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Quality of UHT whole milk marketed in Pernambuco, Brazil

[*Qualidade do leite UHT integral comercializado em Pernambuco, Brasil*]

"Scientific Article/Artigo Científico"

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Abstract

The objective of this study was to evaluate the physical-chemical properties and microbiological quality of Ultra-high temperature (UHT) whole milk marketed in Pernambuco state, Brazil. In total, 390 samples of UHT whole milk were purchased from commercial establishments located in the mesoregions of Pernambuco, and transported to the Laboratory of Meat and Milk Inspection at Federal Rural University of Pernambuco. The physical-chemical analyzes and the microbiological evaluations were carried out following specific legislation. All 13 brands tested met the determined physical-chemical standards. However, it was found that 105 (26.9%) samples were in disagreement with microbiological standardization. It is important to highlight the need of further development of integrated actions involving the inspection bodies and the processing plants, in order to adjust the product to the quality standards established in the legislation, respecting the rights and health of consumers.

Keywords: ultra-high temperature; physicochemical properties; aerobic mesophyll.

Resumo

Objetivou-se nesse estudo avaliar as propriedades físico-químicas e a qualidade microbiológica de leite UHT integral comercializado em Pernambuco, Brasil. Foram adquiridas 390 amostras de leite UHT integral, provenientes de estabelecimentos comerciais localizados nas mesorregiões de Pernambuco, e transportadas ao Laboratório de Inspeção de Carne e Leite – Universidade Federal Rural de Pernambuco. As análises físico-químicas e a avaliação microbiológica foram realizadas seguindo a legislação específica. Todas as 13 marcas atenderam aos padrões físico-químicos determinados. Contudo, foi constatado que 105 (26,9%) amostras estavam em desacordo com a padronização microbiológica. É de fundamental importância a realização de ações integradas envolvendo os órgãos de fiscalização e as usinas de beneficiamento, a fim de adequarem o produto aos padrões de qualidade estabelecidos na legislação, respeitando-se os direitos e a saúde dos consumidores.

Palavras-chave: ultra-alta temperatura; propriedades físico-químicas; mesófilos aeróbios.

Introduction

Milk is a highly appreciated food product among Brazilians due to its nutritional and energetic value. Ultra-high temperature milk (UHT) is the main dairy product consumed in Brazil as a result of its long shelf-life and feasibility (Rosa et al., 2015).

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UHT milk can be defined as homogenized milk submitted for 2 to 4 seconds to a temperature between 130ºC and 150ºC, in a continuous flow system with immediate refrigeration at a temperature below 32ºC and aseptic packing in sterile and hermetically sealed packages (BRASIL, 2017).

The sensory properties of the product are liquid appearance, white color, odor and specific flavor. The physical-chemical parameters are fat (whole milk: minimum of 3%; semi-skimmed or partially skimmed: between 0.5 and 2.9%; and skimmed: maximum value of 0.5%), titratable acidity "percentage of lactic acid" (0.14 to 0.18); alcohol stability at 68% and defatted dry extract (whole milk: minimum of 8.2%; semi-skimmed or partially skimmed: minimum of 8.3%; and skimmed: minimum of 8.4%). UHT milk should not contain bacteria capable of proliferating under normal conditions of storage and distribution. After milk incubation in a sealed package at 35-37ºC, for 7 days, only the presence of aerobic mesophill/mL is tolerable, on an overall level, but with a legally accepted criterion (BRASIL, 1997).

The failures in the hygienic conditions of the raw material, the environment and in the processing steps, in addition to deviations in the time-temperature binomial employed in the UHT heat treatment, can be revealed by monitoring the product and observing the non-compliance with the specific legislation, through laboratory analysis (Prata, 1998; Pereira et al., 2013; Rosa et al., 2015).

In this context, the objective was to evaluate the physical-chemical properties and microbiological quality of whole UHT milk marketed in Pernambuco, Brazil.

Material and Methods

Sampling

The experiments were conducted at the Laboratory of Meat and Milk Inspection (LICAL) of the Department of Veterinary Medicine (DMV) - Federal Rural University of Pernambuco (UFRPE). The selection of municipalities in Pernambuco (PE) state for UHT milk collection considered cities with a population above 50 thousand inhabitants, in the mesoregions of Pernambuco (Pernambuco, 2020).

UHT whole milk (3% milk fat) samples, with undamaged packaging, were obtained in commercial establishments located in seven municipalities in PE state: Arcoverde, Gravatá, Vitória de Santo Antão, Paudalho, Carpina, Recife, and Olinda. During the acquisition, three batches were selected per brand (1, 2 and 3) and 10 samples per batch (I, II, III, IV and V without incubation; I, II, III, IV and V with incubation). All the samples were transported in cardboard boxes, protected from the sun and heat, to the LICAL.

