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CHAPTER 31

Analysis of lactose content and fat content in "zero lactose" and "light" dairy products

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ABSTRACT

Milk and its derivatives are considered complete foods and the greatest source of calcium that can be absorbed by man. However, a large percentage of people cannot consume this food due to lactose intolerance. Because of this factor, they tend to opt for products labeled "zero lactose", however, some products do not follow the determination of the legislation for lactose content, compromising the health of consumers who trust these brands. The same practice is carried out in products labeled "light", where the consumer chooses the product in the hope that there will be a reduced fat content compared to its original version and finds products totally outside the parameters determined in Brazilian legislation and once again putting the consumer's health at risk. In this work, the lactose content in milks labeled "zero lactose" was analyzed, in addition to the fat content in cream cheese labeled "light" and/or "reduced in total fats" produced by three brands and marketed in the region of Alegre-ES and which were in disagreement with the parameters established by the legislation.

Keywords: Milk, Lactase, Intolerance, Obesity, Cottage Cheese, Legislation.

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INTRODUCTION

The milk and dairy products production chain in Brazil is a sector of great economic and social importance. It is the third largest milk producer in the world, with more than 34 billion liters per year, with production in 98% of Brazilian municipalities, with a predominance of small and medium-sized properties, employing close to 4 million people. The country has more than 1 million milk-producing properties and the projections are that, by 2030, the most efficient producers will remain, who adapt to the new reality of technology adoption, improvements in management and greater technical and economic efficiency.

Milk is a stable, complex emulsion rich in nutrients such as fat, protein, lactose, vitamins, minerals and other ingredients suspended in water (MILK; SIQUEIRA; CARVALHO, 2009).

Lactose is the main carbohydrate in milk, and this and its whey are actually the only sources and is made up of two monosaccharides, glucose and galactose, characterizing a disaccharide. About 40 to 50% of milk is made up of lactose, while whey is made up of 75%. However, the use of lactose in dairy products is limited due to its low sweetening capacity, low solubility and individual food intolerance to this sugar (PESSELA *et al*., 2003).

Lactose intolerance occurs due to a lack of the enzyme β-galactosidase or lactase in the small intestine with the ability to hydrolyze lactose into glucose and galactose and it is estimated that approximately 65% of the population has a reduced ability to digest lactose after childhood, and symptoms and signs persist throughout life (HEYMAN, 2006; PEREIRA *et al*., 2012).

As a result, lactose is not absorbed in the small intestine and when it reaches the colon, fermentation occurs with the production of short-chain fatty acids and the formation of gases such as carbon dioxide, hydrogen and methane through the action of microorganisms in the colonic region and can cause abdominal pain in addition to discomfort such as nausea, colic, flatulence, diarrhea, bloating (MATTAR and MAZO, 2010; FRYE , 2002).

Lactose hydrolysis can lead to the development of new products for consumers intolerant to this carbohydrate, in addition to providing technical advantages such as the reduction of lactose crystals in dairy products and increased sweetness power, demonstrating that lactose hydrolysis is a promising industrial process and can occur by acid or enzymatic route, the enzymatic pathway being the most favorable, as it occurs under mild pH and temperature conditions (30 ºC to 40 ºC), unlike acid hydrolysis with strong acids.

β-galactosidase in free or immobilized form is a commercially important enzyme in this process (OBÓN *et al*., 2000). Currently, there are several products on the market with reduced lactose content, classified by ANVISA (National Health Surveillance Agency) according to lactose concentration. To obtain these products, different processes are used that use enzymatic hydrolysis, separation processes or a combination of these methods. These methods hold promise for the food

industry due to the low availability of lactose and the increase in lactose intolerance in the world's population.

In view of the above, the objective of this study was to evaluate the content of these constituents in dairy products called "light" and "zero lactose" sold in supermarkets in the city of Alegre-ES, in order to verify if these products follow the values described on their packaging and if the results obtained are consistent with the Brazilian legislation in force.

LITERATURE REVIEW

MILK

Milk is considered one of the most complete foods because it is rich in proteins, carbohydrates and mineral salts, as well as one of the sources of absorbable calcium for the human species, in addition to containing conjugated linoleic acid, β-carotene, butyric acid, in addition to vitamins A and D.

