

Loss of UHT milk quality: changes in compositional and physicochemical parameters triggered by different storage conditions

Perda da qualidade de leite UAT: alterações nos parâmetros de composição e físico-químicos em diferentes condições de armazenamento

Pérdida de calidad de la leche UAT: cambios en los parámetros de composición y físicoquímicos en diferentes condiciones de almacenamiento

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Abstract

The objective of this study was to evaluate some compositional and physicochemical changes that occur during UHT (Ultra-high temperature) milk storage for up to four months. A total of 120 samples of UHT milk were collected from two dairy plants in the state of Minas Gerais, Brazil, and stored under two temperatures (20°C and 30°C) during five periods (0, 30, 60, 90 and 120 days). The experimental design was completely randomized, in a 2x2x5 factorial arrangement, with six replicates per treatment (six batches). Evaluated parameters included fat, total protein, casein, lactose, total solids, solids nonfat (SNF) and milk urea nitrogen (MUN) contents, as well as acidity, density, freezing point and indexes of lipolysis and caseinomacropptide (CMP). When compared to the Brazilian requirements for UHT milk, the mean values for acidity, fat and SNF contents were in accordance with the standards until the last day of storage. The other parameters are still not covered by the specific UHT milk legislation in Brazil. There was no change of relative density and lactose content also remained constant, except for a decrease at the fourth month. A significant increase, over storage time, in acidity, MUN, lipolysis and CMP indexes occurred. On the other hand, there was a significant decrease in SNF and protein contents. The results showed that there was a gradual loss of quality

during UHT milk storage, aggravated by the rise in temperature. These findings demonstrate that UHT milk legislation could be improved by adding parameters such as lipolysis and CMP indexes.

Keywords: Lipolysis index; Caseinomacropéptide index; Deterioration; Shelf life; Proximate composition; Quality control; Inspection.

Resumo

O objetivo desse estudo foi avaliar alterações físico-químicas e de composição que ocorreram durante o armazenamento de leite UAT (Ultra Alta Temperatura) por um período de até quatro meses. Um total de 120 amostras foram coletadas de duas indústrias de leite no estado de Minas Gerais e armazenadas sob duas temperaturas (20°C e 30°C) durante cinco períodos (0, 30, 60, 90 e 120 dias). O delineamento experimental foi inteiramente casualizado, em um arranjo fatorial de 2x2x5, com seis repetições por tratamento (seis lotes). Os parâmetros avaliados incluíram os teores de gordura, proteína total, caseína, lactose, sólidos totais (ST), sólidos desengordurados (SNG), nitrogênio ureico do leite (NUL), assim como acidez, densidade, crioscopia, índices de lipólise e de caseinomacropéptido (CMP). Quando comparados ao que é estabelecido pela legislação para leite UAT, os valores médios para acidez, gordura e SNG estavam de acordo com os padrões até o último dia de armazenamento. Os outros parâmetros avaliados não estão contemplados pela legislação. Não houve alteração da densidade relativa e o teor de lactose decresceu apenas no quarto mês. Foi verificado aumento significativo, no decorrer do tempo da estocagem, na acidez, no NUL e nos índices de lipólise e CMP. Por outro lado, houve um decréscimo significativo no teor de SNG e proteínas. Os resultados demonstram que houve perda gradual de qualidade do leite UAT durante o armazenamento, agravada pelo aumento da temperatura. Esses achados sugerem que a legislação de leite UAT pode ser atualizada pelo acréscimo de parâmetros como índices de lipólise e CMP.

Palavras-chave: Índice de lipólise; Índice de caseinomacropéptido; Deterioração; Vida de prateleira; Composição centesimal; Controle de qualidade; Inspeção.

Resumen

El objetivo de este estudio fue evaluar los cambios composicionales y fisicoquímicos durante el almacenamiento de leche UAT (Ultra Alta Temperatura) hasta por cuatro meses. 120 muestras de leche UAT fueron recolectadas de plantas lácteas (estado de Minas Gerais, Brasil), y incubadas a 20°C y 30°C durante cinco períodos (0, 30, 60, 90 y 120 días). El diseño experimental fue completamente aleatorio en factorial 2x2x5 y seis repeticiones (lotes). Los parámetros evaluados incluyeron contenido de grasa, proteína total, caseína, lactosa, sólidos totales, sólidos no grasos (SNG) y nitrógeno ureico en leche (NUL), así como acidez, densidad, punto de congelación y índices de lipólisis y caseinomacropéptido (CMP). En comparación con los requisitos brasileños para leche UAT, los valores medios de contenido de acidez, grasa y SNG estuvieron de acuerdo hasta el último día de almacenamiento. Los demás parámetros aún no estén contenidos en la legislación brasileña de leche UAT. Los índices de acidez, NUL, lipólisis y CMP tuvieron un aumento significativo ($p < 0,05$), durante el tiempo de almacenamiento. Por otro lado, hubo una disminución significativa en los contenidos de SNG y proteína. No hubo cambio de densidad, mientras la lactosa se mantuvo constante durante el almacenamiento, excepto por una disminución al cuarto mes. Los resultados mostraron que hubo una pérdida gradual de calidad durante el almacenamiento de la leche UAT, agravado por la mayor temperatura de almacenamiento. Además, nuestros resultados indican la posibilidad de mejora de la legislación sobre leche UAT con la inclusión de los índices de lipólisis y CMP.