At LICAL, the aseptic containers were cleaned with disposable paper towels, identified, protected in plastic bags and sealed. For the analysis, the samples without incubation were refrigerated and kept at 15ºC and those with incubation were heated in an oven at 35-37ºC, for seven days.

Physical-chemical evaluation

The assessment of physical and chemical characteristics of the UHT milk samples was carried out at LDIC in accordance with Adolfo Lutz Institute (2008).

Samples without incubation at 35-37°C for seven days in an oven

There was performed the assessment of titratable acidity, density, fat, defatted dry extract and alcohol stability at 68% of all the samples without incubation at 35-37ºC, according to specific legislation guidelines (BRASIL, 1997).

Determination of titratable acidity

A 10 mL volume of the sample was transferred to a 100 mL beaker. 5 drops of 1% phenolphthalein solution were added. It was titrated with 0.1 M sodium hydroxide solution, using a 10 mL burette, until a pink color appeared.

% lactic acid = v x f x 0.9 / A;

V = number of mL of the 0.1 M sodium hydroxide solution spent on the titration;

f = correction factor for the 0.1 M sodium hydroxide solution;

A = number of mL of the sample; 0.9 = conversion factor for lactic acid

Determination of density at 15ºC, thermolactodensimeter

The density determination was accomplished by using the thermolactodensimeter (TLD). Approximately 500 mL volume of the sample was transferred to a beaker and the TLD was immersed into the beaker. After resting for 1 to 2 minutes, the density was read at 15ºC. The

TLD was lifted and its rod was dried with absorbent paper, from top to bottom.

The TLD was dipped again until close to the previously observed trace. Both density and temperature of the milk were read as soon as TDL stabilized.

Determination of fat content, Gerber's method

A 10 ml volume of sulfuric acid (d = 1.82 g/ml) was carefully added in the butyrometer, subsequently a 11 ml volume of homogenized milk sample was added by letting it to slowly flow down the glass walls and a 1 ml volume of amyl alcohol was also added to the butyrometer. After being closed with an appropriate stopper, the butyrometer was agitated by inverting it, so the three liquids were completed mixed. Then, butyrometer went through 5 min of centrifugation (1000 to 1200 rpm) and, after that, it was transferred to a water bath at 65ºC for 5 min.

The reading on the scale was directly correlated to the percentage of fat.

Obtaining the total dry extract (EST), Ackermann's disk

The demarcation of the internal disk regarding to fat content was taken to meet the value concerning to the corrected milk density (shown in the intermediate circle).

The reading of the value was accomplished where the arrow in the external circle was.

Obtaining defatted dry extract (DDE)

The deffated dry extract (DDE) is determined, by subtracting the content of fat from the total dry extract (TDE): %DDE = TDE - %fat.

Alcohol stability at 68% (v/v)

A 2 ml volume of milk and a 2 ml volume of ethyl alcohol at 68% (v/v) were added to a test tube.

It was stable to alcohol when it did not from clots in the mixture.

Samples incubated at 35-37° C for seven days in an oven

There was performed the assessment of titratable acidity and alcohol stability at 68% of all the samples with incubation at 35-37ºC, according to specific legislation guidelines (BRASIL, 1997).

Microbiological evaluation

The preparation of UHT milk samples, without and with incubation at 35-37ºC for seven days in an oven, and the microbiological tests were performed according to Brazil (2018).

Samples without and with incubation at 35-37 ° C for seven days in an oven

The count of aerobic mesophyll microorganisms from the samples without and with incubation, for seven days at 35-37ºC, was performed using decimal dilutions with 0.1% peptone water (10^{-1} to 10^{-5}), in a continuous flow chamber. The technique used was the pour-plate on standard counting agar (SCA), in duplicate, followed by incubation at 30ºC for 72 hours, in an oven, and the results were expressed in UFC/mL.

The evaluation of microbiological quality followed the guidelines of the specific legislation (BRASIL, 1997).

Morphotintorial evaluation, Gram staining method

Three colony-forming units of aerobic mesophyll, isolated in each sample, were selected and submitted to the Gram staining method in order to assess their morphotintorial characteristics, according to Oliveira (2012).

Statistical analysis

Statistical analysis was descriptive and measures of dispersion were used, when necessary, according to Sampaio (1998) and Altman (1991).

Results and Discussion

A total of 390 samples were obtained and tested. All of them were stable to alcohol at 68% and with physical-chemical values within the parameters established by the legislation (BRASIL, 1997).

The arithmetic averages of fat, defatted dry extract, density and acidity can be seen in Table 1.