The relationship between its different components is very stable and can be used to indicate if there has been any tampering with its components. Under normal conditions it contains approximately 87.5% water, 3.8% fat, 4.6% lactose, 3.1% protein and 0.8% minerals/vitamins. The main physicochemical parameters that determine milk quality are: pH (20 ºC) from 6.5 to 6.7; titratable acidity from 15 ºD to 18 ºD; density from 1.028 g/mL to 1.034 g/mL; fat 3 g/100 g and freezing temperature between -0.510 ºC and -0.550 ºC (BRASIL, 2008).

Despite high rates, government economic plans and deregulation of the economy, milk production in the last 50 years has had an exponential and significant growth (VILELA *et al*., 2017). The largest milk-producing region in Brazil is the southeast region, which produced about 9,995.024 thousand liters in 2020, contributing with 39.2% of the national production. The southern region occupies the second position, with its share of around 37.9% of cow's milk production in Brazil (CONAB, 2021). Brazil is the world's third largest producer of cow's milk, and its share in world dairy production is only 4.4%, behind countries such as the European Union, which has a share of around 29.3%, the United States with 19%, India with 17.8%, among others (CONAB, 2021).

LEGISLATION

Normative Instruction No. 76, of November 26, 2018 of the Ministry of Agriculture, Livestock and Supply - MAPA (BRASIL, 2018) is the one in force in the country. It contains some technical parameters that must be followed in order to improve the quality of the milk, such as conservation, sensory, physicochemical and microbiological parameters.

According to the current legislation, Normative Instruction 76 (BRASIL, 2018), pasteurized milk defined as type A can be classified in terms of fat content as whole, semi-skimmed and skimmed, and must be automatically packaged in a closed circuit.

In this same NI, there are several physicochemical parameters to be followed for pasteurized whole milk, such as the minimum required fat content of 3.0 g/100 g, and the anhydrous lactose content must be 4.3 g/100 g of milk sample. In pasteurized skimmed milk, it is determined that the fat of the same has a maximum of 0.5 g/100 g. These parameters determined by the Ministry of Agriculture, Livestock and Supply aim to maintain a standard of quality of dairy products.

The Technical Regulation on Identity and Quality of Milk UAT (BRASIL, 1997a) points out other parameters to be followed to ensure the quality of milk, its titratable acidity must be between 0.14 and 0.18, minimum protein content of 2.8 g/100 g and must be refrigerated up to a maximum of 4 °C after pasteurization, in addition, for UAT milk there is only the microbiological parameter of mesophilic aerobes.

CREAM CHEESE

Milk and its derivatives are a group of foods that have proteins of high biological value and essential nutrients such as calcium, phosphorus, vitamins A, D, B2, biotin and other components (PHILIPPI, 2014). There are numerous dairy derivatives and among them is cream cheese, which is a type of processed cheese, widely consumed in our country, being manufactured throughout the national territory, but with variations in technology and characteristics (SILVA *et al*., 2012).

According to Ordinance No. 359, of September 4, 1997 of MAPA (BRASIL, 1997b, p.1), "Cream cheese is that obtained by melting a drained and washed curd mass, obtained by acid and/or enzymatic coagulation of milk, with the addition of cream and/or butter and/or anhydrous milk fat or butteroil".

Studies report that cottage cheese appeared at a time when the most valued milk derivative was butter. At that time, cottage cheese was manufactured from skim milk and through the use of milk that coagulated due to the action of the milk's natural lactic microbiota (MUNCK; CAMPOS, 1984).

At that time, artisanal factories had creamers that separated the cream, and the resulting skimmed milk was allowed to coagulate naturally to obtain the dough that would later be transformed into cottage cheese (MUNCK; CAMPOS, 1984). Today, the food industry obtains cottage cheese through acid and/or enzymatic coagulation of milk (BRASIL, 1997b).

The Technical Regulation for fixing the identity and quality of cottage cheese (Ordinance No. 359, of 09/04/1997, p. 24), calls cottage cheese the product in which the dairy base does not contain fat and/or protein of non-dairy origin.

