FERNANDA RIBEIRO LEMOS

PERFIL MINERAL E DE MACRONUTRIENTES EM PRODUTOS LÁCTEOS

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Engenharia Química para obtenção do título de *Magister Scientiae*.

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Hiasmyne Silva de Medeiros

Angélica Ribeiro da Costa
(Coorientadora)

Jane Sélia dos Reis Coimbra
(Orientadora)

Aos meus pais

DEDICO

“ Eis o meu segredo. É muito simples: só se vê bem com o coração. O essencial é invisível para os olhos. ”

Antoine de Saint-Exupéry

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À todos aqueles que, de alguma forma, contribuíram para a realização dessa pesquisa,

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RESUMO GERAL

LEMOS, Fernanda Ribeiro, M.Sc., Universidade Federal de Viçosa, fevereiro de 2019. **Perfil mineral e de macronutrientes em produtos lácteos.** Orientadora: Jane Sélia dos Reis Coimbra. Coorientadoras: Angélica Ribeiro da Costa e Rita de Cássia Superbi de Sousa.

Os componentes lácteos, como gordura, proteína, minerais, carboidratos e ácidos orgânicos auxiliam as atividades metabólicas dos indivíduos, além de serem relevantes para as características sensoriais de produtos à base de leite. Dentre esses constituintes, minerais como sódio, cálcio, potássio e fósforo podem trazer benefícios para o desenvolvimento e crescimento das espécies, mas se consumidos em excesso podem contribuir para o surgimento de doenças, como a Doença Renal Crônica (DRC). Dentre as fases do processamento do leite de qualquer espécie, o tratamento térmico é uma das principais etapas, pois visa à eliminação de microrganismos patogênicos. Neste trabalho, os leites bovino, bubalino, caprino e humano foram submetidos à pasteurização *Long-Temperature Long-Time* (LTLT), 63 ºC / 30 min, e juntamente com alguns produtos lácteos como soro doce, soro ácido, bebida isotônica, bebida láctea, doce de leite, requeijão, muçarela, creme intermediário da produção de manteiga e manteiga, foram estudados com os seguintes objetivos: *(i)* conhecer a composição química do leite de diferentes espécies e dos derivados lácteos através de análises de gordura, proteína, cinzas e carboidrato, e *(ii)* avaliar o efeito do tratamento térmico sobre a estrutura, superfície e os constituintes do leite utilizando a microscopia confocal de varredura à laser, microscopia eletrônica de verredura e a cromatografia de íons, respectivamente. Esta foi utilizada para quantificar minerais, ácidos orgânicos e carboidratos por se tratar de um ensaio analítico que apresenta vantagens sobre as demais técnicas clássicas, como preparo de amostra simplificado e rápido. Os resultados mostraram que o tratamento LTLT para os leites bovino, bubalino, caprino e humano não influenciou significativamente ($p > 0,05$) a quantidade de proteínas, gordura, cinzas e minerais em relação ao leite *in natura* de cada espécie, porém o carboidrato lactose se alterou ($p < 0,05$) em todos os leites LTLT. Comparando os leites in natura e os leites pasteurizados LTLT, foi observado alguns grânulos no leite LTLT sem homogeneização. Esse processo para o leite bovino pasteurizado mostrou que há uma maior uniformidade nos glóbulos de gordura para este leite, o que reduz a coalescência neste produto e aumenta a aceitabilidade por parte do

consumidor. Dentre os lácteos, foi notável que a muçarela apresentou alto teor em sódio, sendo 4,73 vezes mais do que o valor de referência para este produto. Ainda para a muçarela, o nitrato, que é utilizado como conservante, apresentou concentração superior a cinco vezes o limite máximo permitido pela legislação.

GENERAL ABSTRACT

LEMOS, Fernanda Ribeiro, M.Sc., Universidade Federal de Viçosa, February, 2019. **Mineral and macronutrients profile in dairy products**. Advisor: Jane Sélia dos Reis Coimbra. Co-advisers: Angélica Ribeiro da Costa and Rita de Cássia Superbi de Sousa.

Dairy components such as fat, protein, minerals, carbohydrates and organic acids helps the metabolic activities of the individuals, as well as being relevant to the sensorial characteristics of milk-based products. Among these constituents, minerals such as sodium, calcium, potassium and phosphorus can have benefits for the development and growth of the species, but if consumed in excess, they can contribute to the emergence of diseases, such as Chronic Renal Disease (CRD). Among the stages of the processing of milk of any kind, the heat treatment is one of the main steps, since it aims at the elimination of pathogenic microorganisms. In this research, bovine, buffalo, goat and human milk were submitted to the Long-Temperature Long-Time (LTLT) pasteurization at 63 ºC / 30 min, and along with some dairy products such as sweet whey, acid whey, isotonic beverage, intermediate cream of butter production and butter were studied with the following objectives: (i) to know the chemical composition of milk of different species and dairy products through fat, protein, ash and carbohydrate analyzes, and (ii) to evaluate the effect of the heat treatment on the structure, surface and the constituents of the milk using confocal laser scanning microscopy, scanning electron microscopy and ion chromatography, respectively. This technique was used to quantify minerals, organic acids and carbohydrates because it is an analytical test that presents advantages over the other classic techniques, such as preparation of simplified and fast sample. The results showed that the LTLT treatment for bovine, buffalo, goat and human milk did not significantly influence ($p > 0.05$) the amount of proteins, fat, ashes and minerals in relation to the in natura milk of each species, but the carbohydrate lactose was altered ($p < 0.05$) in all LTLT milk. Comparing the in-milk and LTLT pasteurized milk, some granules were observed in LTLT milk without homogenization. This process for pasteurized bovine milk showed that there is a greater uniformity in the fat globules for this milk, which reduces the coalescence in this product and increases the acceptability by the consumer. Among the dairy products, it was notable that the dish was high in sodium, being 4.73 times more than the reference value for this product. Still for the

muçarela, the nitrate, which is used as preservative, presented concentration superior to five times the maximum limit allowed by the legislation.

INTRODUÇÃO GERAL

Os minerais são substâncias encontradas como íons inorgânicos, sais ou constituintes de moléculas orgânicas, como proteínas, gorduras, carboidratos e ácidos nucleicos. Podem se apresentar como íons metálicos ou sob a forma de um sal (íons metálicos associados a um ou mais grupos de elementos químicos, como por exemplo carbonato, fosfato, sulfato, cloreto, dentre outros). Já os minerais orgânicos, também chamados de quelatos, são formados pela ligação de um íon metálico com um ligante orgânico, como aminoácidos ou carboidratos, comumente por ligações covalentes (LESSON & SUMMERS, 1997).

Nos produtos lácteos, observa-se a existência dessas três formas de minerais que influenciam a nutrição, preparação, processamento e estocagem do leite e seus derivados. A fração mineral corresponde de 0,8 % a 0,9 %, sendo composta majoritariamente pelos cátions cálcio, magnésio, sódio e potássio, e pelos ânions fosfato inorgânico, citrato e cloro. Estes íons estão em maior ou menor grau associados entre si ou a proteínas. Dependendo do tipo de íon, pode estar na fase aquosa ou parcialmente associado às micelas de caseína (GAUCHERON *et al*., 2004).

Os alimentos naturais contêm maior quantidade em minerais, tanto os de origem animal quanto os de origem vegetal. Leite, ovo, carne vermelha, peixe, nozes, folhas verdes, legumes, sementes e frutas propiciam importantes quantidades de minerais que contribuem para o bom funcionamento e desenvolvimento do organismo. Os minerais essenciais têm participação na formação de ossos e dentes (a exemplo de cálcio e fósforo), mantêm o equilíbrio eletrolítico de fluidos corporais e auxiliam vitaminas e proteínas nos mais diversos processos metabólicos (DEMORADAN, 2010).

O corpo humano necessita de aproximadamente vinte tipos de minerais essenciais presentes em vários tipos de alimentos, de origem animal e vegetal. Estes elementos podem ser classificados em macrominerais (cálcio, fósforo, potássio, cloro, sódio, magnésio e enxofre) e são requeridos em quantidades acima de 100 mg dia^{-1}. Além desses, há também a classe dos microminerais (zinco, silício, boro, iodo, ferro, cobre, manganês, níquel, selênio, flúor, cromo, cobalto e molibdênio, alumínio, arsênio, estanho, lítio) que são requeridos em quantidades

abaixo de 100 mg dia^{-1}. Todos os minerais essenciais encontram-se presentes no leite em diferentes concentrações (MAHAN E ESCOTT-STUMP, 2005).

No leite, a existência de íons divalentes como Mg^{2+} e Ca^{2+} se dá principalmente por complexos, abrangendo grandes quantidades de citrato de cálcio e citrato de magnésio e, em menor quantidade, de $Ca(H_2PO_4)_2$. O leite contém apenas 2 mols L^{-1} de Ca^{2+} livre. Da mesma forma, citratos estão presentes como íons complexos, ao passo que a parte do fosfato ocorre como $H_2PO_4^-$ e HPO_4^{2-}. Íons univalentes como Na^+, K^+ e Cl^- estão presentes, quase totalmente, como íons livres (DEMORADAN, 2010).

Em se tratando da aplicação de minerais pela indústria alimentícia, o uso excessivo de sódio apresenta sérios riscos à saúde do consumidor. Ingerido em quantidades corretas, esse mineral atua na transmissão de impulso nervoso e na contração muscular; mas se consumido além do necessário, pode desregular o equilíbrio osmótico, acarretando no aumento de água na corrente sanguínea. Pode causar o inchaço assim como a elevação da pressão arterial, que pode sobrecarregar o coração (TERMERO, 2013; PRETO, 2017).

Durante as últimas décadas, tem sido avaliado o comportamento desse mineral em pacientes com doença renal crônica (DRC). Esse tipo de doença acomete o rim, que é o principal órgão responsável pela estabilidade e equilíbrio de eletrólitos no corpo. As principais causas da DRC incluem a hipertensão arterial, diabete *mellitus* e as glomerulonefrites, lesões que acometem os vasos sanguíneos, responsáveis pela filtração do sangue no rim. Essa enfermidade está associada a perda das funções regulatórias, excretórias e endócrinas do rim e sua evolução clínica está associada a altas taxas de mortalidade (KDOQI, 2002).

Além dos minerais citados, a presença de nitrato pode também ser notada em lácteos. O nitrato de sódio ou de potássio pode ser utilizado como conservante durante a produção de queijos para impedir o que é chamado de estufamento tardio, comumente provocado pela bactéria *Clostridium tyrobutiricum*. A legislação brasileira estabelece o teor máximo permitido de nitrato em queijos de acordo com média umidade (até 35,9 %) e alta umidade (36 a 45,9 %) de 50 mg kg^{-1} (MAPA, 1996; GONÇALVES *et al.*, 2011).

Como consequência do uso de fertilizantes na agricultura, o nitrato pode estar presente nos alimentos de origem animal e vegetal e, assim, permear do solo para o pasto utilizado para alimentação bovina. Dessa forma, depois de ingerido pelos

animais, pode ser excretado através do leite. A Organização das Nações Unidas para a Agricultura e Alimentação (FAO) e a Organização Mundial da Saúde (OMS) determinaram um limite máximo para a ingestão diária aceitável (IDA) de nitrato em 3,7 mg kg^{-1} de peso corpóreo (FAO, 2001). Assim, uma pessoa de 80 kg pode ingerir até 296 mg nitrato dia^{-1}.

A legislação brasileira não determina valores máximos para a presença de nitrato em leite; contudo, considera inadequado para consumo o leite que contenha esses elementos (MÍDIO & MARTINS, 2000).

O processamento de leite e produtos lácteos possui longas tradições em nutrição humana, sendo a principal fonte de cálcio na alimentação, cuja importância está relacionada às suas funções no organismo, pois contribui para a formação do tecido ósseo, promove o crescimento, regula o sistema nervoso e aumenta a resistência a infecções (MADRUGA, ARAÚJO & BERTOLDI, 2009).

A composição nutricional do leite o leva a ser base de muitos produtos, gerando renda e emprego no país. A diversidade de produtos lácteos disponíveis para consumo no mercado é outro aspecto relevante que deve ser considerado no estudo sobre consumo de leite. Tal variedade pode influenciar na escolha do produto a ser consumido, particularmente em relação à origem, conteúdo de gordura e lactose. Ademais, os produtos lácteos são fontes de vitaminas lipo e hidrossolúveis, como a vitamina A e vitaminas do complexo B, respectivamente, e engloba quantidade razoável de vários minerais essenciais como fósforo e potássio (CRUZ *et al.,* 2016).

O consumo de lácteos vem crescendo progressivamente, e fatores como aumento populacional, associado ao aumento de renda e às mudanças de hábito dos consumidores por produtos mais saudáveis, provocaram alterações no mercado de alimentos processados, de forma que o mercado deva acompanhar essas novas mudanças (SIQUEIRA, 2015).

Nesse sentido, o mercado de produtos lácteos com baixo ou zero teor em lactose tem crescido nos últimos anos, já que muitos indivíduos têm intolerância a esse carboidrato, devido à falta ou diminuição da enzima lactase, que hidrolisa a lactose em galactose e glicose, mais facilmente absorvidas pelo intestino. O mesmo não ocorre diretamente com a lactose. Essa acumula-se no trato intestinal e serve como alimento para bactérias da flora intestinal. No intestino grosso

começa a ocorrer fermentação, que causa formação de gases, cólicas, inchaço abdominal e diarreia (MATTAR & MAZO, 2010).

Os produtos elaborados à base de leite se constituem de diversas aplicabilidades e possuem diferenças nutricionais como, por exemplo, iogurte, requeijão, diversos tipos de queijos, soro doce, soro ácido, bebida láctea, manteiga, bebida isotônica e doce de leite. Por isso, o processo de elaboração é intrínseco a cada produto. Por exemplo, a formação de ácido lático (ácido orgânico obtido através da fermentação da lactose) em leite fluido é imprópria, pois aumenta a acidez e facilita a coagulação do leite. Porém, para a fabricação de iogurte a presença de ácido lático é desejável, pois além de resultar em ácido lático como produto principal, há a formação de pequenas quantidades de subprodutos que influenciam nas características sensoriais da bebida. Além disso, a diminuição de lactose devido à fermentação facilita o consumo de iogurte para indivíduos intolerantes a esse carboidrato (AWAD *et al*., 2009).

Técnicas clássicas são empregadas para a determinação de mineriais em leite e produtos lácteos. Espectrofotometria, potenciometria, espectroscopia são alguns ensaios comumente utilizados. Porém, a cromatogria de troca iônica se apresenta como um método em que é possível qusntificar todos os constituintes de interesse de uma só vez.

De modo a quantificar minerais, carboidratos e ácidos orgânicos, a técnica de cromatografia de troca de íons foi utilizada. Apresenta-se como vantajosa sobre as técnicas analíticas clássicas, já que envolve um preparo de amostra simples, prático e rápido, sem a utilização de vários reagentes para cada determinação, em que podem ser quantificadas, simultaneamente, as concentrações de cátions e ânions, por exemplo (METROHM, 2019).

OBJETIVOS

Objetivo geral

- Determinar e comparar a influência do processamento sobre o teor de minerais, citrato e carboidratos em produtos lácteos utilizando a técnica de cromatografia de íons.

Objetivos específicos

- Adaptar uma metodologia para preparo de amostras em produtos lácteos para quantificação de minerais (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Cl^{-}, PO_4^{3-}, NO_3^{-}, SO_2^{4-}), citrato ($C_6H_5O_7^{3-}$) e carboidratos ($C_6H_{12}O_6$ e $C_{12}H_{22}O_{11}$) pela técnica de cromatografia de íons.
- Determinar a composição centesimal dos produtos lácteos.
- Analisar a estrutura e superfície do leite cru e pasteurizado LTLT.
- Comparar o teor de cada constituinte analisado em leite cru e após a pasteurização lenta a fim de avaliar perdas minerais durante o tratamento térmico.
- Determinar minerais, citrato e carboidratos nos produtos lácteos: leite cru, leite pasteurizado integral, soro de leite doce (proveniente da produção de muçarela), soro de leite ácido (proveniente da produção de requeijão), leite de cabra fresco, leite de cabra pasteurizado, leite de búfala fresco, leite de búfala pasteurizado, leite humano fresco, leite humano pasteurizado, queijo muçarela, creme (produto intermediário da produção de manteiga), manteiga, doce de leite, bebida láctea, iogurte integral, requeijão cremoso e bebida isotônica produzida com permeado de soro de leite.