The findings from this study were similar to those reported by Ramos-e-Silva and Panetta (2002), Robim et al. (2012) and Silva et al. (2019). Robim et al. analyzed 58 samples of UHT whole milk marketed in Rio de Janeiro, and did not find non-conformities in the physical-chemical properties. Ramos-e-Silva and Panetta (2002) evaluated physical-chemical parameters of quality of long-life whole milk and identified all samples within the limits of the legislation. Silva et al. (2019) investigated the quality of UHT milk produced in Pernambuco and they did not find

samples with physical-chemical values in disagreement with the recommended guidelines.

Table 1. Average values of the physical-chemical characterization (fat, defatted dry extract, density and acidity) of UHT whole milk marketed in Pernambuco state, considering the brands analyzed.

La	Brand													P 370
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	
F	3.4	3.2	3.1	3.0	3.1	3.1	3.1	3.0	3.0	3.0	3.0	3.0	3.0	Mi.3.0
DDE	8.44	8.4	8.38	8.36	8.38	8.38	8.38	8.36	8.36	8.36	8.36	8.36	8.36	Mi.8.2
D	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	$M^1$1.03
A (WI)	0.16	0.16	0.16	0.15	0.17	0.16	0.18	0.16	0.17	0.15	0.16	0.16	0.16	$M^2$0.16
A (I)	0.17	0.17	0.17	0.16	0.17	0.17	0.18	0.17	0.17	0.15	0.17	0.17	0.17	$M^3$0.16
N. A	30	30	30	30	30	30	30	30	30	30	30	30	30	

La = laboratory analysis; P.370 = physical-chemical standard established in Ordinance No. 370 of 09/04/97 (Ministry of Agriculture, Livestock and Supply); Mi = minimum; F = arithmetic mean of the fat percentage; DDE = arithmetic mean of the percentage of defatted dry extract; D = arithmetic mean density at 15ºC in g/mL, A (WI) = arithmetic mean of acidity in grams of lactic acid / 100 ml of milk without incubation at 35/37ºC in an oven; A (I) = arithmetic mean of acidity in grams of lactic acid/100 ml of milk with incubation at 35 / 37ºC in an oven; M^1 = arithmetic mean density at 15ºC in g/mL (1.028 – 1.034g/mL); M^2 = arithmetic mean of acidity in grams of lactic acid / 100 ml of milk without incubation at 35/37ºC in an oven (0.14 to 0.16); M^3 = arithmetic mean of acidity in grams of lactic acid/100 ml of milk with incubation at 35 / 37ºC in an oven.

In contrast, Bersot et al. (2010) investigated the quality of 150 UHT milk samples produced in Paraná and they described 4.3%; 7.4%; 29% and 50.7% of the samples showed acidity, density, fat and ESD, respectively, in disagreement with the standards established in regulation, with the exception of alcohol stability at 68%.

By looking at Table 1, it can be appreciated that 13 brands were found and analyzed in the test, and they showed physical-chemical values in accordance with the legislation. Similar results were obtained by Camara and Weschenfelder (2014), who evaluated the physical-chemical characteristics for UHT whole milk from five different brands, and the five were within the recommended guidelines.

However, in a research with four different brands of UHT whole milk marketed in Foz do Iguaçu (RS), Luiz et al. (2010) reported 25% of them unstable to alcohol at 68% and 75% of the samples showed DDE percentages below the recommended. Similarly, Martins et al. (2008) described six brands of UHT milk harvested during the production process which 100% of them had a percentage of DDE below the recommended.

As for the microbiological analyzes, it was found that 105 (26.9%) milk samples have aerobic mesophyll values above 100 CFU/mL (Table 2).

The excessive identification of microorganisms in this research is in contrast with the findings of Cioglia and Freitas (2017) who, when assessing the microbiological quality of whole UHT milk marketed in Ouro Preto (MG), did not find contamination of the samples.

However, similar results were obtained by Pereira et al. (2013) after the analysis of 30 samples of UHT whole milk marketed in Londrina (PR), where 40% presented values in disagreement with the legislation. Coelho et al. (2001) analyzed 80 samples of UHT whole milk in Belo Horizonte (MG) and found 41.2% of contamination by microorganisms. Souza et al. (2014), when evaluating samples of whole UHT milk processed in Minas Gerais, identified contamination in 35% of the samples.

When analyzing Table 2, it can be seen that 10 (76.9%) brands had excessive contamination by aerobic mesophyll. Different results were found by Bersot et al. (2010), where all brands evaluated had inappropriate levels of contamination. However, this study had similar results to Pereira et al. (2013) who confirmed 73.3% of the analyzed brands of UHT milk with counts above that allowed by legislation.