Palabras clave: Índice de lipólisis; Índice de caseinomacropéptido; Deterioro; Vida útil; Composición proximal; Control de calidad; Inspección.

1. Introduction

Milk is one of the most complete foods, with high content of proteins, carbohydrates, and important fatty acids, vitamins and minerals. However, due to several constraining factors, such as logistics, seasonality, among others, many countries face an increasing demand for dairy products with extended shelf life, while maintaining their sensorial, nutritional and safety characteristics (Liu *et al.*, 2020). Ultra-high temperature (UHT) meets some of these aspects, as this heat treatment is applied to milk to a temperature between 130°C and 150°C, for the period of two to four seconds, through a continuous flow process, immediately cooled to a temperature below 32°C, and filled under aseptic conditions (Brasil, 2017). The result is a less perishable product that can be stored at room temperature for a long time, usually four to six months.

UHT is a commercial continuous process whose temperatures are enough to inactivate the vast majority of microorganisms. Nevertheless, a successful product still requires a good quality raw milk (Oliveira *et al.*, 2019). Psychrotrophic bacteria like *Pseudomonas* spp. are able to produce proteolytic or lipolytic enzymes, which degrade

components of raw milk, leading to failures in flavor and texture. Extracellular proteases and lipases may also affect shelf life of processed milk because some are able to withstand even UHT treatment (Alves *et al.*, 2016; Sørhaug & Stepaniak, 1997). Physicochemical and enzymatic changes during transportation and storage can cause the deterioration of UHT milk, which results in undesirable flavors, sedimentation and gelation, all of which can be detected by consumers. Proteolysis of milk casein by the enzymes originating from psychrotrophic bacteria are suspected as the major contributors to inferior UHT milk quality (Matéos *et al.*, 2015). So, after UHT processing, milk storage over a long period at room temperature might cause changes in milk characteristics, which affect its quality (Kilic-Akyilmaz *et al.*, 2022; Lieske, 2011; Reddy *et al.*, 1991; Volk *et al.*, 2021).

In this context, knowledge of the milk composition and quality standards is necessary, since the non-adherence to these parameters can lead to unsuitable products (Beloti *et al.*, 2015; Suvartan *et al.*, 2021). Although Brazilian regulation (Brasil, 1996) establishes the microbiological and physical-chemical parameters for the control of UHT milk, some important physicochemical analyzes are not usually required for its inspection and include freezing point, density, total solids, protein content, among others (Lieske, 2011). The lack of more parameters to investigate the quality of this milk may result in inspection failure and, sometimes, food safety risks, reduced shelf life and rejection by consumers. Therefore, it is necessary to update the parameters of the UHT milk to include important analyzes for a thoroughly investigation of its quality. Some of these, such as the indexes of lipolysis and caseinomacropetide (CMP) may be considered, since they quantify the degree of milk degradation (Oliveira *et al.*, 2018; Recio *et al.*, 2000; Recio *et al.*, 1996).

Thus, the aim of this work was to evaluate the quality of UHT milk in conditions similar to retailing, through analysis of physicochemical and compositional parameters and to discuss their usefulness to inspect the quality of this type of milk.

2. Methodology

2.1 Milk Samples

UHT milk samples (standardized with 3% fat) were collected directly from two dairy plants, located in Minas Gerais state, Brazil. The dairy industries were chosen based on the known historical of raw milk quality, respectively, good (brand A) and average quality (brand B). The samples of each batch were collected during a three months period, within up to 10 days after processing. The collection day of the samples was established as day zero. Six different batches of UHT milk from each plant were analyzed, and for each batch, ten aseptic packages of UHT milk, containing one liter each, were collected. The samples were transported to the Laboratory of Milk Quality Analysis¹ (School of Veterinary Medicine, Universidade Federal de Minas Gerais, Brazil).

In laboratory conditions, ten packages of UHT milk from each batch were incubated at two temperatures (20°C and 30°C), with five packages (sub-samples) for each temperature. They represented, therefore, each day of sampling for analysis: 0, 30, 60, 90, and 120 days of storage. The total number of samples were 120, considering the number of industries (2), storage temperatures (2) and periods (5), and number of replicates (6).