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CHAPTER 1

Macronutrient Profile and Ash Content of Dairy Products Consumed in Brazil

Macronutrient profile and ash content of dairy products consumed in Brazil

Abstract

The present research was designed to evaluate: *(i)* the chemical composition of milk of four species (bovine, caprine, buffalo, and human), at crude condition and after low-temperature-long-time (LTLT) treatment, *(ii)* the effect of LTLT pasteurization (63 ºC / 30 min) in chemical composition of each type of milk, and *(iii)* the chemical composition of 10 dairy products consumed in Brazil (isotonic beverage, dairy beverage, yogurt, cream, butter, mozzarella, sweet whey, acid whey, curd cheese, and dulce de leche), and *(iv)* the structure and surface of all types of milk mentioned with and without heat treatment. Human milk exhibited the highest carbohydrate percentage (6.75 %), and buffalo milk presented the maximum contents of fat (7.05 %) and protein (4.23 %). The evaluated condition of the heat treatment did not affect the nutritional quality of any species of studied milk. It was observed by the structural analysis using confocal laser scanning microscopy that despite the formation of granules in pasteurized LTLT milk, the stability of milk proteins (caseins) is not changed drastically, as is the case of UHT milk. It was also investigated by surface analysis by scanning electron microscopy, pasteurized LTLT homogenized bovine milk. This industrial process favors the standardization of fat globules that prevent coalescence so that the cream does not separate from the milk. The compositions of fat, protein, moisture, ash, and carbohydrate of dairy products make them rich sources of nutrients, which offers excellent opportunities for both the development of dairy material with new properties and to stimulate the trade of differentiated nutritional dairy products.

Keywords: chemical composition; dairy; heat treatment; milk.

1. Introduction

Milk, one of the most versatile products in the food industry, is consumed in its original fluid form and/or as transformed into salted dairy products (like cheeses), sweets (such as yogurts), and ingredients for formulations (like cream and butter)[1,2].

The consumption of raw milk has increased in recent years. Raw milk presents better flavor, reduced susceptibility to allergies, and higher nutritional quality[1,2]. However, there is a biological risk to consumers due to the lack of heat treatment, which can eliminate pathogenic microorganisms. Infections caused by microorganisms, such as *Campylobacter* spp., *Salmonella* spp., and human pathogenic verocytotoxin producing *Escherichia coli* were recorded after consumption of raw milk[3,4].

In the context of the milk production chain, quality standards are required to ensure the processing of safe dairy food with suitable nutritional properties for the consumer, as well as the increase in shelf life, industrial yield and competitiveness in the domestic and external markets[5].

The global production of milk in 2016 was 799 million tons according to FAO[6], 83 % of this volume was of cow's milk, 14 % of buffalo, 2 % of goat, 1 % of sheep, and less than 1 % of camel[7]. In 2017, milk world production reached 811 million tons, 1.4% higher than in 2016[6,7].

The market of goat and buffalo milk has also grown in Brazil. Currently, the country has a population of almost 10 million goats, and nearly its half is in properties of small producers[1]. There was a 16.1% increase in goat's herd between 2006 and 2017 and the production reached 270 million liters of milk. Presently, the buffalo herd in Brazil is estimated at 1.8 million animals[1].

Milk and its derivatives deserve special mention because they constitute a group of foods of great nutritional value, since they are considerable sources of proteins of high biological value, besides containing vitamins and minerals. The habitual consumption of these foods is recommended, mainly, so that the daily adequacy of ingestion of calcium, a nutrient that, among other functions, is essential for the formation and maintenance of structure of the organism[6].

For newborns, the administration of breast milk is enough to power them in the first months of life, because it contains all the nutrients needed to sustain growth and development of the babies[8]. In this scenario, human milk banks (HMB) have an outstanding social function of providing human milk to babies who are not able to get it from their mothers. In Brazil, there are 27 human milk banks[9].

In order to contribute with the improvement of the quality of milk and dairy products, the study of their chemical compositions is necessary, once their chemical characteristics can change with a number of factors, such as species, race, age, diet, and diseases of the animals, seasons, period of lactation, frauds, adulterations, type of processing, storage, number, interval, and milking process[10]. Moreover, standardization of the milk analysis parameters must also occur because the thermophysical properties vary with change in, for example, temperature[11]. Therefore, it is necessary to observe how the structural characteristics of the milk change with the heat treatment. Thus, the confocal laser scanning microscopy technique was applied.

Thus, the objectives of this research were to determine the chemical composition of the different types of raw and pasteurized milk and dairy products consumed in the Viçosa city, province of Minas Gerais, Brazil, and to evaluate the effect of slow pasteurization on the milk chemical composition for different species.

2. Experimental Section

2.1 Materials

Bovine milk from Holstein cows with six months of lactation was donated by FUNARBE dairy industry (Viçosa, Brazil). Caprine milk from Saanen goats with three months of lactation was donated by the Caprine sector of the Universidade Federal de Viçosa (Viçosa, Brazil). Buffalo milk from Murrah breed with three-four months of lactation was purchased in a rural property (Piranga, Brazil). Dairy beverage and the UHT bovine milk were purchased from Quatá® brand, both treated in ultra-high temperature (UHT) pasteurization. The isotonic beverage formulated with sweet whey was granted by the Department of Food Technology (Viçosa, Brazil). Human milk, from healthy women, with four months of lactation, was donated by the human milk bank of the São Sebastião Hospital, (Viçosa, Brazil).

Dairy products such as cream (intermediate product from butter manufacture), butter salted, curd cheese, creamy dulce de leche, whole yogurt without fruit, sweet whey (from mozzarella manufacture), and acid whey (from curd cheese manufacture) were donated by FUNARBE dairy industry (Viçosa, Brazil). Dulce de leche is a type of milk jam thickened by concentrating a mixture of milk and sucrose under heat[12].

The products were divided into two dairy groups, as can be seen in Table 1. The first was about bovine, buffalo, goat, and human milk. The other products composed the second group: sweet whey, acid whey, isotonic, dairy beverage, yogurt, cream, butter, curd cheese, mozzarella, and dulce de leche.

Table 1. Dairy products arranged in two groups according to presentation and discussion of chemical composition results.

Group 1 - milk	Group 2 - other dairies
Raw bovine	UHT bovine milk
LTLT bovine	Sweet whey
HTST bovine	Acid whey

Raw buffalo	Isotonic
LTLT buffalo	Dairy beverage
Raw goat	Yogurt
LTLT goat	Cream
Raw human	Butter
LTLT human	Mozzarella
	Curd cheese
	Dulce de leche

The reagents used in all the quantifications were analytical grade. Deionized and ultrapure Milli-Q water (18.2 mΩ cm^{-1}) (Millipore, USA) was used to prepare all solutions and solutions and dispersions.

Pretreatment of the material. The donors did the cleaning, milking, collection, and frozen of the milk samples that were transported using isothermal box sealed and packaged with ice. Subsequently, they were separated by species (raw bovine milk, pasteurized bovine milk, raw buffalo milk, raw caprine milk and raw human milk) and stored, in a freezer at -20 ºC, until the quantification of the chemical composition.

All milk samples were evaluated at the crude condition and after the pasteurization by using low-temperature long-time (LTLT) condition, at 63 ± 1 ºC / 30 min, named slow pasteurization. For this heat treatment was used a thermostatic bath (Tecnal, TE-184, Brazil) in the UFV laboratory. Only the cow milk was evaluated also in high-temperature short-time (HTST) at 72 ± 1 ºC / 15 s (fast pasteurization) and ultra-pasteurization (UHT). UHT bovine milk was purchased from the Quatá® brand. Both HTST and UHT milk were standardized at 3% fat, according to information on the product label. The other dairy derivatives were not submitted at pretreatment.

2.2 Methods

The chemical composition of dairy products was evaluated for fat, protein, moisture, and ash composition. The carbohydrate content was determined by the difference of 100% and the sum of the values for protein, lipid, moisture, and ash. The quantifications for fat, protein, moisture, and ash followed the methodologies of the Association of Official Analysts Chemical (AOAC)[13] and the International Dairy Federation (IDF)[14]. The apparatus used was an oven (Marconi, MA 032/3, Brazil), an analytical balance

(Shimadzu, AUY220, Japan), and a specific centrifuge (Quimis, Q222G, Brazil) for butyrometer (Lena, Brazil).

Protein quantification[13]: The total nitrogen was quantified by using the distillation technique, according to the modified procedure of Kjeldahl. An ammoniacal nitrogen distiller (Tecnal, TE - 0364, Brazil) was used. One gram of sample was used for all determinations, except for butter, for that 0.5 g was used because it is very fatty and has a high foaming capacity at high temperatures. These samples characteristics favor the operation of the digestion block (at 350 ºC).

Thus, 1 mL of mineral oil was used as an antifoam solution and, in order to contribute to the process, the heating of the block digester (Tecnal, TE - 040 / 25, Brazil) was controlled to avoid the sample projection. After distillation, the amount of protein in the samples was calculated by equation 1:

$$\%p = (V \cdot N \cdot f)_{HCl} \cdot 14 \cdot \frac{0{,}638}{P_s} \quad (1)$$

In which, % p = is the percentage of protein, V is the HCl volume (mL), N is the HCl normality (N), f is the correction factor of HCl normality, P_s is the sample mass (g).

Fat quantification[13]**:** Except butter, all samples were analyzed for the percentage of fat by the Gerber method.

Milk, whey and isotonic beverage: The percentage of fat was obtained by direct reading on the graduated stem of the milk butyrometer and indicated as a percentage of mass.

Cheese and curd cheese: The quantity of fat was obtained using the cheese butyrometer. The percentage of fat was read directly on the butyrometer scale.

Dulce de leche: The sample was prior five folds diluted with Milli-Q water. Equation 2 was used to find the percentage of fat in dulce de leche:

$$\%f = v \cdot 5 \quad (2)$$

In which, % f = percentage of fat, v is the value obtained on the butyrometer scale.

Cream: The fat analysis was performed using a butyrometer for cream. The percentage of fat was read directly on the butyrometer scale.

Yogurt and dairy beverage: the percentage of fat was calculated by equation 3:

$$\%f = v \cdot 10 \quad (3)$$

In which, % f = percentage of fat, v is the value obtained on the butyrometer scale.

Butter: before the quantification of butter content, the composition of volatile substances was calculated by equation 4:

$$\%vs = \frac{N \cdot 100}{P} \quad (4)$$

In which, % v s is the percentage of volatile substances, N is the mass of volatile substances (g), and P is the sample mass (g).

The amount of insoluble materials in ether was calculated by equation 5:

$$\%i = \frac{B \cdot 100}{P} \quad (5)$$

In which, % i is the percentage of insoluble, B is the insoluble mass (g), and P is the sample mass (g).

Then the percentage of fat in butter was calculated according to equation 6:

$$\%f = 100 - (P + N) \quad (6)$$

In which, % f = percentage of fat, P is the content of the volatile substances (%), and N is the content of insoluble compounds in ether (%).

Moisture[13]: For determination of moisture in all products, 5 g of the sample were used. Sugary dairy products can develop a hard crust in their surface during heating, which prevents water exit. In order to avoid this phenomenon in the moisture quantification, sand was treated and mixed to the sample to increase the surface of evaporation and to hinder the crust from forming. The liquid samples were preheated in the oven, at 90 ± 1 °C, until pasty products consistency were obtained. After that, the moisture determination occurred at 105 ± 1 ºC for 4 h. Then, the samples were placed in a desiccator and weighed. The operations of desiccator dehumidification and weighting were repeated until constant mass. The percentage value was calculated with equation 7:

$$\%m = \left(\frac{m_A - m_B}{m_C}\right) \cdot 100 \quad (7)$$

In which, % m = percentage of moisture, m_A (g) is the (mass sample + capsule + sand) before weighing, m_B (g) is the (mass sample + capsule + sand) after weighing, and m_C (g) is the initial sample mass.

Ash[13]: For the ash quantification, the liquid samples were taken to the oven at 90 ºC for 4 h. in crucibles until products of pasty consistency were obtained, and then incinerated in a muffle oven (Fanem, 142, Brazil), at 550 ± 1 °C until constant mass. For butter samples, the temperature of incineration was lower, of 500 ºC, because it is a fatty sample. The ashes resulting from the incineration were placed in a desiccator and weighed. These operations were repeated until constant mass. For ash determination, equation 8 was used.

$$\%a = \left(\frac{m_{s1} - m_{s2}}{m_{si}}\right) \cdot 100 \quad (8)$$

In which, % a = percentage of ash, m_{s1}(g) is the (sample mass + crucible mass) before weighing, m_{s2}(g) is the (sample mass + crucible mass) after weighing, and m_{si}(g) is the initial sample mass.

Confocal laser scanning microscopy: For the methodology adapted from Wang et al. (2016), 42 mg mL-1 of the Nile Red fluorophore were used in acetone P.A and 2 μg mL -1 of fluorescein isocyanate (FTIC) were added in distilled water. Both fluorophores are branded Sigma-Aldrich, United States.

The volume of 10 μl of sample was placed in the sampler; and then stained with 3 μl of the fluorophores mixture (1: 1). This incubation process occurred for 2 minutes at room temperature. The samples were then observed in the Zeiss laser scanning confocal microscope, LSM510 META, Germany, using a 20-fold objective.

Scanning Electron Microscopy: To prepare the sample, bovine, goat, buffalo and human milk, both raw and pasteurized LTLT, were placed in a closed environment with silica gel for 48 h. After drying, the samples were covered with gold powder using the Quorum Metallizer, Q150R S, United Kingdom, and observed under Leo 1430VP scanning electron microscope (Carl Zeiss, Jena, Thuringia, Germany).

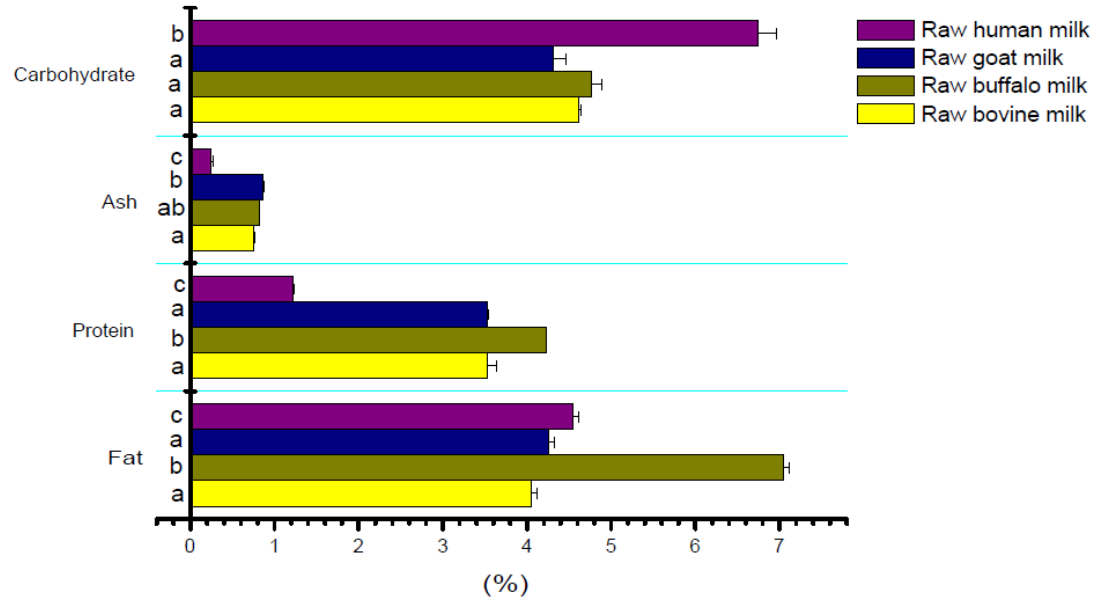
Statistical analysis: The experimental tests were performed in two replicates, each performed in triplicate. All results were presented as mean and standard deviation. For the bovine, goat, buffalo, and human milk, analysis of variance and Tukey's Test at 5 % of significance was applied using Software R.

