillard reaction, together with caramelization, is a non-enzymatic browning involving several chemical reactions, and occurring in food and living organisms (glycation). The non-enzymatic browning reactions originate compounds responsible for the aroma, flavor and color of foods that are essential to their acceptance and consumption (BASTOS *et al.*, 2011). Appropriate heat treatment may ensure microbial safety, and avoid changes of nutritional value and formation of Maillard reaction products, including hydroxymethylfurfural (HMF) (CAIS-SOKOLIŃSKA; DANKÓW, 2004).

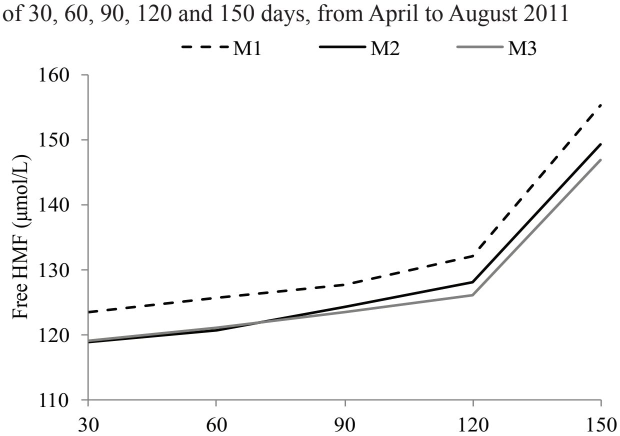
The Total and Free HMF contents during 150 days of storage of all brands are shown in Figures 1 and 2, respectively.

Figure 1: Total hydroxymethylfurfural (HMF) contents (μmol/ L) of three brands of UHT milk analyzed during the storage period of 30, 60, 90, 120 and 150 days, from April to August 2011



Source: Research data.

Figure 2: Free hydroxymethylfurfural (HMF) contents (μmol/ L) of three brands of UHT milk analyzed during the storage period of 30, 60, 90, 120 and 150 days, from April to August 2011



Source: Research data.

An increase in HMF contents was observed until the last evaluation period (150 days) for all samples of the present study. The brand M1 showed the highest Total HMF content within 30 days (130.32 μ mol / L) and 150 days (166.43 μ mol / L). The brand M3 showed the lowest Total HMF content (Figure 1). However, all brands presented values above the ideal, which should not exceed 10 μ mol / L (CAIS-SOKOLIŃSKA; DANKÓW, 2004).

In milk, the Total HMF is formed by lactose degradation by boiling in oxalic acid (VAN BOEKEL, 1998). The main changes in milk are the loss of nutritional value by impairment of lysine, formation of compounds affecting flavor, formation of antioxidant, antibacterial, mutagenic, antimutagenic and anticarcinogenic compounds; reducing the formation of antigens, and polymerization of proteins (BASTOS *et al.*, 2011; VAN BOEKEL, 1998).

Free HMF is formed by the decomposition of lactulose-lysine during the Maillard reaction (CLAEYS *et al.*, 2003). In this study, Free HMF contents also increased over the 150 days of analysis for all brands. The brand M1 presented higher contents since the beginning of the study, ranged from 123.54 μ mol / L (30 days of storage) to 155.27 μ mol / L (150 days of storage). The lowest values were observed for the brand M3 (Figure 2). According to Claeys *et al.* (2003), free HMF contents may be lower than the Total HMF of the samples. In addition, the authors point out that free HMF contents may not be a reliable measure for the Maillard reaction.

4 Conclusion

For all brands, we observed changes in density, cryoscopic index, nonfat milk solids and fat content as compared to the quality limits established by Brazilian law. Regarding the microbiological quality, the samples attended the standards for aerobic mesophilic counts. The sedimentation occurred in all samples, with an increase over the 150 days of storage. The gelation occurred in two of three brands evaluated. The Total and Free HMF contents were above the ideal limits, increasing during milk storage, indicating loss of nutritional value.

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