Currently, there are products available in the retail market analogous to traditional cream cheese, called "cream cheese with starch" and "cream cheese with starch and vegetable fat". However, the analogues found on the market have a wide variety of composition and functional properties, and the sensory quality characteristics are very different from the original product, which has caused dissatisfaction among consumers (CUNHA; DAYS; VIOTTO, 2010).

One of the food substances used in the manufacture of analogous cheeses is starch. Starch is a reserve polysaccharide found in most vegetables. It has great application in the food industry, mainly as a thickener and stabilizer (DEMIATE; KONKEL; PEDROSO, 2001).

Until 2005, products added with vegetable fat were called dairy specialties, foods or products based on cottage cheese. After studies, the DILEI/DIPOA (Department of Inspection of Milk and Dairy Products/Department of Inspection of Products of Animal Origin) suspended the approval of dairy specialties and determined that these products should be called "cottage cheese with...", and the name should be complete and highlighted (VAN DENDER, 2006).

Another substance often added in the manufacture of cream cheese is vegetable fat, used to replace milk fat. In a study carried out with different concentrations of vegetable fat, it was observed that the replacement of part of the cream (25% and 50%) by hydrogenated vegetable fat promoted significant changes in texture, sensory properties, color and, mainly, in the microstructure of the processed cheese (CUNHA *et al*., 2010).

Although vegetable fat has certain operational advantages compared to dairy fat, its greatest disadvantage is the loss of sensory attributes (flavor and aroma) in the final product, requiring the complement with flavored bases or condiments flavored cheese, cream and cottage cheese (RODRIGUES, 2006).

FAT PRESENT IN DAIRY PRODUCTS AND PUBLIC HEALTH

With an exponential growth curve, obesity is a public health issue today. Certain surveys indicate that about 50% of the Brazilian population is overweight, and the cause of this increase is multifactorial, and may be linked to genetic and emotional issues, among others (SILVA; ANDRADE; PUCCI, 2022). The weight factor affects other comorbidities such as insulin resistance, arterial hypertension, hyperglycemia and metabolic syndrome (DA COSTA; DE SOUZA; SANCHES, 2020).

The excess fat present in an unbalanced diet can lead an individual to obesity, a disease that is characterized by the accumulation of adipose tissue in the human being that ends up exposing him to serious pathologies leading to the risk of life in addition to the emotional factors already proven such as depression, suffering and social distancing behaviors (ADES; KERBAUY, 2002)

In 17 years, the obese population in Brazil has had an extremely significant increase, from 12.2% in 2002 to 26.8% in 2019 (AGÊNCIA IBGE NOTÍCIAS, 2020), data that end up worrying doctors and nutritionists in the country due to the high danger of this disease that is linked to major chronic non-communicable diseases such as diabetes, cancer and cardiovascular diseases, these diseases have been gaining strength year after year, causing irreversible lesions in the life of the obese (DUNCAN *et al*., 1993).

The World Health Organization (WHO, 2000) states that there is an "epidemic of obesity on a global scale". In the United States, about 25% of children are overweight or obese, a worrying situation for the future considering that an overweight of 5% already exposes a human being to risks.

Milk and dairy products have saturated fatty acids that help raise cholesterol levels in the blood plasma (SCHERR; RIBEIRO, 2011), in addition to having a high caloric load in their composition that, associated with an unbalanced diet, can lead to chronic non-communicable diseases such as those mentioned above.

Currently, dairy products such as cream cheese have about 6.5 g of total fats in their nutritional table, which represents, according to the label, 11% of the daily consumption value in a tablespoon, a value to be rethought since the human being has an average of five to six meals a day, There are high chances that the daily fat values for a normal human being will be exceeded.