2.2 Physicochemical and compositional analysis

Physicochemical analyzes were done in triplicate, and included: titratable acidity (AOAC, 1990), density at 15°C using a Quevenne lactodensimeter (Brasil, 2019) and freezing point (FP - °C) using the thermistor cryoscope method (ISO/IDF, 2009).

¹ Accredited ISO 17025

For milk composition, 40 mL-aliquots of each milk sub-sample were added to sampling bottles containing bronopol (2-bromo-2-nitropropane-1,3-diol) as preservative, and analyzed for infrared determination of fat, total protein, casein, lactose, total solids, solids nonfat (SNF) and milk urea nitrogen (MUN) contents, using a Lactoscope/CombiScope™ FTIR electronic equipment (Delta Instruments®, Drachten, The Netherlands). The instrument was checked and adjusted using a series of raw milk samples (VALACTA, Canada) based on partial least square regression (PLS) models previously developed (ISO/IDF, 2013).

Milk samples were screened for chemical contaminants (acid neutralizers, formaldehyde, hydrogen peroxide), and other adulterants (starch, sucrose, chlorides) (Brasil, 2019), and for microbial inhibitors using Delvotest® SP NT analytical kit (DSM®, The Netherlands).

2.3 CMP index

CMP was analyzed by high performance liquid chromatography (HPLC) using a Class 6.1 Chromatograph (Shimadzu®, Japan) equipped with a Zorbax GF-250 gel separation column (Agilent®). The mobile phase was composed of a phosphate buffer solution, at pH 6.0 and UV detection at 205 nm. Standard solutions of CMP containing at least one point below 30mg/L and a point above 75mg/L in whole fat milk, were prepared: 0mg/L, 15mg/L, 30mg/L, 45mg/L, 60mg/L, 75mg/L and 90mg/L. A CMP index plot versus the peak area was constructed based on the chromatogram, and the linear regression line was calculated using values with $R \geq 0.95$. Milk aliquots of 10 mL were precipitated with 5 mL of 24% trichloroacetic acid (TCA), slowly added under constant stirring, allowed to stand for 60 minutes, and then filtered on qualitative filter paper. A 20µL portion of filtrate was injected into the chromatograph, with mobile phase flow of 1.5mL per minute (Brasil, 2019; Olieman & Riel, 1989).

2.4 Lipolysis index

The lipolysis index was determined by the titration of extracted free fatty acids (FFA) (Deeth *et al.*, 1975). In a test tube, 3 mL of milk were mixed with 10 mL of isopropanol, petroleum ether and sulfuric acid solution (40:10:1 v/v), 6 mL of petroleum ether P.A., 4 mL of distilled water and two drops of methylene blue solution for phase differentiation. The sample was then shaken and allowed to stand for 5 minutes. The clear supernatant was withdrawn with a pipette and its volume recorded and transferred to an Erlenmeyer flask. To the supernatant were added 2 drops of methanolic solution of phenolphthalein (1% w/v) and the free fatty acids were titrated with KOH methanol solution (0.02 N). The FFA content was given in µ equivalent mL⁻¹ of milk and calculated by the following equation:

$$FFA (\mu \text{ equivalent mL}^{-1}) = \frac{T * N}{P * V} * 1000$$

Where:

T = net titration volume;

N = normality of the KOH methanol solution;

P = proportion of the upper layer titrated (i.e., volume of aliquot withdrawn/total volume of upper layer);

V = volume of milk sample (mL).

2.5 Experimental design and statistical analysis

A completely randomized design was used in a 2x2x5 factorial scheme, with 20 treatments (industries x storage temperatures x storage periods), and six replicates per treatment (six batches), with a total of 120 samples. A linear regression

model with significance of 5% was used to monitor the effect of storage on milk physicochemical parameters, and lipolysis and CMP indexes, as well as to analyze the influence of different temperatures (20°C and 30°C) and brands (A and B) in each of the variables evaluated during the milk storage periods. Tukey Test was used for post-hoc comparison in the treatments with type I error controlled at the significance level of 5% (Dean *et al.*, 2017). Used statistical softwares included SPSS 22, IBM; Minitab 19.2020.1, Minitab; and JMP 16.0.0, SAS.