3. Results and Discussion

3.1 Milk

Regardless of the species, milk is composed mainly by water, fat, protein, carbohydrate, and ash. Figure 1 shows the observed chemical composition of bovine, caprine, buffalo, and human raw milk species. Figures 2, 3, 4, and 5 present a comparison between the chemical composition of raw milk and low-temperature long-time (LTLT) processed milk of, respectively, bovine, buffalo, caprine, and human milk. The moisture was presented separately from others milk constituents.

Figure 1. Chemical composition (%) of raw milk between species.



Means followed by the same letters, in the lines, do not differ statistically by the Tukey test, at 5% of significance.

According to the Figure 1, the chemical composition of bovine and goat milk had no significant difference ($p > 0.05$). Their fat contents are lower as compared to human and buffalo milk. Unlike ruminants, humans require a higher proportion of fat concerning their body weight during breastfeeding. This energy is needed to develop the brain and other nervous system components[16]. The buffalo milk presented fat content almost twice higher than the other types of studied milk. A high content of fat affects the processing and yield of certain products, making it suitable for producing cheeses, butter, among others[17].

The results in Figure 1 showed similarities between the protein levels of bovine and goats raw milk. The buffalo milk has the highest content of protein in the data set evaluated. Kapadiya et al.[21] reported protein levels of buffalo milk that corroborate with this result. The human milk presented the lowest protein content according to the Figure 1. Claeys et al.[18] also find a similar value for protein in human milk.

Arora[17]also reported a high protein content in buffalo milk whose concentration of caseins and globular proteins are higher than in the bovine milk. Unlike cow's milk, the majority of buffalo milk caseins is present in the micellar form. The casein micelles are bigger than the bovine ones. These characteristics of proteins assign differentiated qualities to processed cheeses and a lower rennet coagulation time of buffalo milk[22].

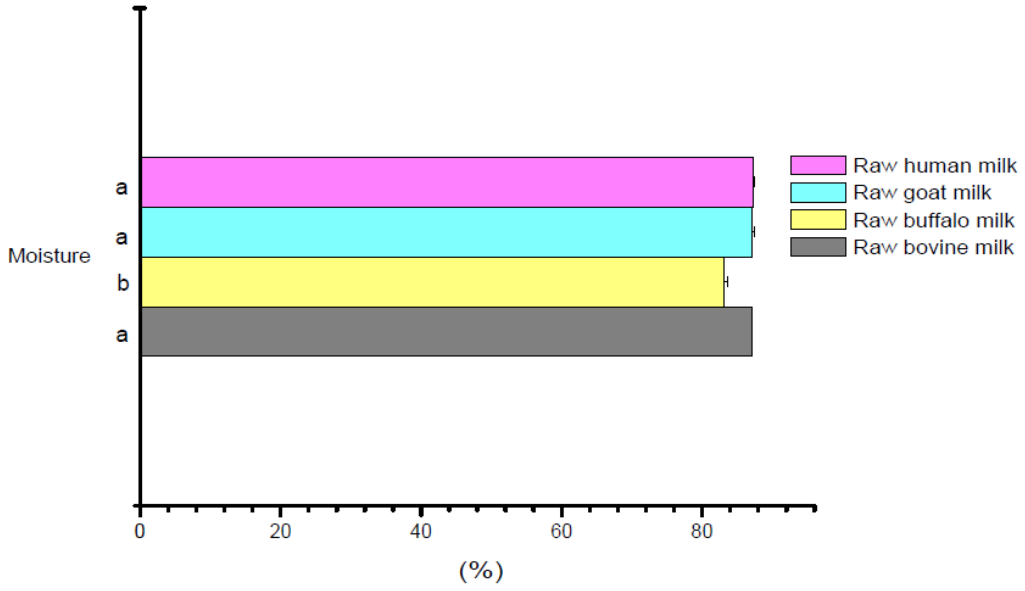
The protein content of the different milk is directly related to the growth rate of each species[16]. This information explains the lower concentration of proteins in the raw human

milk presented by the Figure 1 since it is the species with the lowest growth rate among the analyzed ones. A calf doubles its weight after birth in only 40 days, while a human newborn doubles its weight after 180 days[24]. However, according to Wu et al. [24], human colostrum, the first milk secretion that occurs between 0 and 7 days, has considerably higher protein contents than those presented in mature human milk.

The Figure 1 reveals that the ash content of bovine milk was lower than goat milk. Statistically, buffalo milk does not differ between the two mentioned above, presenting an intermediate value. Despite the statistical differences, the levels of ashes of these three type of milk are higher than human milk. Besides, with the exception of human milk, all other milk types presented in Figure 1 come from ruminant species. According to Claeys *et al.*[20], non-ruminant animals have lower ash contents than ruminants. Non-ruminant animals such as equidae also exhibit low ash content, but still higher than the average for human milk in the literature.

As shown in the Figure 1, buffalo milk presented higher mineral content compared to bovine milk, corroborating the results of Ahmad *et al.*[29], who also reported the similarity between the bioavailability of Ca and P for both kinds of milk. The authors suggest that the Ca and P amount associated with casein micelles is higher in buffalo than in bovine milk. This positive variation affects cheese processing using buffalo milk, as well as the quality of these products[17].

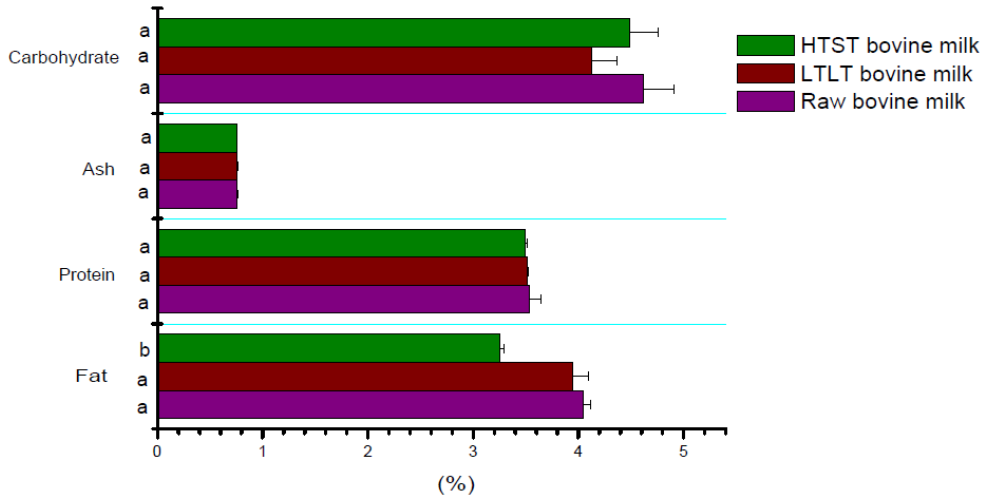
Figure 1.1. Moisture (%) of raw milk between species.



Means followed by the same letters do not differ statistically by the Tukey test, at 5% of significance.

The moisture content for bovine, goat and human milk did not differ significantly ($p > 0.05$), since the total solids content for these compounds was 12.95 %, 12.95 % and 12.76 %, respectively. However, for buffalo milk, the amount of moisture varied significantly ($p < 0.05$), with 16.86 % of total solids. What explains this fact is that buffalo milk has a higher content of protein, fat and minerals when compared to the other milk investigated[21].

Figure 2. Chemical composition (%) of raw and pasteurization bovine milk LTLT and HTST.



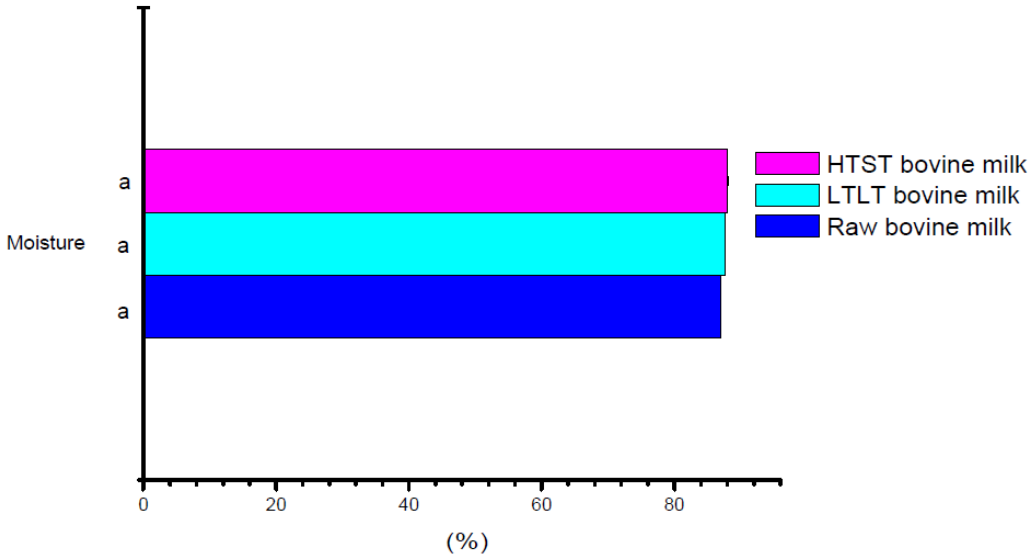
Means followed by the same letters, in the lines, do not differ statistically by the Tukey test, at 5% of significance.

There was no statistically significant difference between the components of raw milk and LTLT milk bovine ($p > 0.05$), showing that the heat treatment did not influence the fat, protein, moisture, ash and carbohydrate contents. However, in comparison to the HTST processing, the fat suffered a significant decrease, most probably by the standardization of this component at 3 % fat.

According to the Figure 2, the difference in protein content between raw and LTLT bovine milk was not significant. The HTST milk also did not present significant difference in comparison to the raw and LTLT milk of bovine milk. This is because the heat treatments frequently used do not affect the nutritional and digestibility properties of milk proteins significantly.

However, functional properties (eg, emulsification, gelation and solubility) evaluated in electrophoresis tests are associated with the secondary and tertiary structures of the proteins. The way proteins interact with other components present in milk can be affected by the heat treatment applied and also by the addition of minerals such as calcium[28,29]. Bovine milk is normally processed on an industrial scale before consumption, being submitted to the standardization process of fat content. Milk from other species is handcrafted handled and, therefore, presents a greater variability of fat content, due to different factors, such as animal breed, diet, etc.[18]. High-temperature short-time (HTST) is standardized; consequently, presented lower fat content than the raw and LTLT according to the Figure 2. This fact makes it difficult to evaluate the effect of heat treatment among all bovine milk, and it is pertinent to compare the raw and LTLT only.

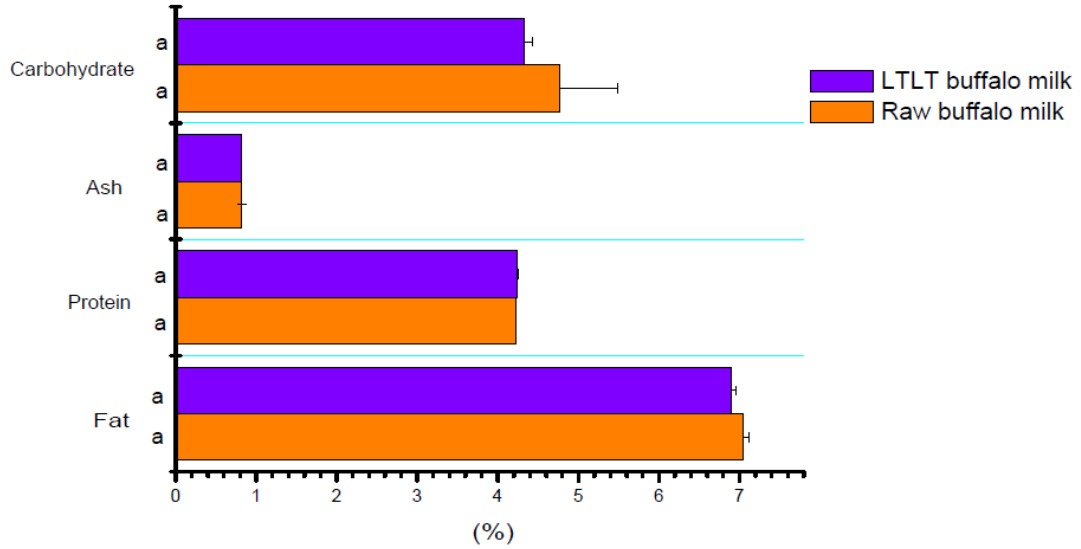
Figure 2.1. Moisture (%) of raw and pasteurization bovine milk LTLT and HTST.



Means followed by the same letters do not differ statistically by the Tukey test, at 5% of significance.

There was no statistical difference ($p > 0.05$) for the moisture content in raw milk in relation with milk heat treatment, indicating that the water content did not appear to be altered by either type of LTLT and HTST pasteurization.

Figure 3. Chemical composition (%) of raw and pasteurization buffalo milk LTLT.



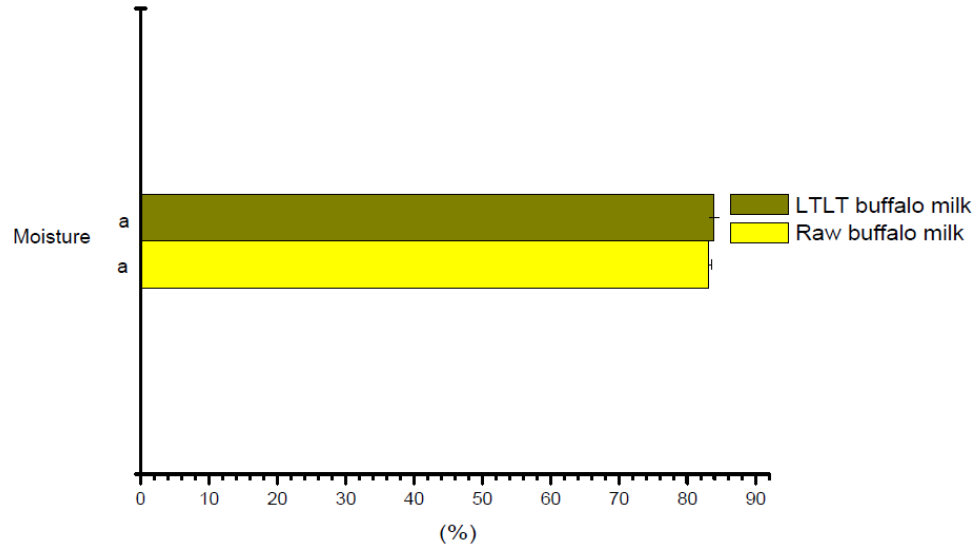
Means followed by the same letters, in the lines, do not differ statistically by the Tukey test, at 5% of significance.

The difference in fat content between raw milk and LTLT milk for buffalo milk was not significant ($p > 0.05$). Thermal degradation of milk lipids is generally not observed, because the temperature required for non-oxidative decomposition of fatty acids (higher than 200 ºC) is well outside the range in which milk products was heated. Thus, it can be assumed that LTLT heat treatment applied in the studied milk of different species does not present a substantial effect on the milk fat[19]. Studies have shown that factors such as the diet and breed of animal affect the fat content more than heat treatments[20,21].

It is worth mentioning that, although the LTLT pasteurization does not induce a significant variation of fat content, heat treatments can affect the fat distribution, as well as its interaction with proteins present in the milk.

According to the Figure 3, the difference in carbohydrate content between raw milk and industrially processed LTLT milk for each species was not significant ($p > 0.05$). This result agrees with the literature since in different studies LTLT pasteurization did not affect the lactose and oligosaccharides concentration[37,38].