LACTOSE INTOLERANCE

Faedo et al. (2013) describe that lactose is used by mammals, being converted into glucose and galactose soon after hydrolysis by the enzyme lactase in the small intestine, enabling intestinal absorption. People with lactase deficiency do not have this ability, and when they consume milk, lactose is not hydrolyzed, leading to increased intestinal osmotic pressure and, consequently, water retention. In these cases, lactose enters the large intestine, where it is fermented by the gas- and water-producing microflora, or hydrolyzed by bacteria into short-chain organic acids. Unabsorbed gas can cause bloating, and the acid produced can irritate the intestinal wall and increase peristalsis, which, combined with water from the intestines, can cause diarrhea.

Woltmann, Simon, and Silveira (2013) emphasize that in most people, lactase levels drop significantly after weaning, meaning that some individuals with very low lactase activity experience undesirable digestive symptoms after milk intake. Unlike other disacrases such as sucrase and maltase, lactase is not an adaptive enzyme, that is, its level does not change based on the amount of carbohydrates ingested. The term hypolactosia refers to decreased lactase activity, which can be a primary or secondary condition. Primary can be of two types, HPTA (primary adult-onset hypolactasia) and congenital hypolactatemia (a rare congenital lactase deficiency). Secondary is due to the involvement of the mucosa of the small intestine in conditions such as acute gastroenteritis,

giardiasis, celiac disease, inflammatory bowel diseases, among others (WOLTMANN, 2013). In Figure 01 below we have two examples: in scheme A the flow of lactose with normal enzyme activity in the small intestine, where the carbohydrate is hydrolyzed into its respective corresponding monosaccharides and absorbed. In regimen B, lactose, which is not hydrolyzed by the enzyme, remains in the intestine. Fermentation occurs generating short-chain fatty acids, carbon dioxide and hydrogen gas, which results in abdominal bloating, flatulence, cramps and diarrhea (WOLTMANN, 2013).

HYDROLYSE THAT LACTOSE

Lactose hydrolysis is a promising process for the food industry, as it makes it possible to develop new products for intolerant consumers, reducing crystallization of this carbohydrate in dairy products and increasing its sweetening power (FISHER, 2010). There are two methods of hydrolysis of lactose: the acid method and the enzymatic method.

Acid method

It reacts very quickly, but involves dilute strong acid solutions, such as sulfuric and hydrochloric acid, pH (1.0 - 2.0) and high temperatures (100 °C to 150 ºC); Therefore, it is a restriction on the food industry, as it causes changes in the flavor and color of food, in addition to causing denaturation of milk proteins.

Enzymatic method

It can be applied to milk or whey without the need for further processing. Hydrolysis is catalyzed by *β-galactosidase* and proceeds under mild pH and temperature (30 °C to 40 °C). This not only reduces changes in heat-sensitive compounds, but also requires lower energy consumption, decreases environmental corrosive effects, and the formation of unwanted by-products. The products obtained through this process maintain their properties, increasing the relative sweetness capacity. Hydrolysis of lactose catalyzed by *β-galactosidase* primarily produces an equimolecular mixture of glucose and galactose, however, galactose can polymerize or bind lactose to form oligosaccharides (HYDE; HUSSAIN, 2009; MAHONEY, 1998)

Enzymatic hydrolysis maintains the desired nutritional and quality properties of the products, as well as adding sweetness to them. Therefore, it can be seen that enzymatic hyrolysis of lactose is more convenient for the food industry (GROSOVÁ; ROSENBERG; REBROTH, 2008). As a result, a great deal of effort has been devoted to the research and development of viable biotechnological processes to obtain low-lactose dairy products.

ENZIMA *B-GALACTOSIDASE*

Segundo Mahoney (2003) a enzima *β-galactosidase* (β-D-galactosideo-galactohidrolase, E.C.3.2.1.23):

> it is usually called lactase since it catalyzes the hydrolysis of lactose into its constituent sugars (PARK; OH, 2010). All enzymes generically referred to as lactases are *β-galactosidases*, but the reverse is not true. Certain *β-galactosidases*, including some plant cells and mammalian organs, other than those in the gut, have low or no lactose hydrolysis activity since their catalytic function is the breakdown of other galactosyl groups, such as glycolipids, glycoproteins and mucopolysaccharides.