3. Results and Discussion

3.1 Physicochemical and compositional parameters of UHT milk

The average results of the physicochemical and compositional analyzes carried out along the storage are presented in Table 1. When compared to the Brazilian standards for UHT milk, average values for acidity, fat and SNF contents remained within limits for up to 120 days. However, 24 samples (20%) had a fat content lower than the minimum of 3% during storage. For the other parameters, there are no reference values established in the specific legislation of UHT milk. According to Brazilian legislation, raw milk must have a minimum protein content of 2.9g 100g⁻¹; relative density at 15°C between 1.028 and 1.034, freezing point between -0.512°C and -0.536°C, minimum lactose content of 4.3g 100g⁻¹, minimum SNF of 8.4g 100g⁻¹ and total solids of 11.4g 100g⁻¹ (Brasil, 2017; Brasil, 2018). Therefore, the UHT milk would still be complying with minimum standards for raw milk except for fat content due to fat standardization, and freezing point, because of lower freezing temperatures due to the addition of stabilizers.

Fat and total solids contents did not change significantly during the storage time, irrespective of brand and storage temperature. Industry origin (brands) was an influential factor on CMP index, with higher values in the brand with lower quality raw milk (Brand B) ($p < 0.05$).

Significant differences were found on storage temperatures influence (20°C; 30°C) over freezing point (-0.5294°C; -0.5265°C), lactose (4.56g 100g⁻¹; 4.52g 100g⁻¹), protein (3.34g 100g⁻¹; 3.32g 100g⁻¹), SNF (8.88g 100g⁻¹; 8.82g 100g⁻¹), respectively. For CMP index values were 99.20mg 1000mL⁻¹ and 131.4mg 1000mL⁻¹ respectively ($p < 0.05$).

Freezing point is an important tool to detect fraudulent addition of water to milk, but the addition of stabilizers, usually used to avoid protein gelation, decreases the milk freezing point. This will limit the use of the milk freezing point as fraud indicator for UHT milk (dos Santos *et al.*, 2018; Martins *et al.*, 2008). Stabilizer salts are usually added to milk before UHT processing to decrease viscosity and gel formation during storage, known as age gelation, which is a progressive increase in the viscosity of milk (Anema, 2019). The limit of stabilizers addition in UHT milk allowed in Brazil is 0.1% of stabilizing salts (sodium citrate and/or sodium phosphates) (Brasil, 1997), which may reduce the freezing point of milk by about 0.016°C. Usually, no significant changes in density were observed. Hence, prediction of the milk freezing point reduction after stabilizer addition is only possible if the initial freezing point of raw milk is previously known. So, this is a failure point for inspection, since the decrease of 0.016°C due to addition of stabilizers might disguise potential water addition of levels close to 3%. For the extreme values of allowed freezing point levels, fraudulent water addition to milk close to 4.5% would not be detectable (Beloti *et al.*, 2015).

Milk acidity gradually increased during the days of storage, maybe due to conversion of lactose into different organic acids (Fox & McSweeney, 1998) or mainly due to free fatty acids from lipolysis (Figure 1) (Costa, 2010; Deeth, 2006; Fernandes *et al.*, 2008; Hassan *et al.*, 2009; Laučienė *et al.*, 2019). However, the samples remained within the legal values (0.14 to 0.18g lactic acid 100mL⁻¹), except for 15 samples, which presented acidity above legal limit (3 samples, 2.5%, at 60 days; 3 samples, 2.5%, at 60 days, and 9 samples, 7.5%, at 120 days). Of these samples, 11 (9.2%) were stored at 30°C and

four (3.3%) at 20°C; 13 (10.8%) were from dairy plant A and 2 (1.7%) from B. Findings of up to 33.3% of UHT milk samples with high acidity has been reported elsewhere (Tamanini *et al.*, 2013).

Table 1. Physicochemical and compositional parameters of UHT milk collected in two dairy industries, stored at 20°C and 30°C during 0, 30, 60, 90, and 120 days (n=120).

Parameter	Days of storage				
	0	30	60	90	120
Fat (g 100g ⁻¹)	3.12 ^{ab}	3.09 ^{ab}	3.00 ^b	3.07 ^{ab}	3.13 ^{ab}
Total protein (g 100g ⁻¹)	3.36 ^a	3.33 ^b	3.32 ^b	3.31 ^b	3.31 ^b
Casein (g 100g ⁻¹)	2.58 ^{ab}	2.55 ^b	2.58 ^{ab}	2.59 ^a	2.57 ^{ab}
Lactose (g 100g ⁻¹)	4.55 ^a	4.56 ^a	4.56 ^a	4.56 ^a	4.49 ^b
Total solids (g 100g ⁻¹)	12.04 ^a	11.96 ^{ab}	11.84 ^c	11.92 ^{bc}	11.93 ^b
Solids nonfat (g 100g ⁻¹)	8.91 ^a	8.87 ^{ab}	8.84 ^b	8.84 ^b	8.80 ^c
Milk Urea Nitrogen (mg 100mL ⁻¹)	21.3 ^c	22.2 ^{bc}	24.7 ^{ab}	24.8 ^{ab}	25.3 ^a
FP (°C)	-0.541 ^{ab}	-0.540 ^b	-0.541 ^{ab}	-0.544 ^a	-0.541 ^{ab}
Acidity (g lactic acid 100mL ⁻¹)	0.154 ^c	0.157 ^c	0.171 ^b	0.172 ^{ab}	0.178 ^a
Density at 15°C (g mL ⁻¹)	1.032 ^a	1.032 ^a	1.032 ^a	1.032 ^a	1.032 ^a
CMP index (mg L ⁻¹)	18.6 ^d	63.89 ^c	125.6 ^b	156.5 ^{ab}	186.3 ^a
Lipolysis (milliequivalent L ⁻¹)	0.20 ^c	0.23 ^{bc}	0.24 ^b	0.25 ^b	0.34 ^a