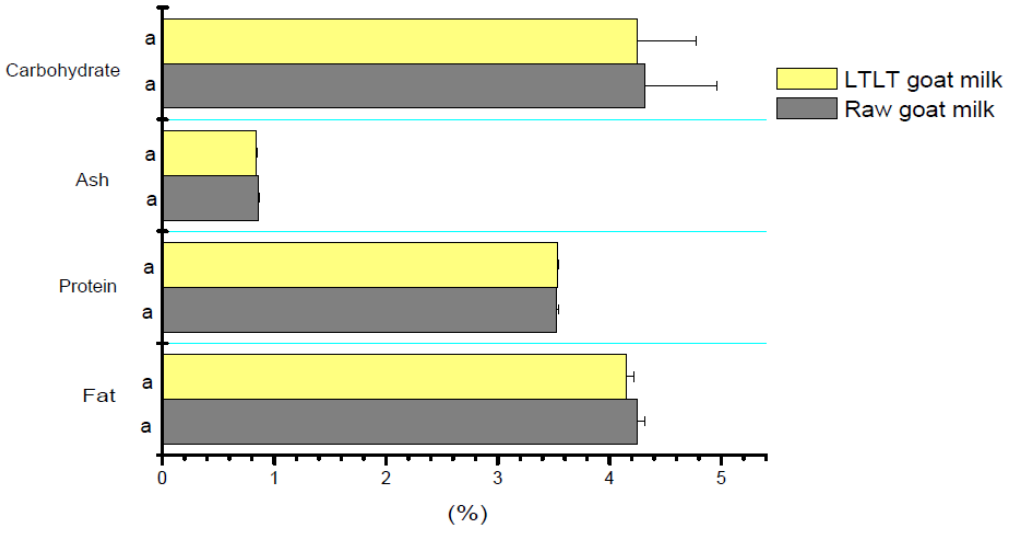
Figure 3.1. Moisture (%) of raw and pasteurization buffalo milk LTLT.



Means followed by the same letters do not differ statistically by the Tukey test, at 5% of significance.

There was no significant moisture variation ($p > 0.05$) of buffalo milk in relation to LTLT pasteurized buffalo milk.

Table 4. Chemical composition (%) of raw and pasteurization goat milk LTLT.



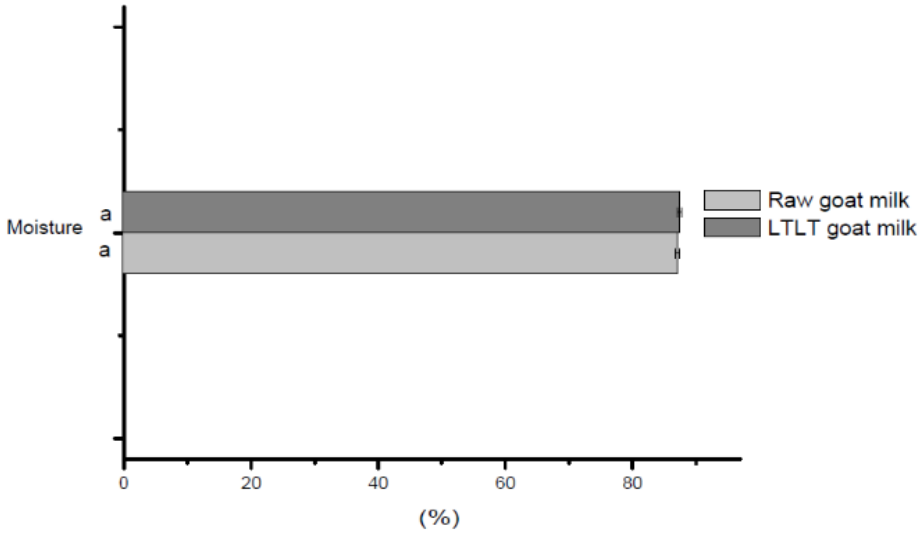
Means followed by the same letters, in the lines, do not differ statistically by the Tukey test, at 5% of significance.

According to the Figure 4, the difference of ash content between the raw and LTLT milk for goat milk was not significant ($p > 0.05$). These results agree with the study of Claeys *et al.*[18] in which raw and sterilized bovine milk did not present significant differences in the mineral content. According to Gaucheron[30], these minerals availability depends on

their chemical form. Severe treatments at temperatures above 90 °C can cause irreversible changes in these forms.

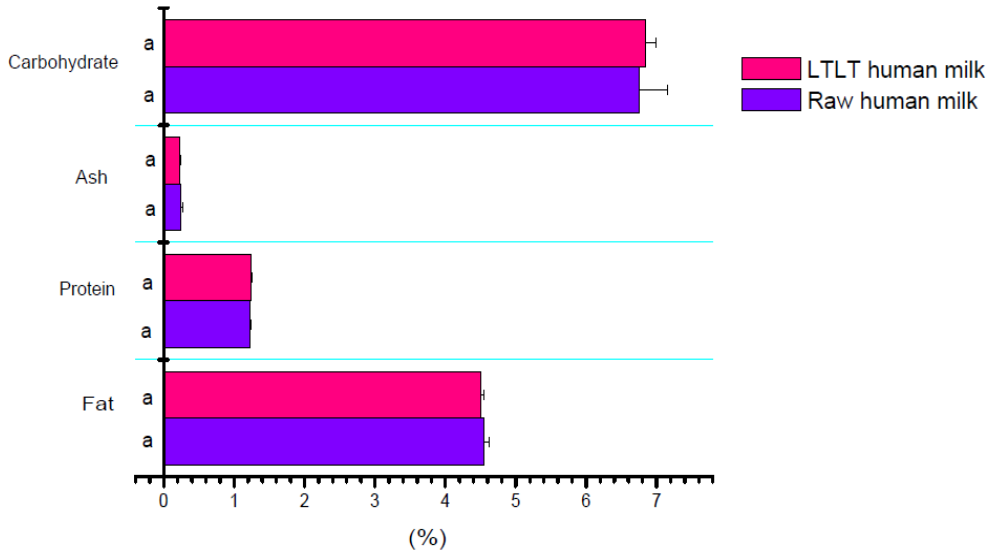
Thermal treatments, considered non-aggressive, such as LTLT applied in this research probably did not affect the bioavailability of minerals of the different milk. A comparative study between two groups of newborns fed with raw bovine milk and LTLT (63 ºC / 30 min), even thermal treatment applied to the different species in this study, showed no difference in the bioavailability of Ca, P, and Na[31].

Figure 4.1. Chemical composition (%) of raw and pasteurization goat milk LTLT.



Means followed by the same letters do not differ statistically by the Tukey test, at 5% of significance.

Figure 5. Chemical composition (%) of raw and pasteurization human milk LTLT.



Means followed by the same letters, in the lines, do not differ statistically by the Tukey test, at 5% of significance.

According to Harzer & Bindels[27], a relation between protein content in the milk and the growth rate in species is also associated with the mineral content. Their results showed the occurrence of main minerals present in milk ash (as calcium and phosphorus), at high concentrations, in the milk of species with a great growth rate. Human species, compared with the other species, has a low growth rate and demands milk with low protein content. Therefore, the human being needs a little quantity of minerals, since a lower content of calcium and phosphorus is required to meet its bone growth.

The minerals bioavailability can be connected to the type of protein present in milk that is divided into two categories, the caseins, and whey proteins. While bovine milk exhibits a protein ratio of 80:20 for caseins: whey proteins, human milk has a protein ratio of 20:80 caseins: whey proteins. Casein is a protein that interferes negatively in the mineral's bioavailability, such as in the calcium (Ca) and phosphorus (P) bioavailability. The percentage of casein in human milk is low, so the bioavailability of Ca and P is high. Thus, despite a small mineral content in human milk, the low casein concentration allows the demand for these minerals to be met[28].

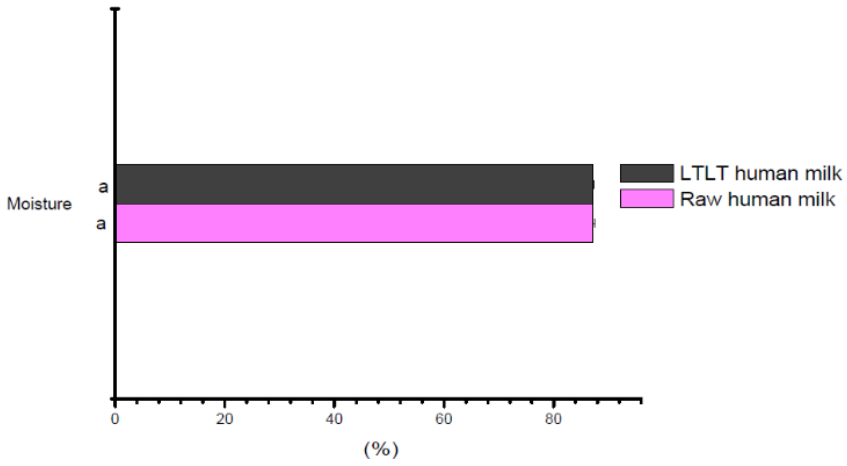
The bovine, goat, and buffalo milk had no significant difference ($p > 0.05$) regarding the carbohydrate content. Milk of human presented a higher content than the other species ($p < 0.05$). Gantner *et al.*[32] evaluated the carbohydrate content of milk from different species and reported a higher carbohydrate content in milk from non-ruminant than that from ruminants. Among non-ruminants, human milk is the one with the highest carbohydrate content, which agrees with our data. Lactose is the main carbohydrate present in milk and provides approximately 40 % of the energy needs of the newborn, providing enough

energy for the development of the central nervous system[33]. According to Wu et al.[24], of all mammals, human milk has the highest lactose concentration. Due to its nutritional importance, among all macro components, lactose is the one with the lowest variation in human milk during the first 12 months of lactation[34].

Small amounts of glucose and galactose (monosaccharides precursors of lactose) and small oligosaccharides fractions (sugars formed by 2 to 6 monosaccharides) are found in milk, besides the lactose. The percentage of oligosaccharides varies among species, both in quantity and quality[33]. Clayes *et al.*[18] reported the concentration of oligosaccharides of bovine, caprine, and buffalo milk inferior to 0.3 g L^{-1}, whereas for human milk the range was between 5 and 10 g L^{-1}. According to Morrow *et al.*[35], oligosaccharides are the third largest component present in breast milk after lactose and lipids. Certainly, oligosaccharides contribute to the higher carbohydrate concentration in human milk. This large difference concerning other species is due to their role in the growth of intestinal flora, in modulating and stimulating the immune system, and in protecting against pathogenic microorganisms[36].

Heat treatments at temperatures higher than 90 ºC, may induce the lactulose formation and the Maillard reaction occurrence. However, according to Bertino et al.[37], during thermal processing, only a small lactose fraction (0.5 % for UHT and 1 % to 2 % for sterilization) is converted into lactulose. Above 100 °C, thermal treatments can also lead to the lactose degradation in acids, especially lactic acid and consequently cause an increase in the milk acidity[9,39].

Figure 5.1. Moisture (%) of raw and pasteurization human milk LTLT.



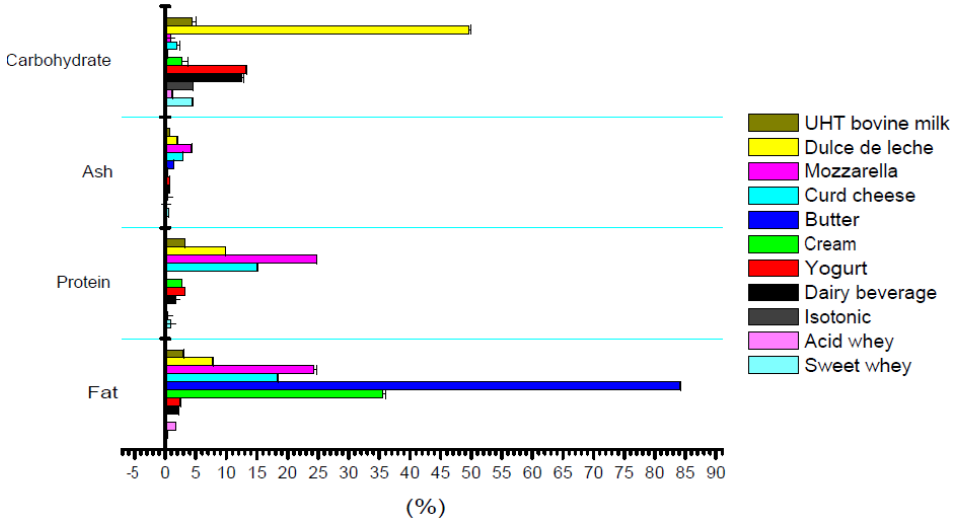
Means followed by the same letters do not differ statistically by the Tukey test, at 5% of significance.

3.2 Dairy Derivatives

Cream, butter, mozzarella, curd cheese, sweet whey, acid whey, isotonic, yogurt, dairy beverage, and dulce de leche

The Figure 6 shows the results of the chemical composition of the evaluated dairy products, expressed as a percentage. The data were expressed as mean ± standard deviation.

Figure 6. Chemical composition (%) of dairy products.



UHT bovine milk: The UHT milk was not added together to the other milk because it came from another cattle herd, since it was obtained by another company. Because it was a standardized milk at 3% fat, the value for this component was 2.95%.

Cream: A large amount of protein (2.71 %) and carbohydrates (2.80 %) were quantified in the cream, probably due to the presence of its aqueous phase, that is the buttermilk. This intermediate product had a lower ash content (0.40 %), inferior to the butter (1.32 %) since it is not submitting to salting with sodium chloride. The fat content nearly 35 % was within the expected limits for this intermediate product. According to Deosarkar *et al.*[40], for a good industrial yield, the cream should present fat content between 35 and 40 %, quantity which is appropriate to be used in the batting stage. Values above this amount will promote fat loss in buttermilk and below that there will be less use of the equipment capacity[40].

Butter: A low amount of protein (0.17 %) and carbohydrate (0.20 %) were measured in the butter. The continuous phase (85 %), composed mainly of lipids, has a low concentration of these two components. Besides, when butter is washed with water, buttermilk and the remaining components of the dispersed phase that still exists are remove[41]. The ash represented an amount of 1.3 % of minerals such as magnesium, calcium, potassium, and phosphate. Sodium and chloride may be present in larger quantities due to the salting process of the butter, which is made with sodium chloride. The moisture and fat contents of 14 % and 84 %, respectively, were within the range suggested by Galli & Risé[42]. For these authors, butter should be prepared exclusively by

batting and kneading the pasteurized cream to obtain 15 % of moisture and, at least, 80 % fat mass, in the case of salted butter. Elshemey[43] studying the fat behavior of some foods by using measurements of the X-ray scattering reported a fat percentage in butter of 88 %. Thus, our results are in the literature[42,43] range.

Mozzarella: This dairy product exhibited: 24.68 % of protein; a high percentage of moisture, of 47.02 %; 24.25 % of fat being considered a lean cheese accordingly to the fat content (up to 25 %); and the highest mineral content, of 4.21 %, when compared with the other studied products. This high mineral composition is due to the addition of calcium chloride to facilitate the step of casein coagulation, to the inclusion of sodium chloride into the salting stage, and the large quantity of calcium phosphate bound to the casein micelles which make up the mass of the cheese. The carbohydrate percentage of 0.83 % was low, in concordance with the cheese characteristics[44]. Acidification of milk is necessary to increase the power of coagulation and the consistency of the rennet, aiding in the serum withdrawal step. With the formation of lumps, milk components (like proteins, fat, lactose, and minerals) are trapped in a three-dimensional gel structure. However, a part of the lactose is metabolized in lactic acid and other sub-products by yeast, bacteria, and enzymes producing the cheese's characteristic taste[44].

Curd cheese: The curd cheese[45] is a typical Brazilian cheese added that contains cream and melting salt. This creamy and stretchy cheese is produced by melting the curdled dough that was previously removal of whey and washed. The process results in acidic coagulation, thus generating acid whey. The maximum moisture content for this product should be 65 %. The curd cheese evaluated in the present research revealed a high moisture content (62.05 %) and lower ash content (2.88 %) when compared to the mozzarella, probably due to the difference between the manufacturing process of both types of cheeses.

Sweet whey: The sweet whey[46] resulting from the production of mozzarella was majority composed of water (93.75 %), followed by carbohydrates (4.39 %), which were present in the mozzarella and were withdrawn after the washing step. This whey is poor in protein (0.97 %) that is found in large quantity in the mozzarella mass. Minerals, such as chloride, sodium, potassium, and magnesium, are present as ions in the aqueous phase of the milk. Thus, one can expect to find minerals in the cheese whey as was observed (0.56 %).