According to Neri *et al*., (2009), this enzyme is also responsible for the synthesis of GOS, when there is a high concentration of lactose in the system, through a galactosylation reaction not conducive to hydrolysis. When the concentration of this disaccharide is low, hydrolysis occurs, producing glucose and galactose. *β-galactosidase* is one of the most studied and reported enzymes in the literature. It is found in almonds, apricots, peaches, pears and other plants, in the intestines of mammals, or is produced by a variety of microorganisms (DWEVEDI; KAYASTHA, 2009; MAHONEY, 2003).

METHODOLOGY

SAMPLE COLLECTION

The samples were purchased in local stores in the city of Alegre-ES, available in supermarkets. Three samples of different brands named A, B and C of UHT Semi-skimmed Milk "Lactose-Free" and three samples of different brands of Light Cream Cheese were acquired that were named E, F and G. The samples were taken to the Food Technology Laboratory (LTA) of the Federal University of Espírito Santo, UFES-Alegre, ES where the analyses were performed.

FAT ANALYSIS

Fat determination was performed by the Gerber method, using 3 g of the samples. Each sample was weighed in the butyrometer cup and completed with 5 mL of distilled water, 10 mL of sulfuric acid (H2SO4; density 1.825) and 1 mL of isoamyl alcohol. Subsequently, the butyrometer was placed in a water bath at 65 ºC for 3 min. until the sample is dissolved and centrifuged at 1200 rpm for 10 min. Then, it was completed with distilled water up to a value of approximately 30% in the meter of the butynometer itself to take the reading (BRASIL, 2006).

LACTOSE ANALYSIS

The determination of lactose in the milk sample was made by the determination of reducing sugars expressed in lactose, by oximetry. 10 mL of the homogenized and fluid sample were pipetted

in a 250 mL volumetric flask. Then, 5 mL of potassium ferrocyanide solution (15%) and 5 mL of zinc acetate solution (30%) were added to this sample, shaking and completing the volume of the flask with distilled water. The sample was sedimented for 15 minutes and then taken for filtration in a paper filter. The filtered sample was transferred to a burette. In a 250 mL erlenmeyer solution, 5 mL of Fehling A, 5 mL of Fehling B, 40 mL of water and a small amount of glass pearls were added, and heated until boiling. Then, the sample solution placed in the burette was dripped without agitation until the supernatant fluid was slightly bluish. Subsequently, if the boiling was maintained, 1 drop of methylene blue (1%) was added, continuing titration until the discoloration of the indicator and the appearance of a brick-red color (SILVA *et al*., 2014).

RESULTS AND DISCUSSION

The lactose analysis, as described in the methodology, was performed through oximetry, thus, in the titration of the Fehling solution, 9.6 mL of the standard glucose solution (0.5 g) was spent, which resulted in the Fehling solution titer equal to 0.048.

The milk samples analyzed were named A, B, C and D, and sample D was whole milk, therefore containing lactose, used as a control. All samples were acquired in the local market of the city of Alegre-ES, and were stored at around 2º to 3º degrees Celsius.

The lactose content of each milk sample analyzed can be seen in Table 1, in which it can be observed that the results obtained were above 2% lactose for brands B and C and 4.17% for brand A, all of which were called "Lactose-Free" on their respective labels. Brand D, which had the denomination of "Whole Milk" on its packaging, had an average value of 5.30% lactose.

Table 1 - Lactose content (%) of the four brands of milk analyzed in this study (mean value ± standard deviation).

Milk brands	**Teor de Lactose (%)**
The	4.17 ± 0.25
B	2.43 ± 0.16
C	2.58 ± 0.08
D (Control)	5.30 ± 0.24

Source: The auhor (2022).

The brands A, B and C analyzed are in disagreement with what is determined by the legislation that provides for "zero lactose" products that the content of the same is a maximum of 0.1% (BRASIL, 2017).

In a study carried out by Píccolo et al. (2019), it was also possible to observe that the milks analyzed presented parameters above those established by the current legislation mentioned above.

Milks labeled "lactose-free" had between 4% and 5% lactose in their composition, since whole milk usually has around 4.7% lactose, a fact of concern in relation to consumer health, making it necessary, therefore, to take legal action against the companies that manufacture these products.