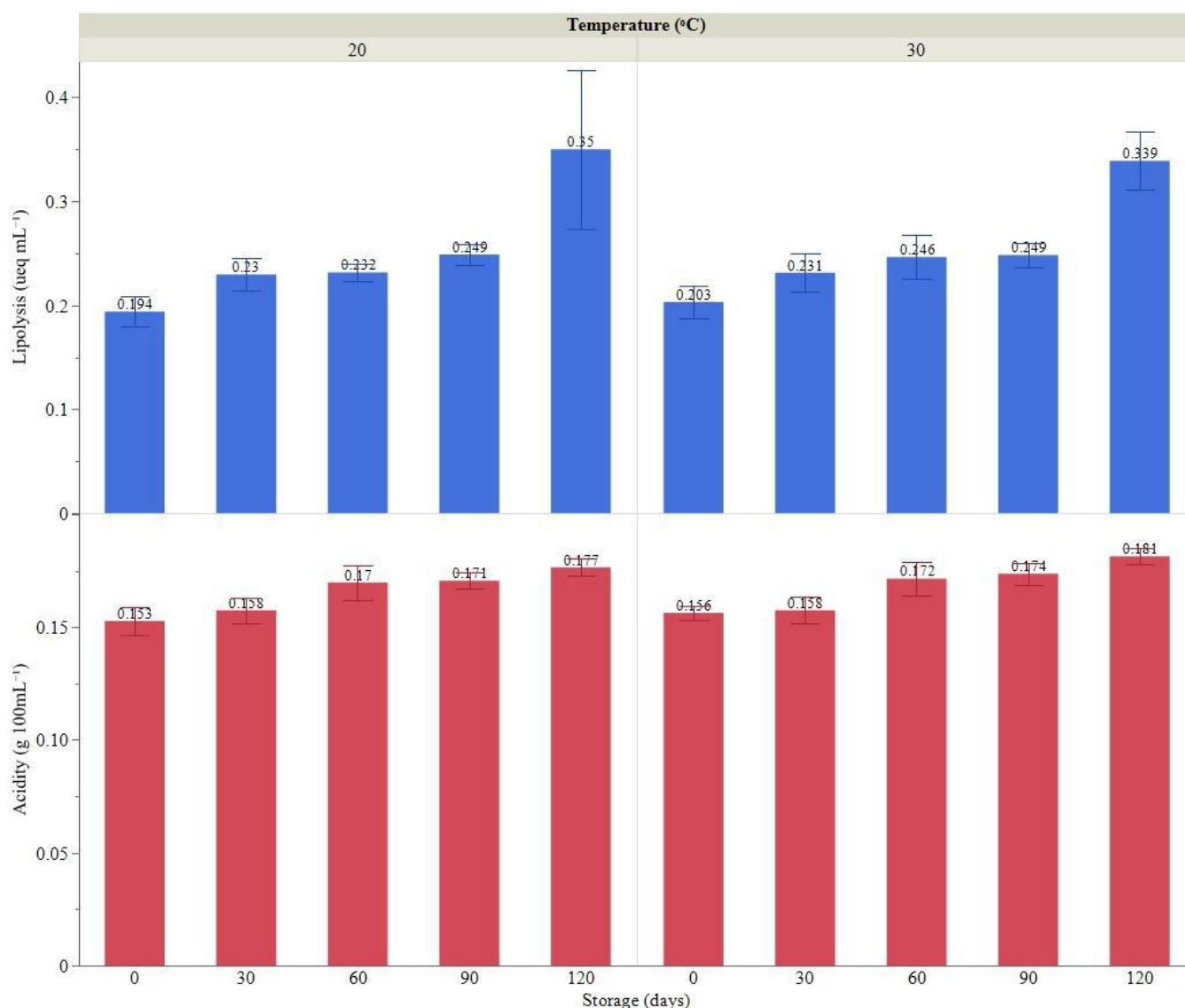
Source: Authors.