Acid whey: The acid whey[46], obtained from the production of curd cheese, contains more fat and moisture than the sweet whey and has a lower content of minerals, carbohydrates, and protein according to Table 7.

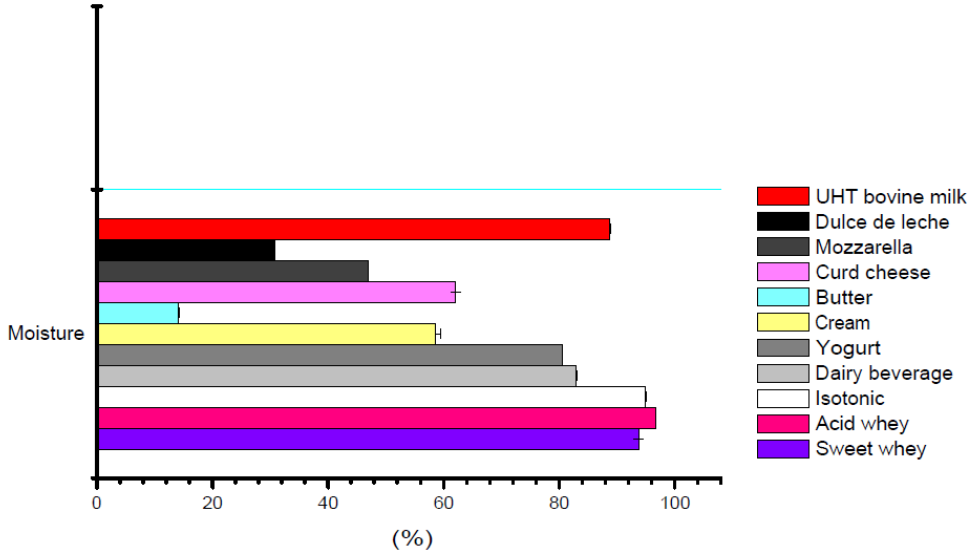
Isotonic beverage: Isotonic is a permeate-based electrolytic beverage obtained from the process of ultrafiltration of sweet whey[46]. The fat content was null, as expected, due to the sports purposes that this electrolyte is addressed. The carbohydrates present in the sweet whey remained in the beverage in aiming to supply the body energy replenishment. Notably, during the mid and high-intensity exercise series lasting at least 60 min., carbohydrate supply can help maintain glucose levels to deliver energy more quickly to the body's cells[47].

Yogurt: This milk derived beverage exhibited 3.18 % of protein, 2.45 % of fat, and 80 % of moisture. Sucrose is generally added to this product representing nearly 11 % of the total beverage composition. There is high consumption of the lactose since this carbohydrate is the substrate for lactic acid bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, some microorganisms responsible for the coagulation, texture, and sensorial characteristics of yogurts[48].

Dairy beverage: This UHT dairy beverage[46] showed higher moisture than yogurt meaning it has less quantity in total solids, and similarity in the amount of fat, carbohydrates, and minerals. The dairy beverage contains at least 51 % of milk base, being a big part of this percentage correspondent to the sweet whey. Since sweet whey has a smaller amount in protein than the yogurt, the dairy beverage also presented reduced content of protein regarding with yogurt.

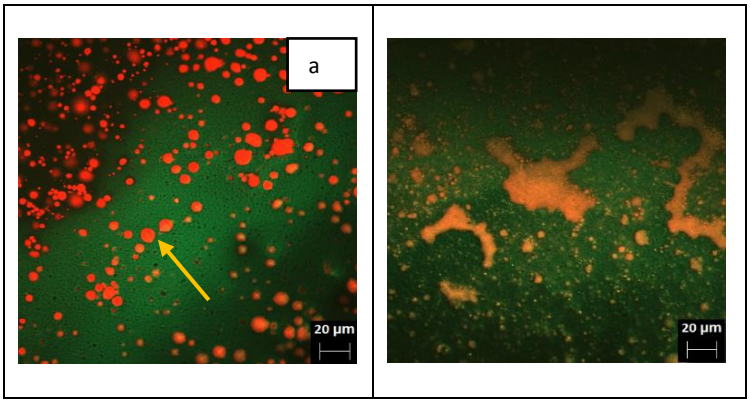
Dulce de leche: The creamy dulce de leche[12] is a dairy product made from concentrated and sugary milk, at atmospheric pressure and temperature proximately to 100 ºC. Almost half of the product composition is of carbohydrates, which include a small part of the lactose from the milk and a large part of sucrose (49.6 %). The moisture content was nearly 30 %, and the amount of fat, protein, and ashes was almost triple of bovine milk due to the water evaporation from milk.

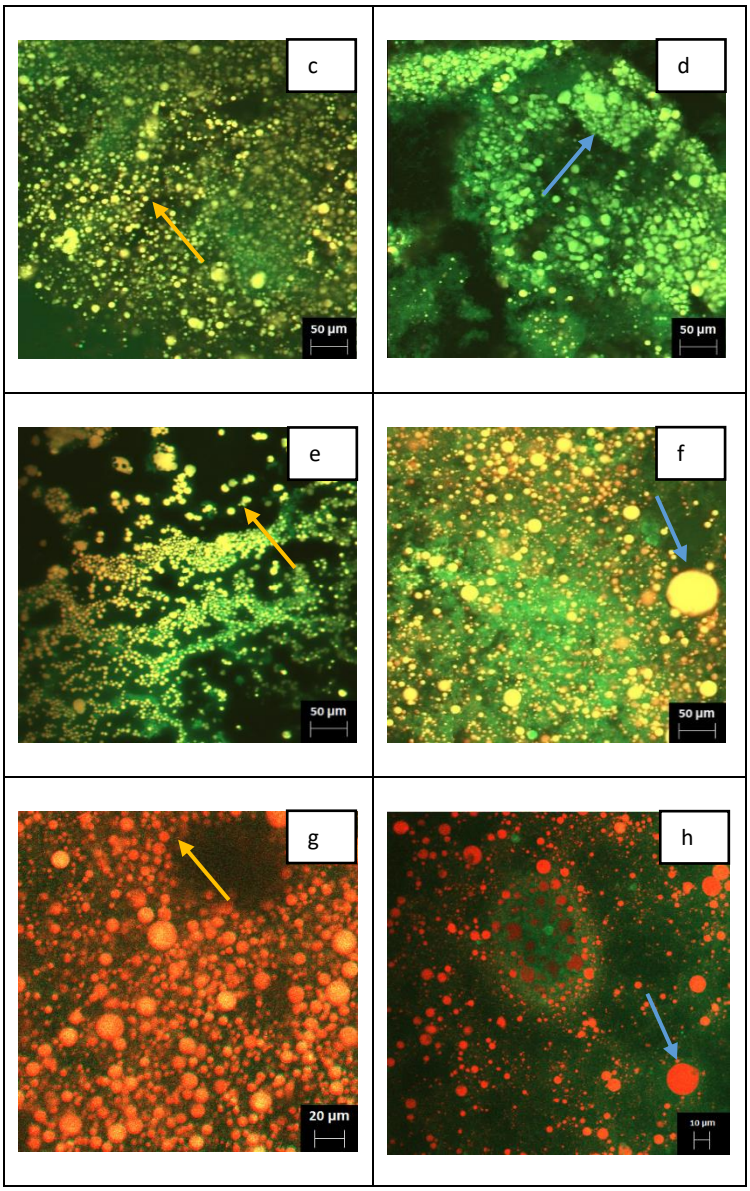
Figure 6.1. Moisture (%) of dairy products.



Structural analysis

Figure 7. Confocal laser scanning microscopy for milk of different species before and after LTLT pasteurization.





a = raw bovine milk and b = LTLT bovine milk; c = raw buffalo milk and d = LTLT buffalo milk; e = raw goat milk and f = LTLT goat milk; g = raw human milk and h = LTLT human milk.

Lipids in milk are presented as droplets, consisting mainly of triglycerides and individually wrapped by a lipoprotein membrane. The milk fat globule membrane (MGGL) is the structure that provides integrity to the globule and keeps it in the state of the emulsion in milk.

Other lipids present include phospholipids, cholesterol, free fatty acids, mono and diglycerides[49]. In Figure 7, the fat globules are highlighted by the reddish coloration and the proteins by the greenish coloration. The lipoprotein membrane surrounding the fat globules provides integrity and stability of fat in milk. Any change in the membrane favors the approach and coalescence of the globules that emerge on the milk surface much faster than the isolated globules[50].

More than 80% of milk fat globules are less than 1μm in diameter; despite this, the average size of the globules is around 4 μm[51]. As can be seen from Figure 7.a, raw bovine milk had a larger fat globule size when compared to LTLT homogenized bovine milk (Fig. 7.b). This was probably due to the homogenization process, which causes the fat globules to be broken, giving rise to smaller particles that are covered by protein. Thus, LTLT homogenized bovine milk (Fig. 7.b) presented a smaller fat globule size than buffalo LTLT (Fig. 7.d), LTLT goat (Fig. 7.f) and LTLT human (Fig. 7.h).

The yellow arrows indicate the average size of the fat globules in the raw milk and the blue arrows indicate the average size of the fat globule aggregates after the LTLT pasteurisation. Figure 8e shows that goat milk had the lowest mean diameter of fat globule, which is an advantage for feeding infants and the elderly due to the higher digestibility of this milk.

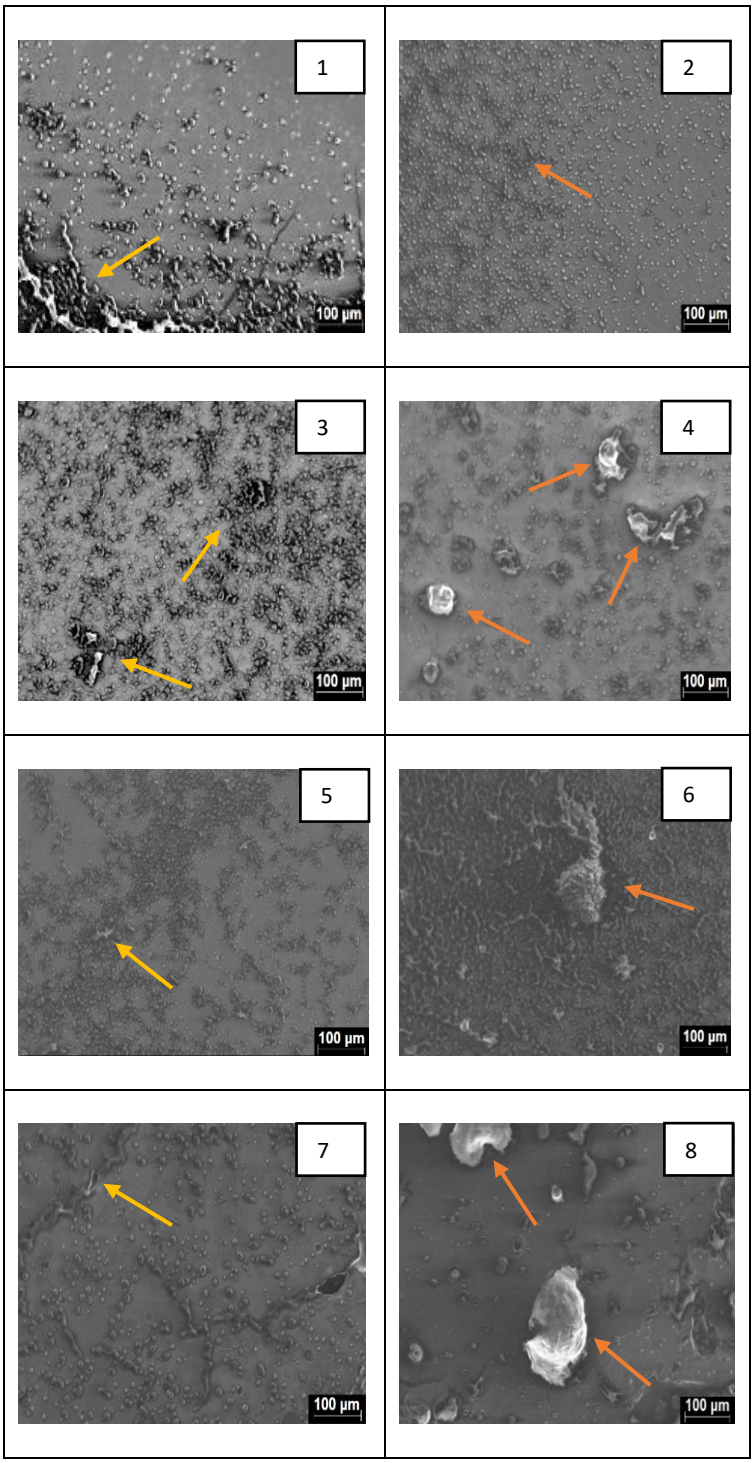
The distribution of milk globules with different diameters can be conditioned by the physiological, as lactation stage, by the type of feed and by the nutritional condition of the animal, milking frequency; microbiological quality of milk and milk products; treatment of milk and by-products, cooling, freezing, mechanical damage, high pressure treatment, heat treatment, homogenization and drying and MGGL research and analysis techniques[52,53,54].

According to the Figure 7, after pasteurization, some structural changes occurred in the analyzed milk in relation to the aggregation of proteins, but in different degrees for each milk species. The same was observed by Ying et al.[55] and Ye et al.[56]. After pasteurization, the protein increase in MFGM may be related to serum protein transfer to MFGM, according to Corredig & Dalgleish57 & Ying et al.[55].

> It was also observed a higher aggregation stage between the proteins for the bovine and caprine species, which may be related to the differences in the physico-chemical properties of the MFGM and the protein structure of each species. It is suggested that quantitative changes of MFGF proteins during pasteurization may be brought about by aggregation with MFGM proteins or skim milk proteins by disulfide bonding or transfer of serum proteins to MFGM Ying et al.[55].

Surface analysis

Figure 8. Scanning electron microscopy (SEM) for different milk before and after LTLT treatment.



The fat globules of the raw milk are highlighted by the yellow arrows and the granules (aggregates of fat globules covered by proteins) resulting from the pasteurization of the milk are highlighted by the red arrow in Figure 8. These granules may indicate a possible destabilization of the casein micelles suspended in milk due to protein denaturation probably caused by the pasteurisation temperature[58].

This temperature does not interfere severely in the processing of pasteurized milk. So the quality of the product can be maintained. Heat treatments above 90 °C, such as those occurring for UHT milk, can cause significant gel formation effects due to the greater destabilization of the proteins, leading to protein sedimentation and, consequently, product rejection by the consumer[59,60].

In all the evaluated milk, it was possible to notice that there was formation of granules after the step of pasteurization. The exception occurred for homogenized LTLT pasteurized bovine milk (Fig. 8.2).

Homogenization is used to modify the functional properties and has no effect on the nutritional value of the product. This process prolongs the stability of the fat emulsion by mechanically reducing the size of the globules to a diameter of about 1 μm to 2 μm. Minor fat globules have more difficulty to flocculate and, therefore, the cream is prevented from separating, which favors the acceptance of the product by the consumer[61].

4. Conclusion

The chemical composition of evaluated dairy products shows them as a rich source of nutrients, which offers excellent opportunities for both the development of dairy material with new properties and to stimulate the trade of differentiated nutritional dairy products. The chemical composition of the raw milk of different species differs considerably, mainly: for the highest contents of fat and protein for buffalo milk, carbohydrate for human, and ash for goat species; and lowest contents of fat for cow milk, protein for human, carbohydrate for goat, and ash for the human species. The LTLT pasteurization, an industrial heat treatment, does not affect the centesimal composition of any of the evaluated raw kinds of milk. Some granules were observed in LTLT pasteurized milk without homogenization, but at pasteurization temperature, the granules formed are not sufficient to cause significant destabilization of the milk proteins and also do not lead to coagulation. The homogenization made only for pasteurized bovine milk showed that there is a greater uniformity in the fat globules for this milk, which reduces the coalescence in this product. Less severe heat treatments, as LTLT, associated with good agricultural and hygienic practices can be a viable alternative for small producers to guarantee the quality of the milk without changing its nutritional quality.