Vasconcelos *et al*. (2020) obtained a positive result for lactose in products labeled "zero lactose", in samples purchased in the city of Belo Horizonte-MG, presenting more than 300% above the provisions of the legislation. Alves and Pedroni (2021) demonstrate that the practice of omitting information to the consumer is not limited to UAT milk but also to powdered milk, where in 3 samples of powdered milk and 2 of dairy compound labeled "zero lactose" lactose content was found above the allowed, being in disagreement with RDC No. 135, of 2017 (BRASIL, 2017).

The lactose intolerant population can suffer serious nutritional deficiencies with the removal of milk and dairy products from their diet, foods that are essential for their perfect physiological functioning but which can be a problem with such high levels of lactose in products labeled "zero lactose".

In addition to hypolactsia, already mentioned previously in this study, Oliva and Palma (2001) state that the ingestion of lactose by intolerant individuals can cause aggression to the musoca of the small intestine and can lead to severe diarrhea, which can lead to protein-calorie malnutrition or even death. Moreira, Padovani and Maffei (1996) refer to diarrhea as responsible for high mortality rates in children under 5 years of age in developing countries.

Fat analyses were performed on three brands of cream cheese called E, F and G in the "Wholemeal" and "Reduced in Total Fat and Calories" versions. The conclusion of the experiment leads us to the same type of practice as in the previous analysis, consumer negligence. In Table 2 we can see that the results obtained in this work are contrary to the provisions of the current legislation that determines that this product must have a minimum 25% reduction of its traditional version (IN nº 75 - BRASIL, 2020). It is important to note that the differences presented in the analyses were small and may mean a danger to the population, especially for individuals with certain pathologies who need to reduce fats in their diet.

Table 2 - Fat content (%) of the three brands of cream cheese, in the Integral and Light versions, analyzed in this study (mean value ± standard deviation).

Brands of Cream Cheese	Mean fat value (%)
E (Full)	33.0 ± 0.76
E (Light)	25.7 ± 1.27
F (Integral)	32.0 ± 1.85
F (Light)	24.8 ± 1.12
G (Integral)	34.0 ± 0.53
G (Light)	28.7 ± 0.69

Source: The auhor (2022).

According to Drake and Swanson (1995), cheeses with low fat concentration tend to have a lower yield when compared to whole cheeses, this is because normally the lipid constitutes about 50% of the total dry extract of these dairy products, which in the point of view of the industry can be an economic loss compared to the amount produced of the same product in the whole version.

Another factor that must be considered is the sensory acceptance of this product because the fat gives texture, flavor and a good presentation in food in general, therefore, increasing the lipid content in the mixture so that this type of cottage cheese resembles its original version is the key to sales success and failure in terms of honesty with the consumer.

Gomes and Penna (2010) observed that it is possible to use new techniques to obtain products with reduced fat content, close in sensory terms to products in the whole version. In the aforementioned study, it was possible to make cream cheese a functional product with the use of inulin and soy protein in the dough and, despite some physicochemical and sensory divergences such as moisture, viscosity and brightness, this type of alternative is indeed viable for sale, benefiting the consumer and the trade with new alternatives to conventional products.

CONCLUSION

Samples of milk labeled "zero lactose" and cream cheese labeled "light" and/or "reduced in total fats and calories" were found sold in supermarkets in the city of Alegre-ES and, probably, are also sold in neighboring municipalities, which are in disagreement with the current legislation, leading the consumer to purchase a dairy product that is inappropriate to what is recommended on the label and to its purpose of consumption.

There has been an increase in consumer demand in recent years for fermented dairy products or products with claims of functionality on their labels, and it is essential that the industries and all

those involved in the production processes are committed in order to ensure the supply of products suitable for consumption, ensuring the sustainability of the sector and the health of the consumer.

And from the point of view of consumer safety and of corresponding to what the legislation determines, it is recommended for further studies to use other more effective analytical techniques for the determination of lactose, specifically for hydrolyzed milks, such as enzymatic methods, near-infrared (NIR) spectroscopy and/or chromatography.

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