Lactose remained practically constant throughout the storage period and had a significant decrease ($p < 0.05$) only at 120 days of storage. Storage temperatures and brands were interferent factors. The decrease in lactose is possibly linked to the Maillard reaction, which occurs mainly during heating of milk and prolonged storage. The Maillard reaction is slow at lower temperatures and practically doubles at each 10°C increase. The reaction extension might be monitored with the emergence of compounds such as furosin, hydroxymethylfurfural and carboxymethylisine (Anema, 2019; Celestino *et al.*, 1997; Suvartan *et al.*, 2021).

Protein concentration decreased and MUN and CMP index increased, but casein concentration measured using FTIR did not change ($p < 0.05$) (Figure 2), maybe due to the wavelengths used in the PLS calibration for this component. Casein hydrolysis has been reported at levels of 0.0042g 100g⁻¹ of UHT whole milk day⁻¹ (decreasing from 2.37% to 1.86% during 120 days), with a half-life estimated as approximately 276 days (dos Santos *et al.*, 2018). Even though levels of casein measured using FTIR were constant, protein concentration decreased, while MUN and CMP increased significantly ($p < 0.01$). Increases in MUN concentration might be the result of non-protein nitrogen formed due to milk proteolysis (Milaneze *et al.*, 2018; Santos *et al.*, 2018) and this finding is corroborated with the increased CMP index, which is used as an indicator of illegal cheese whey addition to milk, and may be an indicator of milk proteolysis (Ferron-Baумы *et al.*, 1992; Milaneze *et al.*, 2018; Recio *et al.*, 2000; Villanoeva *et al.*, 2014).

No illegal substances or microbial inhibitors were found in the samples.

Figure 1. Average acidity ($\text{g } 100\text{mL}^{-1}$) and lipolysis index (milliequivalent L^{-1}) of UHT milk samples stored over 120 days at 20°C and 30°C ($n=120$).