According to Brasil (2001), UHT milk should not have pathogenic microorganisms that cause physical, chemical and organoleptic changes in the product, under normal storage conditions. The Ordinance No. 370 of 1997 (Ministry of Agriculture, Livestock and Food Supply) ratify this information, when it establishes that UHT milk must be free of microorganisms capable of proliferating under normal conditions of storage and distribution, as well as not presenting any sample from a batch of five, with aerobic mesophilic values greater than

100 CFU/mL, after incubation at 35-37ºC (BRASIL, 1997). Conversely, considering the data showed in Table 2, 21 (63.6%) of the lots presented contamination by aerobic mesophyll above the limit, characterizing them as inappropriate for human consumption (BRASIL, 2001).

Table 2. Quantification of samples of UHT whole milk marketed in Pernambuco state, with values above 100 CFU/mL, considering the lots and the brands analyzed.

	WI						I							
Brand	**L1**	**M**	**L2**	**M**	**L3**	**M**	**L1**	**M**	**L2**	**M**	**L3**	**M**	**SA**	**LU**
A	1/5	28.7	1/5	94.7	0/5	0.0	0/5	0.0	1/5	0.0	2/5	1242.9	16.7	3
B	1/5	25.5	2/5	137.3	1/5	20.4	0/5	0.0	3/5	12.43	2/5	17.13	30.0	3
C	3/5	17.3	1/5	10.4	1/5	21.8	0/5	0.0	2/5	15.98	1/5	11.2	26.7	3
D	0/5	0.0	0/5	0.0	1/5	19.8	2/5	425.3	0/5	0.0	0/5	0.0	10.0	2
E	0/5	0.0	0/5	0.0	0/5	0.0	0/5	0.0	0/5	0.0	1/5	10.4	3.3	1
F	5/5	16.7	5/5	20.1	5/5	17.8	5/5	38.1	5/5	13.7	5/5	11.1	100.0	3
G	1/5	13.2	0/5	0.0	0/5	0.0	1/5	17.9	0/5	0.0	0/5	0.0	6.7	1
H	0/5	0.0	0/5	0.0	0/5	0.0	0/5	0.0	0/5	0.0	0/5	0.0	0.0	0
I	2/5	19.0	0/5	0.0	0/5	0.0	5/5	22.1	0/5	0.0	0/5	0.0	23.3	1
J	5/5	29.0	0/5	0.0	0/5	0.0	5/5	10.7	0/5	0.0	0/5	0.0	33.3	1
K	0/5	0.0	0/5	0.0	0/5	0.0	0/5	0.0	0/5	0.0	0/5	0.0	0.0	0
L	5/5	40.1	5/5	27.2	5/5	28.4	5/5	47.9	5/5	30.2	5/5	170.1	100.0	3
M	0/5	0.0	0/5	0.0	0/5	0.0	0/5	0.0	0/5	0.0	0/5	0.0	0.0	0
Total				**50/195**						**55/195**			**26.9**	**21/33**

WI = without incubation; I = with incubation; L1 = lot 1; L2 = lot 2; L3 = lot 3; M = arithmetic mean x 10^1 CFU/mL; SA = samples with values above 100 CFU/mL; LU = lots unfit for human consumption.

It is also important to note in our results a predominance of Gram-positive cocci (37.3%), Gram-positive bacilli (25.6%), Gram-negative bacilli (22.3%) and Gram-negative coconuts (14.8%), according to the Gram staining method. These data corroborate with prior publications (Coelho et al., 2001; Pereira et al., 2013; Silva et al., 2019), where most of the bacteria identified were Gram-positive. In addition, these authors consider Gram-positive and Gram-negative bacteria commonly present in the dairy properties and UHT milk plants. In the same sense, Lee (1984) and Bersot et al. (2010) stated that these microorganisms, as they cannot withstand the high temperatures used in the UHT process, when identified, they suggest environmental contamination. According to Pereira et al. (2013), the presence of these bacteria in UHT milk analysis indicates failures in heat treatment or after, such as insufficient time and temperature, as well as contamination by filling, handling and poorly sanitized surfaces (ICMSF, 1994).

Conclusion

In this study, the identification of samples of UHT whole milk marketed in Pernambuco, with a microbial load above recommendations by technical regulation, implies that failures in heat processing and/or in self-control programs, from obtaining the raw material to the shipping of products, may be related to contamination. Based on this scenario, it is important to highlight the need of further development of integrated actions involving the inspection bodies and the processing plants, in order to adjust the product to the quality standards established in the legislation, respecting the rights and health of consumers.

Conflict of Interest

The authors declare that there is not conflict of interest.

Ethics Committee

This research does not apply, because.

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