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CHAPTER 2

Determination of Minerals, Organic Acids and Lactose in Dairy Products by Ion Chromatography

Determination of minerals, organic acids and lactose in dairy products by ion chromatography

Abstract

The classic analytical assays for the determination of minerals, organic acids and carbohydrates in dairy products can be time consuming, complex, and often require skilled labor for each individual determination. In this sense, ion exchange chromatography is an advantageous technique, since it allows a simple sample preparation to be used for the analysis of cations and anions in the same run. The aim of this research was to determine the mineral composition of organic acids and lactose in milk samples of different species, such as bovine, caprine, buffalo and human, as well as the effect of slow pasteurization (63 ºC / 30 min) on the constituents. Other dairy products of bovine milk, such as dulce de leche, curd cheese, mozzarella, dairy beverage, etc. were also evaluated for mineral content of organic acids and carbohydrates. According to the results, it was possible to observe that the Low Temperature Long Time (LTLT) and High Temperature Short Time (HTST) treatments did not cause significant variations ($p > 0.05$) in the migration of the majority of ions between the aqueous phase and the micellar phase. With the heat treatment LTLT, the concentration of lactose in all the milk decreased ($p < 0.05$). Nitrate was found in all milk species evaluated, around 38-39 mg kg^{-1}. The amount of nitrate found was five times higher (252.8 mg kg^{-1}) than that allowed for this type of product (50 mg kg^{-1}), and the sodium limit was also exceeded (24044 mg kg^{-1}) 4.74 times more than the value found by the Brazilian Table of Food Composition (TACO) (5070 mg kg^{-1}). This result should be understood as an alert to the population for healthier dairy choices in order to reduce sodium, and to configure ion chromatography as an efficient analytical technique.

Keywords: chromatography, heat treatment, dairy products.

1. Introduction

Food and beverages, as well as their raw materials, are subject to chemical analysis to evaluate their composition, quantification of minerals, carbohydrates, calorific value, physico-chemical properties, verification of toxicological variables, determination of contaminants, adulterants, among others[1].

Some already established techniques can be applied for the evaluation of minerals, organic acids and carbohydrates in milk and dairy products. For anions: Inorganic phosphate is usually determined by the method of Fiske and Stubbarow[2], as described in the official methods of the Association of Official Analytical Chemists (AOAC). Chloride can be quantified by titration with $AgNO_3$ using potentiometric or endpoint detection of the indicator. Sulfate is generally precipitated by $BaCl_2$ and determined gravimetrically. For the determination of nitrate, there is the reduction in nitrite and subsequent reaction with sulphanilic acid in acidic medium. The resulting product is determined by spectrophotometry[3].

Among the analyzes for cation determination, calcium can be determined by direct titration with EDTA or by atomic absorption spectroscopy; magnesium by titration with EDTA[4]; ionized calcium is generally determined by the method of Smeets[5], modified by Tessier and Rose[6], or using a calcium ion selective electrode[7]. Sodium and potassium can be determined by flame photometry, atomic absorption spectroscopy or selective ion electrodes.

The determination of the organic acids present in milk and dairy products allows monitoring activity and microbial growth and also for nutritional reasons, as they contribute to the flavor and aroma of the products. Both lactic acid and citric acid in milk are present in the form of lactate and citrate, respectively. Lactate can be determined spectrophotometrically after reaction with $FeCl_2$ or enzymatically (using lactate dehydrogenase); citrate by Marier and Boulet[8] colorimetric method, modified by White and Davies[9], by citrate complexation with copper ions[10] or enzymatic assay involving the use of citrate lyase, malate dehydrogenase, lactate dehydrogenase and NADH[11].

For carbohydrates, one of the methods used to determine lactose is that described by Lane-Eynon which is based on the reduction of the known volume of alkaline copper (Fehling reagent) to cuprous oxide[3].

Detection methods, such as spectroscopy, besides having high instrumentation costs, also require skilled labor. The gas chromatographic method requires time-consuming derivatizations. The time taken to prepare each analysis separately, reagent costs and complexity should also be taken into account.

Ion Chromatography (IC) presents as a dynamic ion exchange method that occurs between the mobile phase and ion exchange groups attached to the carrier material. With anion exchange columns, for example, solutions of ions are used as eluent to separate anions[12].

The use of a conductometric detector in the CI allows the use of two different chromatographic applications: with suppression and without suppression. In the former, the mobile phase leaving the column prior to detection passes through a suppressor unit in which the conductivity of the mobile phase is significantly reduced so that the ions in the sample can be detected without correction of the baseline. Methods using the suppressor column configuration are referred to as chemical suppression ion chromatography[13].

The suppression IC is performed without the use of a suppressor unit, with columns filled with stationary phases for ion exchange of low exchange capacity and diluted eluents, so that the conductivity due to the mobile phase is also low. This method has a lower detectability than suppressive column chromatography[14, 15].

In view of the various analytical techniques described for food analysis, the CI deserves to be highlighted, since it meets the necessary requirements for a good determination, such as precision, speed, reproducibility and sensitivity. In addition, the IC allows the analysis of different analytes, using the same sample, during a single chromatographic run, without the need for complex sample preparation[16].

Quality control of milk and milk products is an area that requires rapid and accurate analytical methods, characteristics common to HF. The large variety of columns and detectors that the CI uses to meet the demand for analysis of cations, anions, organic acids and carbohydrates, as well as other components such as proteins[17].

Complex matrices such as milk and derivatives, which have a high concentration of organic matter, require a rigorous sample preparation, since the

presence of protein and fat in columns of detection of minerals, organic acids and carbohydrates can shorten the useful life of these columns[18].

Among the applications of IC in milk and derivatives, the determination of sugars is outstanding. Recently, a method of IC using anion exchange column and amperometric pulse detector was accepted by the International Dairy Federation and the International Organization for Standardization[19].

CI is also used to detect and quantify anions, cations and organic acids in milk and derivatives using suppression systems and conductivity detectors. A number of studies have shown the advantages of using IC for detection of these analytes[20, 21,22].

In addition to the rapid quantification of these ions and organic acids, the possibility of using only a sample preparation and an equipment is a great advantage. CI also provides a powerful method for analyzing the mineral transformations undergone by milk and its derivatives during technological treatments, such as acidification or ionic strength increase[21]. In the preparation of the sample the pH of the mobile phase is an important variable, since the analyte of interest may be totally or partially solubilized[23].

The aim of this work was *(i)* to use ion chromatography to quantify minerals (sodium, potassium, magnesium, calcium, chloride, phosphate, nitrate and sulfate), organic acids (lactic acid and citrate) and carbohydrates (lactose, sucrose, glucose, fructose and galactose) in bovine, buffalo, goat and human milk, as well as dairy products of bovine origin such as sweet whey, acid whey, isotonic beverage, dairy beverage, yogurt, etc., and *(ii)* to evaluate the effect of slow pasteurization on the nutritional quality of raw milk.

2. Experimental Section

2.1 Materials

Samples of bovine milk were donated by dairy FUNARBE, Viçosa, MG, and samples of goat milk were granted by the goat sector of the Federal University of Viçosa, UFV. The buffalo milk samples were collected from a rural property located in Piranga, MG. The samples of human milk were made available by the Human Milk Bank (HMB) of Hospital São Sebastião, Viçosa.

Dairy products such as cream (intermediate cream of butter production), butter with salt, curd cheese, dulce de leche, whole yoghurt without added fruit, sweet whey (derived from the production of mozzarella) and acid whey (by-

product of curd cheese production) were donated by FUNARBE dairy. The isotonic beverage formulated with whey was granted by the Department of Food Technology, UFV. Whole milk and dairy beverage, both Ultra High Temperature (UHT) heat treatment, were purchased from the Quatá brand. For better exposure of the data, the samples were divided into two dairy groups. The first referred to bovine, buffalo, goat and human milk. The second group was composed of the other dairy products, as can be seen in Table 1:

Table 1. Dairy disposition according to presentation and discussion of the macronutrient profile.

Group 1 - milk	Group 2 - other dairies
Raw bovine	UHT bovine milk
LTLT bovine	Sweet whey
HTST bovine	Acid whey
Raw buffalo	Isotonic
LTLT buffalo	Dairy beverage
Raw goat	Yogurt
LTLT goat	Cream
Raw human	Butter
LTLT human	Mozzarella
	Curd cheese
	Dulce de leche

Reagents: All of the following analytical grade reagents (Sigma-Aldrich Co, Saint Louis, MO, USA) were used: lactic acid (69778), nitric acid (84378), sulfuric acid (320501), sodium carbonate (223484), sodium phosphate (96068), calcium chloride (C4901), lithium chloride (310468), magnesium chloride (M8266), potassium phosphate (P5629), fructose (F0127), galactose (G0750), glucose (G8270), sodium (71690), lactose (L3750), sodium nitrate (15736), sucrose (S9378) and sodium sulfate (239313).

Equipment: The following equipments were used: ion chromatograph (Metrohm, 850, Switzerland), auto injector (Metrohm, 919, Switzerland), amperometric pulse detector (Metrohm 896, Switzerland) (Tecnal, TE-0851, Brazil), centrifuge (Eppendorf, 5430, Germany) and thermostatic bath (Tecnal, TE-184, Brazil).

Pre-treatment of material: The milk of the species in question was evaluated *in natura* and after low temperature long time (LTLT), at 63 ± 1 ºC / 30 min. The bovine milk,

however, was also analyzed after high temperature short time (HTST) at 72 ± 1 º C / 15 s. The bovine milk purchased from the Quatá brand was heat treated in Ultra High Temperature (UHT). The remaining dairy products were used without the need for pretreatment. In the Chapter 2, item 2.1, addresses in a more specific way the obtaining and the equipment used for pasteurization of the milk.

2.2 Methods

Sample preparation for bovine, buffalo, goat and human milk, sweet and acid whey, isotonic beverage, dairy beverage and yoghurt

Quantification of minerals and organic acids: dilutions of 1:100 using ultrapure water obtained by Milli-Q purification system (C79625, Merck KGaA, Darmstadt, Germany) were performed resistivity of 18.2 $m\Omega.cm^{-1}$. The samples were then centrifuged at 7830 g for 15 min to remove the fat from the aqueous phase.

Carbohydrate quantification: dilution of 1:490 using ultrapure water was done and the samples were subsequently centrifuged at 7830 g for 15 min.

At the end of each centrifugation, a 10 mL plastic syringe coupled to a fine needle was used to withdraw the continuous phase from the falcon tube. After collection, to retain protein and remaining fat, the needle was replaced by a hydrophilic cellulose nitrate membrane syringe filter with 0.45 μm pore diameter (Sartorius, 11306-142-G, Goettingen, Germany).

Then the samples were distributed in the self injector of the ion chromatograph, which was dialysed by the equipment (0.2 μm pore), to separate the remaining sample matrix that would be interfering with the chromatographic process. After the separation of the components by the ion chromatograph, it was possible to obtain the concentration values of organic minerals and acids with a dilution of 1:100 (dilution factor equal to 100). Then, to obtain the actual concentration of these components, Equation 1 was used:

$$C = c \times d \quad (1)$$

In witch:

C = Actual sample concentration in mg kg^{-1}; c = Concentration, in mg kg^{-1}, obtained by Magic Net 3.1 software; d = Dilution factor.

For the correct determination of the carbohydrate concentration, Equation 1 with a dilution factor of 490 was used.

Sample preparation for cream, butter, mozzarella, curd cheese and dulce de leche

Quantification of minerals, organic acids and carbohydrates: after 1:100 dilutions were made in ultrapure water, the samples were taken to the heating in a thermostatic bath at 40 ºC / 20 min. Subsequently, the samples were centrifuged (7830 g / 30 min) and filtered with 0.45 μm membrane. Before injecting the solutions of interest, three white samples of ultrapure water were used.

The actual concentration values of minerals, organic acids and carbohydrates for solid samples were obtained through Equation 2:

$$C_a = \frac{v_1}{m_1} \times c \times 100 \quad (2)$$

In which: C_a = Concentration of the analyte in the sample in percent (g 100 g^{-1}); v_1 = Mass of water used for sample dilution, in kg; m_1 = Heavy mass for the dilution of the sample, in mg; c = Concentration of the analyte, obtained by the software, in mg kg^{-1}.

Thus, to obtain the sample in mg kg^{-1}, Equation 3 was used:

$$C_{ra} = C_a \times \frac{10^6 mg}{kg} \quad (3)$$

In which: C_{ra} = Actual concentration of the analyte of interest in the sample, mg kg^{-1}; C_a = Actual concentration of the analyte in the sample, in g 100 g^{-1}.

Operating Parameters of Ion Chromatography

For the quantification of cations: the column Metrosep C 3 and pre-column C4 Guard / 4.0 (Metrohm, Switzerland) of size 150 x 4.0 mm, packaging of silica gel with carboxylic groups were used. The working temperature of the column was at local room temperature (22 ° C), pressure of 5.8 MPa and flow of 0.5 mL min^{-1}. The mobile phase was composed of nitric acid, 5 mmol L^{-1}. For the preparation of all mobile phases, ultrapure water obtained by the Milli-Q purification system was used.

For anions: the column Metrosep A Supp 7 and pre-column A Supp 4/5 Guard (Metrohm, Switzerland) of 250 x 4.0 mm, polyvinyl alcohol packaging with quaternary ammonium groups were used. The working temperature was 45 ° C, pressure of 11,0 MPa and flow of 0.7 mL min^{-1}. The mobile phase was sodium carbonate, 3.6 mmol L^{-1}, and the suppression solution was sulfuric acid, 500 mmol L^{-1}.

For organic acids: the column Metrosep Organic Acids and pre-column Metrosep Organic Acids Guard / 4.6 (Metrohm, Switzerland), 250 x 7.8 mm, polystyrene-divinylbenzene copolymer packaging with sulfonic acid groups were used. The working temperature was 32 ° C, pressure of 4.84 MPa and flow of 0.5 mL min^{-1}. The mobile phase was composed of sulfuric acid, 0.5 mmol L^{-1}, and the suppression solution, by lithium chloride, 40 mmol L^{-1}.

For carbohydrates: the Metrosep Carb 2 column and pre-Carb 1 Guard 14.0 column (Metrohm, Switzerland), 150 x 4.0 mm, polystyrene-divinylbenzene copolymer packaging with quaternary ammonium groups were used. The working temperature was 32 ° C, pressure of 9.85 MPa and flow of 0.5 mL min^{-1}. The mobile phase was 0.200 mol L^{-1} sodium hydroxide.

Analytical curve: It was prepared with Sigma-Aldrich analytical grade reagents and ultrapure water was used for the solutions and dilutions. The analytical curve of cations and anions, organic acids and carbohydrates was composed of seven concentrations, using their respective standards. For each curve, the concentration ranged from 1 to 500 mg L^{-1}. Before starting calibration with the standards for the analytical curve, three "white" samples were injected into the ion chromatograph to make sure that the equipment contained no residual impurities.

Limits of detection and quantification: The detection limit (LD) represents the lowest concentration of the test substance that can be detected, but not necessarily quantified. The LD can be calculated in three different ways: visual method, signal-to-noise ratio method and the analytical curve-based method[24, 25, 26] which was used in this research, calculated by Equation 4:

$$LD = 3\frac{s}{S} \quad (4)$$

In which *s* is the standard deviation of the analyte concentration composing the analytical curve and *S* is the slope (angular coefficient) of the curve.

According to Ribani et al.[25], the quantification limit (LQ) represents the lowest concentration of the analyte that can be reliably measured. Like LD, LQ is expressed as a concentration. For this calculation, Equation 5 was used:

$$LQ = 10\frac{s}{S} \quad (5)$$

In which *s* is the estimated standard deviation of the analyte concentration and *S* is the angular coefficient of the analytical curve[27].

Statistical analysis: All determinations were done with two replicates, each performed in triplicate. All results were presented as mean and standard deviation. For the bovine, goat, buffalo and human milk, Tukey's Variance and Test Analysis was performed at 5% of significance, using Software R.