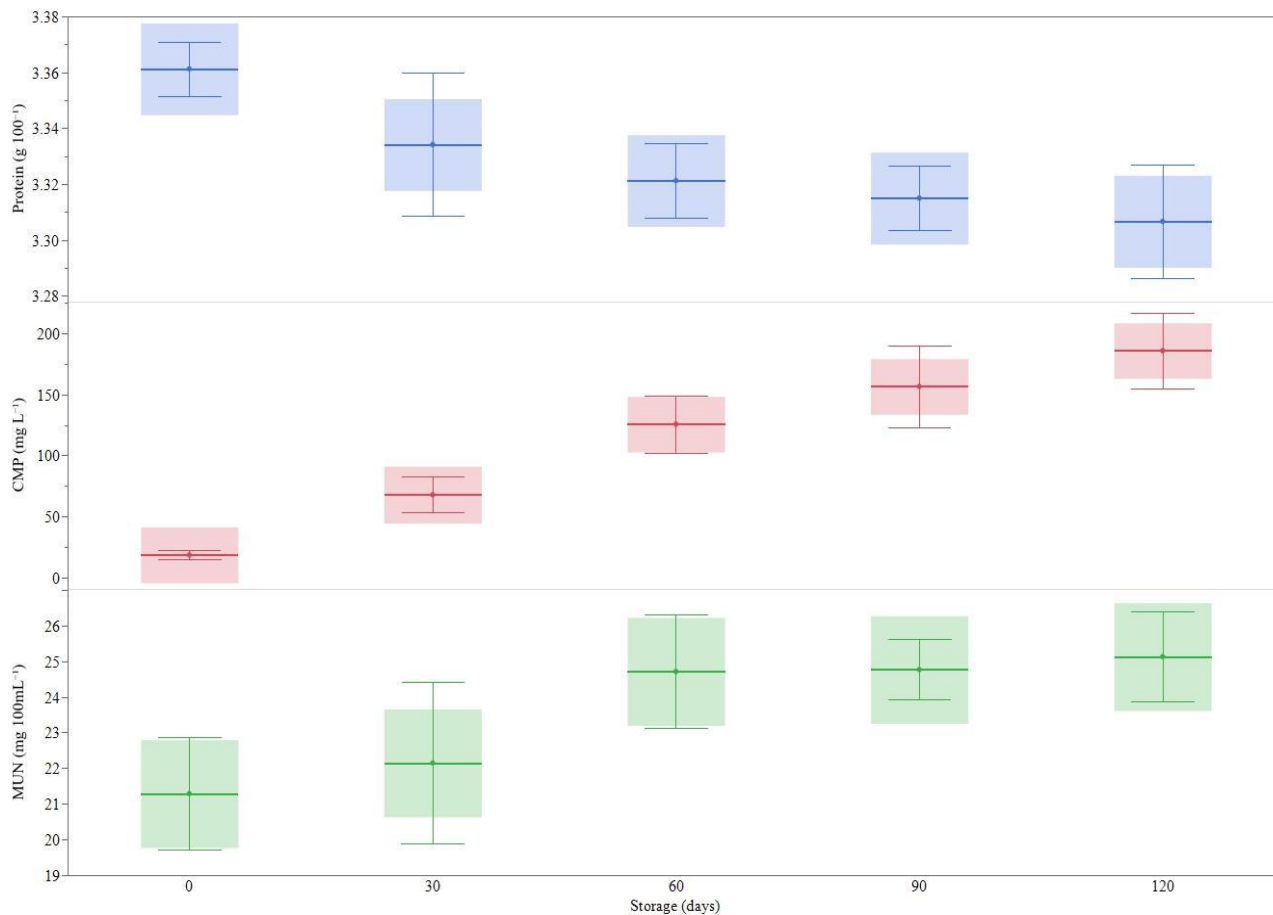


*Each bar is constructed using a 95% confidence interval of the mean. Source: Authors.

3.2 CMP Index

All the samples on the first day of analysis (first day of storage) were compliant to CMP index requirement in Brazil, which must not exceed $30\text{mg } \text{L}^{-1}$ (Brasil, 2006). Hence, increasing CMP index throughout the storage (Figure 2) might be directly linked to proteolysis. At 30 days of storage, CMP index was greater than $30\text{mg } \text{L}^{-1}$ in 87.5% of the samples, and above 75mg in 37.5% of the samples (Table 2). In poor quality raw milk, microbial contaminants, particularly the psychrotrophic microorganisms, produce proteases that cleavage the κ -casein close to the hydrolysis site of chymosin (rennet), producing the pseudo CMP, which is detected together with the CMP using the HPLC method (Olieman & Riel, 1989; Recio *et al.*, 1996; Villanueva *et al.*, 2014). Although this index was devised to detect cheese whey addition to freshly processed milk samples, it proved to be an optional parameter of milk quality, since it is able to detect extensive proteolysis in milk, which can be an indicator of raw milk quality.

Figure 2. Protein and milk urea nitrogen (MUN) concentration, and caseinomacropeptide (CMP) index of Ultra High Temperature (UHT) milk stored at 20°C and 30°C for 120 days (polynomial regression fit with confidence intervals).



*Each bar is constructed using a 95% confidence interval of the mean. Source: Authors.

Considering a 120-days shelf life for UHT milk, the quality of this product must be maintained throughout this period. To minimize quality changes, it is necessary to control the population of psychrotrophic microorganisms in raw milk or the storage temperature, since high temperatures favor the enzymatic activity.

The linear regression model for CMP index during the storage period was:

$$\text{CMP index (mg L}^{-1}\text{)} = 26.3 + 1.41 * \text{days of storage}$$

As a whole, the R square (0.536) indicates that this regression is a good estimator for CMP index during the evaluated period.

Table 2. Number of samples according to caseinomacropепptide index (CMP) range during the storage period (0, 30, 60, 90, and 120 days) of UHT milks.