3. Results and discussion

3.1 Results

Quantification of the milk components previously described is important, as is the reliability of these data. Table 2 shows the retention time (RT), coefficient of determination (r^2), limit of detection (LD) and quantification (LQ) for each analyte of the standard solution of 100 mg L^{-1}. It is possible to observe that the determination coefficients were high, presenting good quality of adjustment of the regression model (r^2> 0.999). The relative standard deviation for all analytes showed a low DP (%), indicating that the dispersion of the points around the analytical curve was low.

The chromatograms of all the products were not inserted in this work, however for demonstration, those referring to the dulce de leche were presented and is in ANNEX.

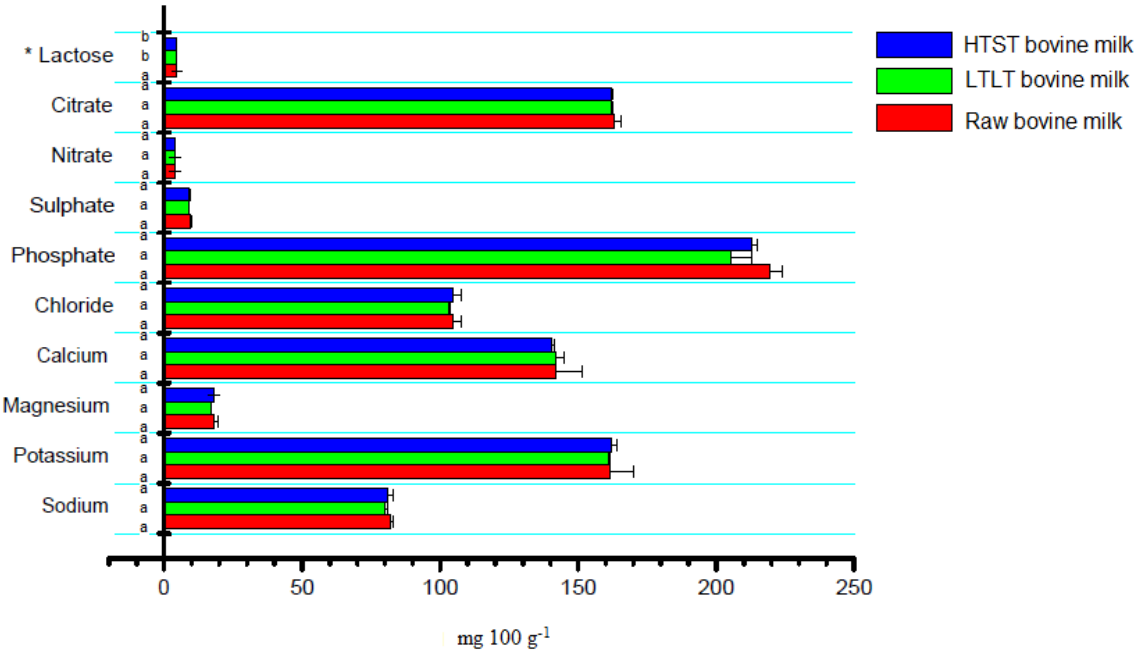
Table 2. Calibration parameters for cations, anions, organic acids and carbohydrates by ion chromatography technique.

	T_R (min.)	**r^2**	**Relative DP (%)**	**LD (mg kg^{-1})**	**LQ (mg kg^{-1})**
Sodium	5,04	0,9999	0,375	0,319	0,966
Potassium	6,72	0,9999	0,248	0,310	0,939
Magnesium	10,19	0,9999	0,514	0,202	0,612
Calcium	12,38	0,9998	0,718	0,854	2,587
Chloride	11,03	0,9997	0,803	0,275	0,833
Nitrate	20,34	0,9995	1,125	1,587	4,809
Phosphate	27,93	0,9996	0,776	0,828	2,509
Sulfate	30,74	0,9999	0,331	0,121	0,366
Lactic acid	13,87	0,9997	0,976	0,889	2,693

Citrate	9,54	0,9998	1,649	1,211	3,669
Glucose	11,06	0,9999	0,481	1,482	4,490
Galactose	11,66	0,9997	1,108	1,614	3,357
Fructose	13,22	0,9996	1,981	0,917	2,778
Lactose	18,85	0,9998	0,710	1,311	3,972
Sucrose	22,05	0,9997	1,692	1,019	3,087

Tables 3, 4, 5, 6 and 7 present, respectively, the concentrations (mg 100 g^{-1}) of minerals, organic acids and carbohydrates for bovine, buffalo, goat, human and other dairy products determined by chromatography of ions.

Figure 1. Concentration of minerals, organic acids and lactose for raw bovine milk and after LTLT and HTST pasteurization.

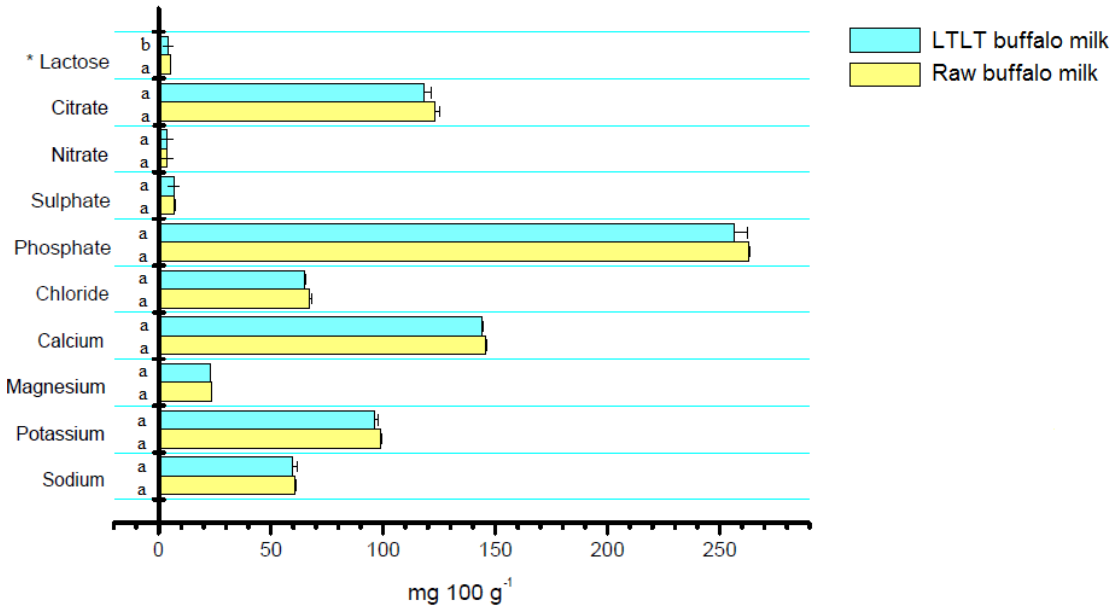


Means followed by the same letters, in the lines, do not differ statistically by the Tukey test, at 5% of significance. (*) value divided by 1000.

In all types of milk there was a significant difference ($p < 0.05$) between the lactose content in the *in natura* milk and LTLT milk. However, according to the Figure 1, the LTLT and HTST milk presented variation when compared to the *in natura* bovine milk. However, when LTLT and HTST milk were compared, there was no significant variation ($p > 0.05$).

There was no significant variation in the phosphate and calcium amounts between the raw, LTLT and HTST milk, evidencing that the binomial time and temperature of pasteurization was not enough to cause changes in the saline equilibrium of the milk samples. The same fact was observed for the buffalo, goat and human milk.

Figure 2. Concentrations obtained for cations, anions, organic acids and lactose in raw buffalo milk and LTLT pasteurization.



Means followed by the same letters, in the lines, do not differ statistically by the Tukey test, at 5% of significance. (*) value divided by 1000.

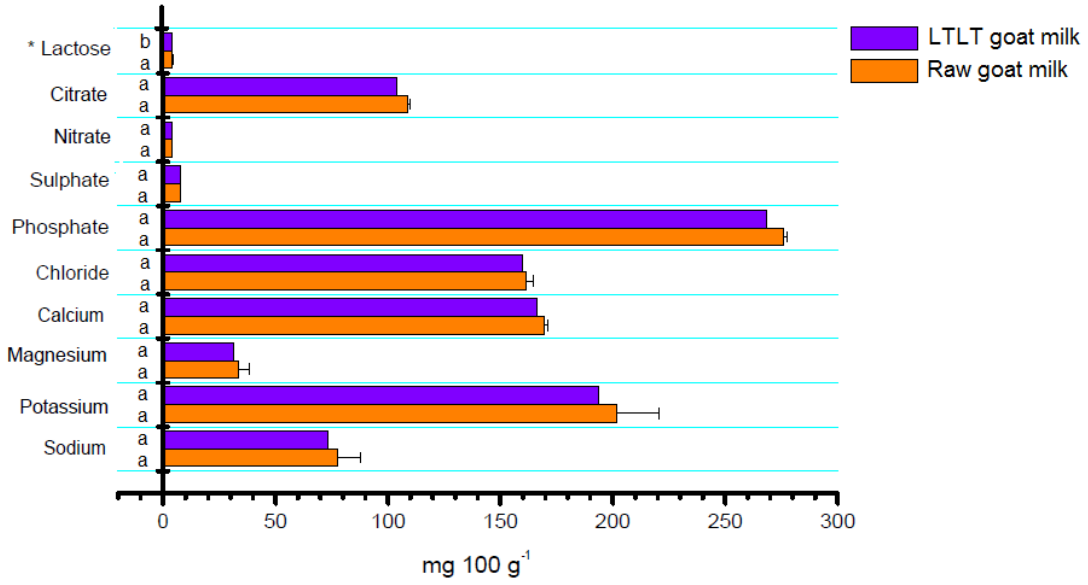
Values of 3.8 mg 100 g^{-1} of nitrate were obtained for raw and LTLT bovine, buffalo, goat and human milk, with no significant difference between them. In Food, Acceptable Daily Intake (ADI) for nitrate is at most 3.7 mg kg^{-1} body weight[28], so that a person weighing 80 kg can ingest up to 296 mg of nitrate daily. If this person takes 1 cup of 200 mL of bovine milk per day, he will have ingested about 2.612 % of the acceptable amount of nitrate. This percentage may seem little, but remember that milk can be consumed in different ways, such as breads, cakes, sweets, cheeses, yogurts, etc. Thus, the amount at the end of the day can reach close to or even exceed the ADI of NO_3^-.

Milk contains nitrate salts in its composition, since they are naturally present in the environment, water and food of plant origin, or are added intentionally during the processing of food of animal origin. In the dairy industry, nitrate salts are used in order to prevent deformation, alteration of flavor and consequently economic damage to the industry due to late cheese stuffing[29].

Due to the use of nitrate salt in the elaboration of these products, the Ministry of Agriculture, Livestock and Supply[30] established the maximum limit for this cheese compound, which should not exceed 50 mg kg^{-1}. However, according to the Figure 2, the concentration of NO_3^- in the mozzarella sample was 252.81 mg kg^{-1} of the product, five times the maximum stipulated for nitrate in cheeses. In similar studies, Pimentel et al.[31]

found high nitrate values in all the analyzed samples (grated cheese samples), with average contents ranging from 5.11 to 159.1 mg kg^{-1}. There is no current legislation on the maximum allowable nitrate in milk.

Figure 3. Concentrations obtained for cations, anions, organic acids and carbohydrates in goat's milk in natura and with LTLT treatment.



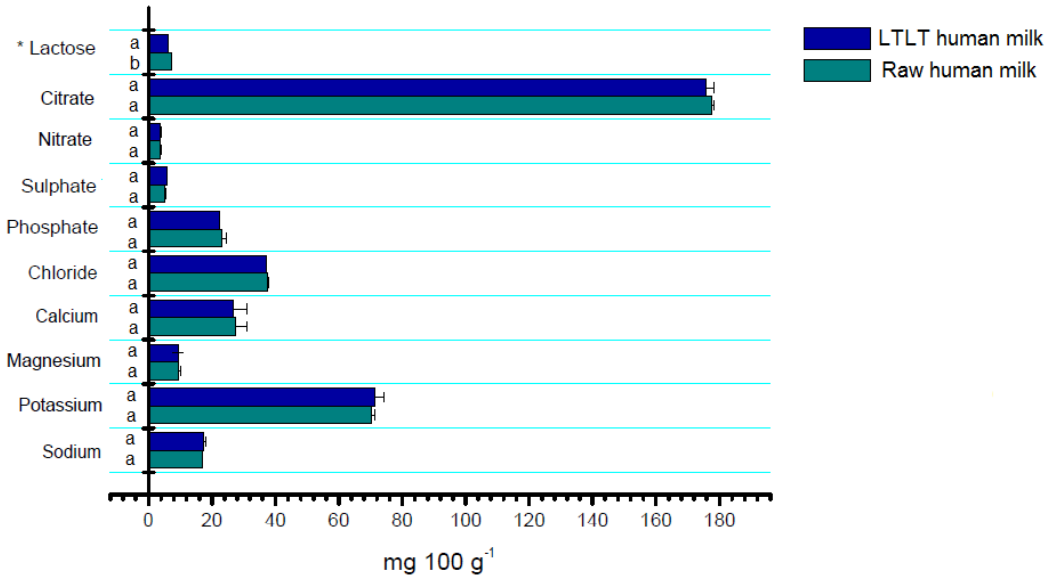
Means followed by the same letters, in the lines, do not differ statistically by the Tukey test, at 5% of significance. (*) value divided by 1000.

There was no significant variation for bovine, buffalo, goat and human raw milk compared to LTLT and HTST treatments for sodium, potassium, chloride, magnesium and sulfate ($p > 0.05$). These minerals, which are soluble in the aqueous phase, do not appear to migrate to the micellar phase as occurs with calcium phosphate, which has decreased solubility with increasing temperature[32, 33, 34, 35, 36, 37].

Soluble salts are present in ionic forms, complex and non-ionizable complexes. Sodium and potassium are totally present as cations. Similarly, chloride and sulfate, which come from strong acids, are present as anions in the natural pH of milk (6,6-6,8). The salts of weak acids (phosphates and citrates) are distributed among various ionic forms. A small part of sodium and potassium is associated with citrate, inorganic phosphate and chloride to form salts in the aqueous phase. According to Gaucheron[38], magnesium, sodium, potassium, chloride, sulfate and citrate are in proportions of 70 %, 93 %, 98 %, 100 %, 100 % and 90 % respectively as anions or salts in the aqueous phase and the UHT

treatment does not appear to have salt balance for these components of milk. According to Fox et al.[39] the heat treatment less pronouncedly affects the salts, with the exception of the calcium phosphate salts, which were discussed separately. Cilliers et al.[40] reported no significant differences in most mineral macros, such as calcium and sodium in HTST pasteurized milk.

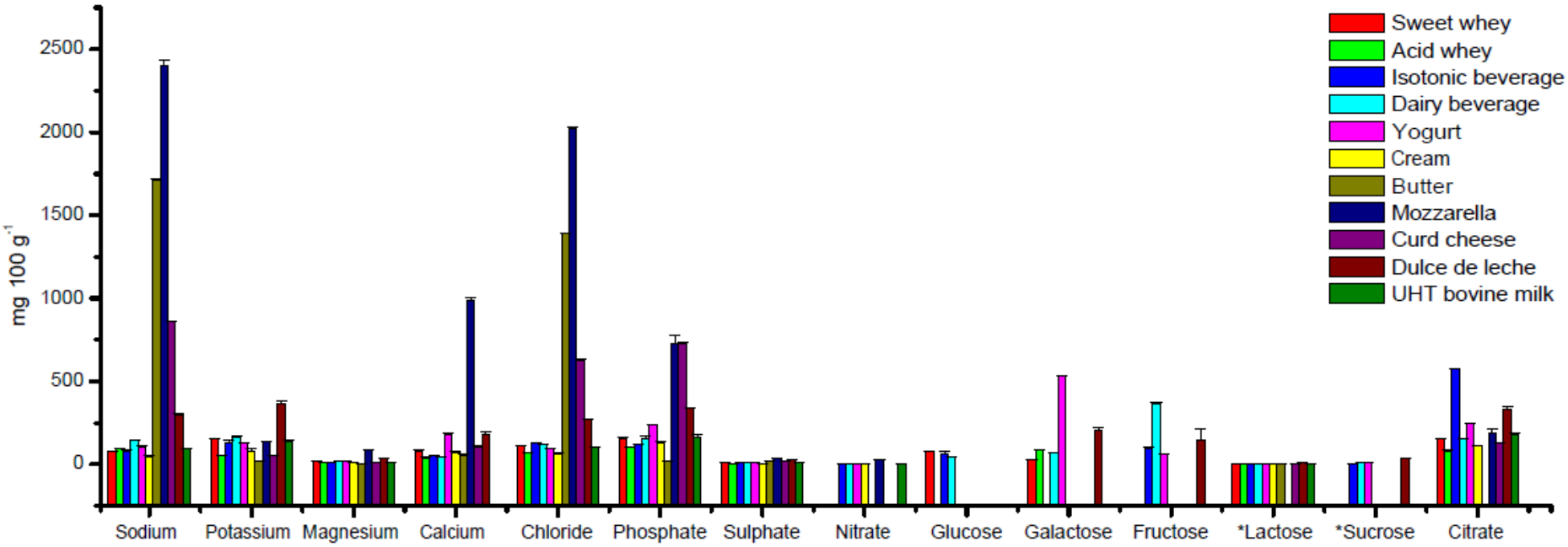
Figure 4. Concentrations obtained for cations, anions, organic acids and carbohydrates in human milk *in natura* and with LTLT treatment.