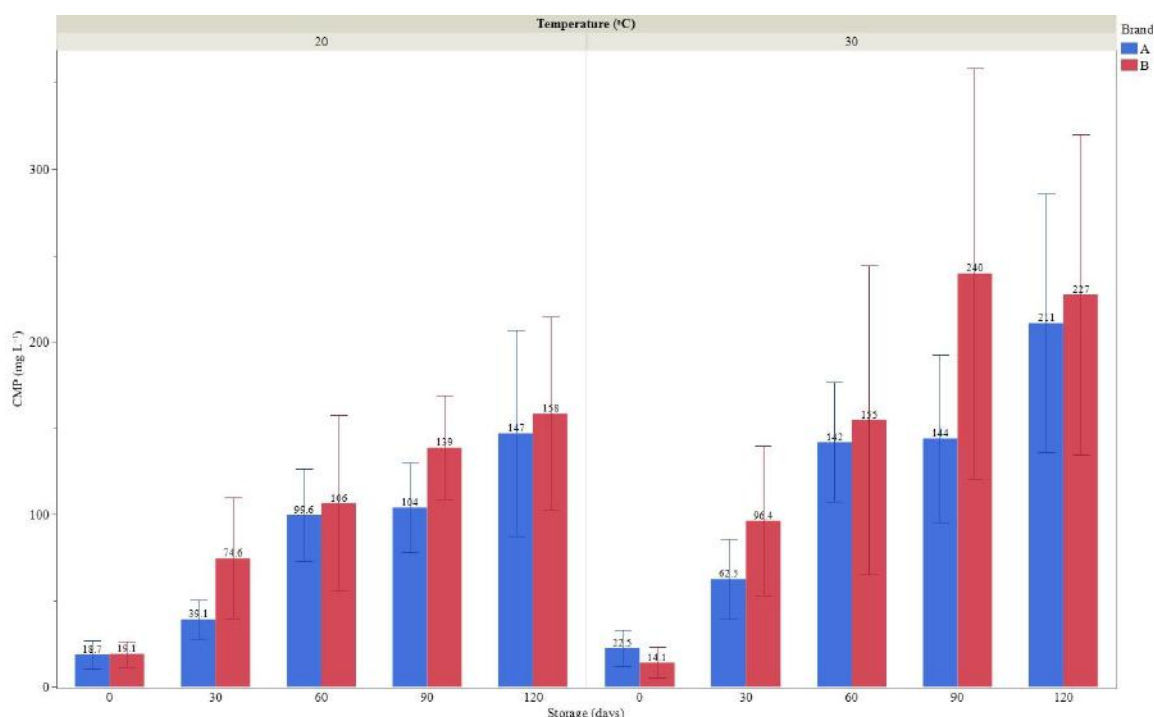
CMP range (mg L ⁻¹)	N. of samples (%)				
	0 day	30 days	60 days	90 days	120 days
≤30	24 (100%)	3 (12,5%)	0 (0%)	0 (0%)	0 (0%)
30-75	0 (0%)	12 (50%)	3 (12.5%)	1 (4.2%)	0 (0%)
>75	0 (0%)	9 (37.5%)	21 (87.5%)	23 (95.8%)	24 (100%)

Source: Authors.

In a study with UHT milk stored for 49 days, was found that after the fourth day, the concentration of CMP was approximately 45.0 mg L⁻¹, with a progressive increase over the days. The authors concluded that even with the UHT process, proteolysis still occurs in milk throughout the shelf-life due to the action of heat-stable proteinases that remain active after thermal processing, even though the bacteria producing the enzymes are destroyed (Friedrich *et al.*, 2010). According to Fox (1992), these enzymes thermal denaturation would only be possible at treatments as high as 142°C for 18 seconds or 120°C for 15 minutes, which would negatively affect the sensorial and physicochemical characteristics of UHT milk.

It was also observed a significant association ($p < 0.05$) of the increase in CMP index with the different temperatures and brands, as shown in Figure 3, with higher CMP values under storage of 30°C ($p < 0.05$). Villanoeva *et al.* (2014) found similar results with increasing CMP index under increasing storage temperatures (-12°C, 6°C, and 21°C) after 60 days of storage.

Figure 3. Average CMP index (mg L⁻¹) of UHT milk samples stored over 120 days at 20°C and 30°C, brands A and B (n=120).



*Each bar is constructed using a 95% confidence interval of the mean. Source: Authors.

3.3 Lipolysis index

A significant increased lipolysis was observed from day 0 (0.20 milliequivalent L⁻¹) to 120nd day (0.34 milliequivalent L⁻¹) (p<0.001) (Figure 1).

The different temperatures and industries (brands A and B) did not affect the milk lipolysis indexes (p>0.05). Milk lipolysis usually occurs due to the action of thermoresistant lipolytic enzymes produced by psychrotrophic bacteria. These reactions continue to occur during the storage period and over time FFAs accumulate and increase in concentration, which makes milk acidity higher (Figure 1) (Deeth, 2006; Laučienė *et al.*, 2019; Souza *et al.*, 2021). Lipolysis index values in whole milk of more than 1.5 mmol L⁻¹ are unacceptable to most people, with the flavor being described as rancid, butyric, astringent or even bitter. Although in this study the values found showed growth over time, they did not reach such high values.

4. Conclusion

The storage of UHT milk during 120 days under laboratory conditions similar to retailing resulted in significant changes in its physicochemical and compositional parameters, consequently leading to gradual loss of quality.

Decreasing protein content was associated with gradual increases in MUN and in CMP index as result of proteolysis. Furthermore, higher acidity and lipolysis index values were observed during the storage period. This reinforces the importance of the quality of the raw milk used for UHT milk manufacture, as well as its expeditious processing, avoiding storage for long periods. These findings suggest that the use of additional parameters to evaluate UHT milk, such as CMP and lipolysis indexes might improve the inspection of this product, since they are proteolysis and lipolysis indicators, respectively.

In this sense, other works must be conducted to further investigate the physicochemical and compositional parameters of UHT milk over the storage period in order to support an update in the legislation referring to this product.

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