Means followed by the same letters, in the lines, do not differ statistically by the Tukey test, at 5% of significance. (*) value divided by 1000.

During the heat treatment of lactose, lactose can decompose giving rise to acidic compounds (acetic, levulic, formic, pyruvic acids), hydroxymethyl furfural, aldehydes, alcohols and reductones. Also, by combining heating with the acid medium, this disaccharide can decompose into its monomers galactose and glucose. However, only the heat treatment is not enough for this process to occur[39]. It was observed that there was no milk species that showed concentrations of galactose and glucose, indicating that lactose hydrolysis did not occur for these milk analyzed.

Figure 5. Composition in minerals, organic acids and carbohydrates for different dairy products.



(*) value divided by 1000.

Lactose is 9.4 times less sweet than sucrose, but when hydrolyzed to glucose and galactose, the monosaccharide combination results in only 1.5 times less sweetness than sucrose[41]. To contribute to the overall sweetness, products such as dairy, isotonic, yogurt and dulce de leche had the addition of sucrose in the process of elaboration, increasing the taste and the acceptability of these products. As with lactose, a part of the sucrose may degrade in its constituent monomers (fructose and glucose). It is thus understood that glucose can be derived from the decomposition of lactose and sucrose. Figure 5 shows that glucose was found in sweet whey, isotonic and milk beverages, while the fructose was quantified in isotonic and dairy beverage, yogurt and dulce de leche. Already galactose was quantified in sweet whey, acid whey, dairy beverage, yogurt and dulce de leche.

UHT milk has been added as a product and is not in the Milk Table to come from any source other than that which originated raw milk, LTLT and HTST. Studies involving raw milk and UHT milk from the same source indicated that the calcium phosphate concentration decreases considerably with the heat treatment[42].

One possible explanation is that this decrease of calcium phosphate in UHT milk[42] occurred due to the severity of this heat treatment. The inorganic phosphate present in the aqueous phase of milk as $H_2PO_4^-$ (dihydrogen phosphate) became less soluble and dissociated into HPO_4^{2-} (hydrogen phosphate)[38, 43]. Thus, HPO_4^{2-} was associated with calcium by the higher affinity to this ion than the $H_2PO_4^-$ form, so that the inorganic calcium phosphate increased in the aqueous phase. The supersaturation of this salt in the continuous phase caused it to migrate to the micellar phase. Thus, at high pasteurization temperatures (highter than 100 °C), the amount of calcium phosphate associated with casein micelles increased significantly, with the reduction of soluble inorganic calcium and phosphorus contents in the aqueous phase[36]. At these temperatures, the changes may be irreversible for the colloidal calcium structure[38, 44].

After severe heat treatment, the heat-induced colloidal calcium phosphate becomes likely insoluble, but some amount of native colloidal calcium phosphate dissolves in the cooling to partially restore the pH and balance of the salts[45, 44, 39].

In previous studies, when comparing raw milk and UHT milk, a significant difference ($p < 0.05$) in the citrate concentration was observed. The practice of citrate addition in the UHT milk processing industry is customary. Sodium citrate is one of the main salts used

as chelators. It is used with the aim of improving thermal stability and increasing the period of storage of dairy products[43].

Accordingly, MAPA, by Ordinance No. 370, of September 4th, 1997, decided to approve the inclusion of sodium citrate in the Technical Regulation for Identity and Quality of UHT Milk (UAT) in concentrations not exceeding 1 mg mL^{-1} for that the product maintains its quality and shelf life of four months[46]. In the present study, the concentration found for UHT milk was 1,831 mg mL^{-1}, but it should be kept in mind that fresh milk already has a certain amount of citrate and UHT milk was only quantified after ultra pasteurization. Therefore, it can not be ascertained to what extent the amount of sodium citrate added to the milk.

Minerals in dairy products:

Figure 6 shows the concentrations of sodium, potassium, magnesium, calcium and phosphate minerals for most of the dairy products of this current research, as well as other references.

Figure 6. Macrominerals and techniques used in this research compared to other authors.

Product (mg 100g^{-1})	References	Na^+	K^+	Mg^{2+}	Ca^{2+}	PO_4^{3-}	Applied technique
Raw bovine milk	Current research	94,0	140,0	12,0	74,0	167,0	IC
	USDA, 2019[47]	49,0	151,0	13,0	119,0	285,2	AAS/ES
	TACO[50]	64,0	133,0	10,0	108,0	251,5	ICP-OES
Raw buffalo milk	Current research	60,0	98,0	23,0	145,5	273,0	IC
	USDA, 2019[47]	52,0	178,0	31,0	169,0	358,7	AAS/ES
	TACO[50]	53,0	182,0	31,5	180,0	368,0	ICP-OES
Raw caprine milk	Current research	77,0	201,0	34,0	170,0	276,0	IC
	USDA, 2019[47]	50,0	204,0	14,0	134,0	340,3	AAS/ES
	TACO[50]	59,0	131,0	13,5	116,0	325,0	ICP-OES
Raw human milk	Current research	16,0	70,0	9,5	27,0	23,0	IC
	USDA, 2019[47]	17,0	51,0	3,0	32,0	43,0	AAS/ES
	TACO[50]	25,0	43,0	2,5	23,0	44,5	ICP-OES
Yogurt	Current research	107,0	130,0	18,0	182,0	239,0	IC
	Kira & Maihara, 2007[48]	33,0	139,6	8,18	93,4	212,2	ICP-OES
	TACO[50]	45,5	116,0	8,0	93,5	155,5	ICP-OES
Dairy beverage	Current research	144,0	167,0	21,0	43,0	158,0	IC
	Kira & Maihara, 2007[48]	66,1	175,8	15,1	71,5	196,2	ICP-OES
	TACO[50]	46,0	62,0	8,0	88,0	193,0	ICP-OES
Butter	Current research	1709,0	22,0	1,0	56,0	22,0	IC
	USDA, 2019[47]	643,0	24,0	2,0	24,0	73,5	AAS/ES
	TACO[50]	579,0	15,0	1,5	9,5	85,0	ICP-OES
Dulce de leche	Current research	298,0	366,0	34,0	179,0	339,0	IC
	USDA, 2019[47]	129,0	350,0	22,0	251,0	193,0	AAS/ES
	TACO[50]	121,0	260,0	16,5	196,0	432,5	ICP-OES
Curd cheese	Current research	858,0	51,0	8,5	107,0	728,0	IC
	Gonzalez, 2014	441,8	96,3	14,6	298,4	948,5	IC
	TACO[50]	558,0	93,0	11,0	284,0	1374,0	ICP-OES
Sweet whey	Current research	78,0	153,0	19,0	82,0	157,0	IC
	USDA, 2019[47]	54	161,0	8,0	47,0	141,0	AAS/ES
	TACO[50]	-	-	-	-	-	-
Acid whey	Current research	94,0	50,0	10,0	40,0	99,0	IC
	USDA, 2019[47]	48,0	143,0	10,0	103,0	78,0	AAS/ES
	TACO[50]	-	-	-	-	-	-
Mozzarella	Current research	2403,0	134,0	89,0	990,0	727,0	IC
	Kira & Maihara, 2007[49]	446,2	74,6	22,0	700,4	471,1	ICP-OES
	TACO[50]	507,0	68,5	23,0	775,0	1423,0	ICP-OES

According to USDA[47], for the minerals calcium, magnesium and phosphorus, they were analyzed by atomic absorption spectroscopy (AAS), and potassium and sodium by emission spectrometry (ES). According to Kira & Maihara[48] and Kira & Maihara[49], the minerals were analyzed by Inductively Coupled Atomic Emission Spectrometry (ICP-OES).

The reference values of the Brazilian Table of Food Composition[50] are not available for sweet whey and acid whey. Figure 5 shows that sodium concentrations found in all dairy

products with the exception of human milk were higher than those reported by other authors[50, 47]. The mozzarella, however, had the highest increase compared to the others: 4.73 times greater than the concentration reported by TACO. The increase of sodium in food serves as an alert for the population and for the food industry, since the consumption of this mineral higher than the Recommended Daily Intake[30] (RDI), which is 2400 mg day^{-1}, may health risks, especially with chronic kidney diseases (CKD). Some of the main causes of CKD include hypertension and diabetes mellitus[51].

Observing the other results in the Figure 6, it is possible to notice that the concentrations of the minerals differed significantly in relation to the quantities obtained by the other references. Some variables can explain these differences, starting with obtaining the raw material. Milk of the same species, as already mentioned, can be differentiated due to several factors (feeding, stress, age, diseases, lactation period, etc.). The techniques of elaboration of these dairy products can also differ from those in which the products evaluated in this work were produced. For example, for the production of curd cheese, the industry can obtain the coagulation in two ways: enzymatic, using a process similar to that of the mozzarella cheese or acid, through yeast. The techniques of preparation and quantification of the sample may also show significant differences in relation to each other, so that, when encountering the concentration of the mineral in the dairy, very different values are found that are sought to compare.

3. Conclusion

The heat treatment was able to significantly alter ($p < 0.05$) the concentration of lactose in bovine, buffalo, goat and human milk when compared to raw and after LTLT pasteurization. Furthermore, it was possible to verify that there was no significant variation ($p > 0.05$) between LTLT and HTST pasteurisation for bovine milk. It was found that sodium, potassium, magnesium, chloride and sulphate did not present significant differences ($p > 0.05$) according to the severity of the heat treatment for the milk, corroborating with several studies that show that these minerals maintain their solubility against heat, unlike the calcium phosphate salt, which decreases the solubility with increasing temperature. It was found that the amount of nitrate in poplar exceeded five times the maximum limit allowed for this cheese preservative. Regarding the other products, it was verified that for all dairy products, except for human milk, the sodium value obtained in this study was higher than that reported by the TACO Table. The most remarkable value was observed in the mozzarella, which had 4.73 times more sodium. The ion exchange chromatography technique proved to be advantageous over the others presented in this study, so that it was possible to quantify various minerals, organic acids and carbohydrates in several dairy products using a relatively simple and unique preparation for all types of samples. practical way and in an automated equipment that presents reliability in its results.

ANNEX

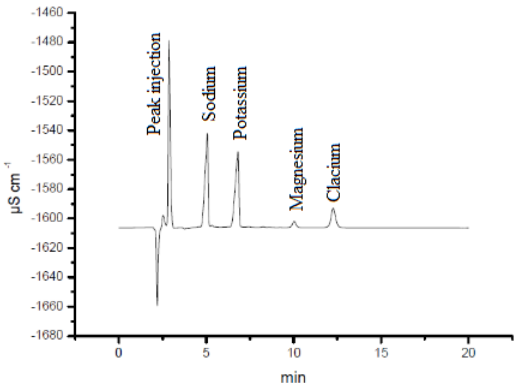


Figure 1. Chromatogram of cations for sweet milk.

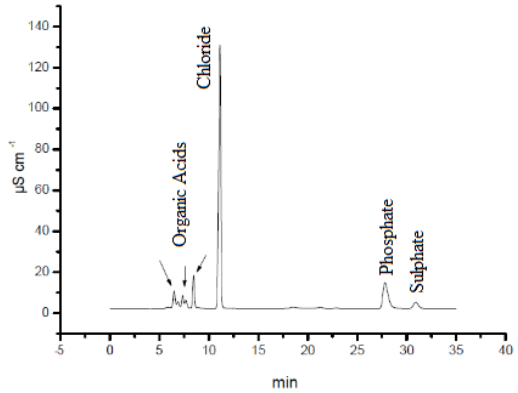


Figure 2. Chromatogram of anions for sweet milk.

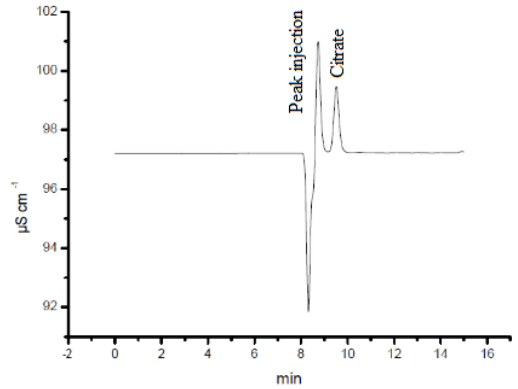


Figure 3. Chromatogram of organic acids for sweet milk.

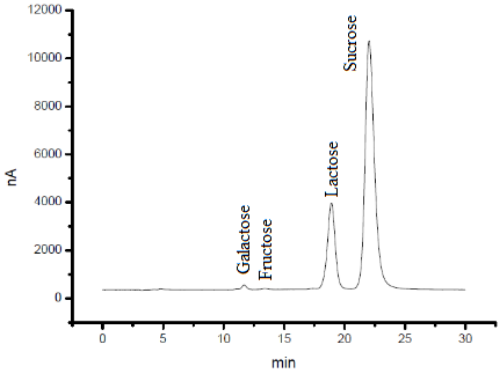


Figure 4. Chromatogram of carbohydrates for sweet milk.

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CONCLUSÃO GERAL

De modo geral, os leites de vaca, búfala, cabra e de mulher apresentaram algumas diferenças significativas em relação à composição química. O leite bubalino se destacou como tendo maior teor em proteína e gordura. Já o leite humano apresentou maior concentração em carboidratos e menor teor em cinzas. O leite caprino apresentou maior quantidade de cinzas e, para proteína, gordura, umidade e carboidratos se assemelhou ao leite bovino. Alguns fatores como raça, idade, período de lactação, doenças, alimentação, dentre outros, podem influenciar nestes resultados. Quanto aos leites *in natura* e LTLT de cada espécie avaliada nesta pesquisa, não foram observadas alterações significativas para o conteúdo de gordura, proteína, umidade, carboidrato e cinzas. Porém, as análises cromatográficas de lactose para os leites bovino, bubalino, caprino e humano indicaram que esse carboidrato sofreu variações significativas com o tratamento LTLT em todas as espécies de leite. Em leites pasteurizados LTLT sem passar pelo processo de homogeneização, entretanto na temperatura de pasteurização (63º / 30 min.), os grânulos formados não conseguem provocar uma desestabilização notável das proteínas do leite e também não levam à coagulação. A homogeneização que ocorreu apenas para o leite bovino pasteurizado mostrou que os glóbulos de gordura desse produto são menores e mais regulares, o que diminui a coalescência neste leite. Minerais como sódio, cloreto, potássio, magnésio, sulfato e nitrato não variaram suas concentrações de forma significativa em relação à pasteurização LTLT, indicando que a solubilidade desses minerais não diminui com o aumento da temperatura, como ocorre para o fosfato de cálcio. Os demais lácteos tiveram seus resultados confrontados com os obtidos por outros autores. O mineral sódio foi o elemento que mais ultrapassou os valores de referência. Para doce de leite esse aumento excedeu em 246,30 %; para manteiga, 295,2 %; bebida láctea ultrapassou em 313 % e muçarela, o aumento foi de 474 % em relação aos valores da Tabela Brasileira de Composição de Alimentos.