Research, Society and Development, v. 9, n. 10, e4619108590, 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i10.8590

Production of naturally ‘‘lactose free” fresh cheese”

Produção de queijo fresco naturalmente “zero lactose”

Producción de queso fresco natural "sin lactosa"

Received: 09/18/2020 | Reviewed: 09/24/2020 | Accept: 10/02/2020 | Published: 10/04/2020

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Abstract

Lactose intolerance is a disorder that affects a large part of the population, in this sense, it becomes feasible the study of manufacturing techniques that make it possible to guarantee the elaboration of products with low lactose content. Taking this into account, the present study aimed to evaluate the lactose content and physical-chemical characteristics of washed mass cheeses during the maturation period. Pasteurized whole milk with initial lactose percentage of 4.55 g/100 g was used and after coagulation, cutting and stirring, a partial (10% of the initial volume) whey removal was followed by addition of the same volume of hot water at 75 °C. Cheeses were analyzed at 0, 7, 15, 30 and 60 days of maturation for lactose content, acidity, pH, water activity, moisture, fixed mineral residue and chlorides. A lactose content of less than 0.005 g/100 g was obtained shortly after manufacturing (fresh product). The starter culture used and the process of washing the mass likely contributed to the low lactose content of the cheeses. Thereby is presented a viable alternative for the production of naturally lactose-free cheeses.

Keywords: Intolerance; Delactosing; Fermentation.

Resumo

A intolerância a lactose é um distúrbio que atinge grande parte da população, nesse sentido, torna-se viável o estudo de técnicas de fabricação que possibilitem a garantia de elaboração de produtos com baixo teor de lactose. Diante deste fato, este estudo objetivou avaliar o teor de lactose e características físico-químicas de queijos de massa lavada durante o período de maturação. Utilizou-se leite integral pasteurizado com teor de 4,55 g/100 g de lactose e após a coagulação, corte e mexedura, foi realizada uma pré-dessora, com a retirada de 10% do volume inicial, substituído pelo mesmo volume de água quente a 75 °C. Os queijos foram analisados nos tempos 0, 7, 15, 30 e 60 dias de maturação quanto ao teor de lactose, acidez,

pH, atividade de água, umidade, cinzas e cloretos. Obteve-se teor de lactose inferior a 0,005 g/100 g logo após a fabricação (queijos frescos). O tipo de cultura *starter* utilizada e o processo de fabricação, principalmente a lavagem da massa, possivelmente contribuíram para o baixo teor de lactose dos queijos. Com isto, demonstra-se uma alternativa viável para a produção de queijos naturalmente isentos de lactose.

Palavras-chave: Intolerância; Delactosagem; Fermentação.

Resumen

La intolerancia a la lactosa es un trastorno que afecta a gran parte de la población, lo que torna factible estudiar nuevas técnicas de fabricación de queso que permitan garantizar la elaboración de productos con bajo contenido en lactosa. Así, este estudio tuvo como objetivo evaluar el contenido de lactosa y las características fisicoquímicas de los quesos de pasta lavados durante el período de maduración. Se utilizó leche entera pasteurizada con un contenido de 4.55 g/100 g de lactosa y después de la coagulación, corte y agitación se realizó una remoción del 10% del volumen inicial, reemplazado por el mismo volumen de agua caliente a 75 ° C. Los quesos se analizaron a los 0, 7, 15, 30 y 60 días de maduración para el contenido de lactosa, acidez, pH, actividad del agua, humedad, cenizas y cloruros. Poco después de la fabricación se obtuvo un contenido de lactosa inferior a 0,005 g/100 g en los quesos frescos. El tipo de cultivo iniciador utilizado y el proceso de fabricación, principalmente el lavado de la masa, contribuyeron posiblemente al bajo contenido de lactosa de los quesos. Esto demuestra una alternativa viable para la producción de quesos naturalmente libres de lactosa.

Palabras clave: Intolerancia; Eliminación de lactosa; Fermentación.

1. Introduction

Lactose intolerance is a common disorder worldwide. This intolerance is result of low levels of the enzyme lactase in the intestine and consequent inability to digest lactose. It is characterized as a metabolic disorder when people are unable to digest significant amounts of lactose due to a genetic insufficiency in the production of the enzyme lactase (Facioni et al., 2020). People with lactose intolerance present abdominal symptoms, such as diarrhea, nausea, disorders and pain, consequence of ingestion of lactose, normally associated with dairy products. Recent studies show that the risk and intensity of symptoms after lactose

intake depends on the dose of lactose, lactase activity, intestinal microbiota and sensitivity of the gastrointestinal tract (Martínez Vázquez et al., 2020).

In recent years is notable the growth in the number of dairy products without lactose or with reduced lactose content, in order to promote dairy consumption also by people who are lactose intolerant, with no later unpleasant symptoms (Gille et al., 2018). In this sense, Dekker et al. (2019) emphasized that for the entire milk production chain, from production of raw material to the industry, products with low lactose content ensure market expansion by mobilizing groups of different consumers who are not attended by the common milk products.

Lactose decreases its content in cheese by important production stages, such as whey separation and bacteria fermentation (Lima Tribst, Piza Falcade, Carvalho, Ricardo de Castro Leite Junior, & Meirelles de Oliveira, 2020). Matured cheeses are naturally lactose free, while fresh cheeses may have a content varying from 0.1 to 4.6 g/100 g (Dickel et al., 2016; Gille et al., 2018). Zero lactose milk products are normally obtained by adding enzyme lactase (β-galactosidase). However, manufacturing technology can significantly influence the lactose content of cheese (Szilagyi & Ishayek, 2018).

This work had as objective to quantify the lactose content and to evaluate the physical-chemical characteristics of a cheese produced with a washing step of the cheese curd, during the maturation period.

2. Methodology

2.1 Raw Material Characterization

The cheeses were produced in a dairy industry in São Jorge do Oeste, in the Southwest region of Paraná State - Brazil. Refrigerated raw milk was used from selected properties close to the industry (25°41'41.4"South, 52°53'58.1"West). Milk milking, storage and transportation procedures followed the current legislation (Brasil, 2018).

Raw milk characterization with respect to protein, lactose, total solids and defatted dry extract were performed using three samples, using infrared methodology (ISO 9622, 2013); Somatic Cell Count (SCC) and Standard Plate Count (SPC) according to Methodology of Flow Cytometry (IDF, 1995). Acidity analyzes, cryoscopy, fat content, density, and search for acid neutralizer were performed according to the methodology described in normative

instruction (Brasil, 2018). Betalactamic and Tetracyclines Antibiotic residues were searchedusing Immunoenzymatic Methodology (*Twinsensor BT*®, Unisensor, Bélgica).

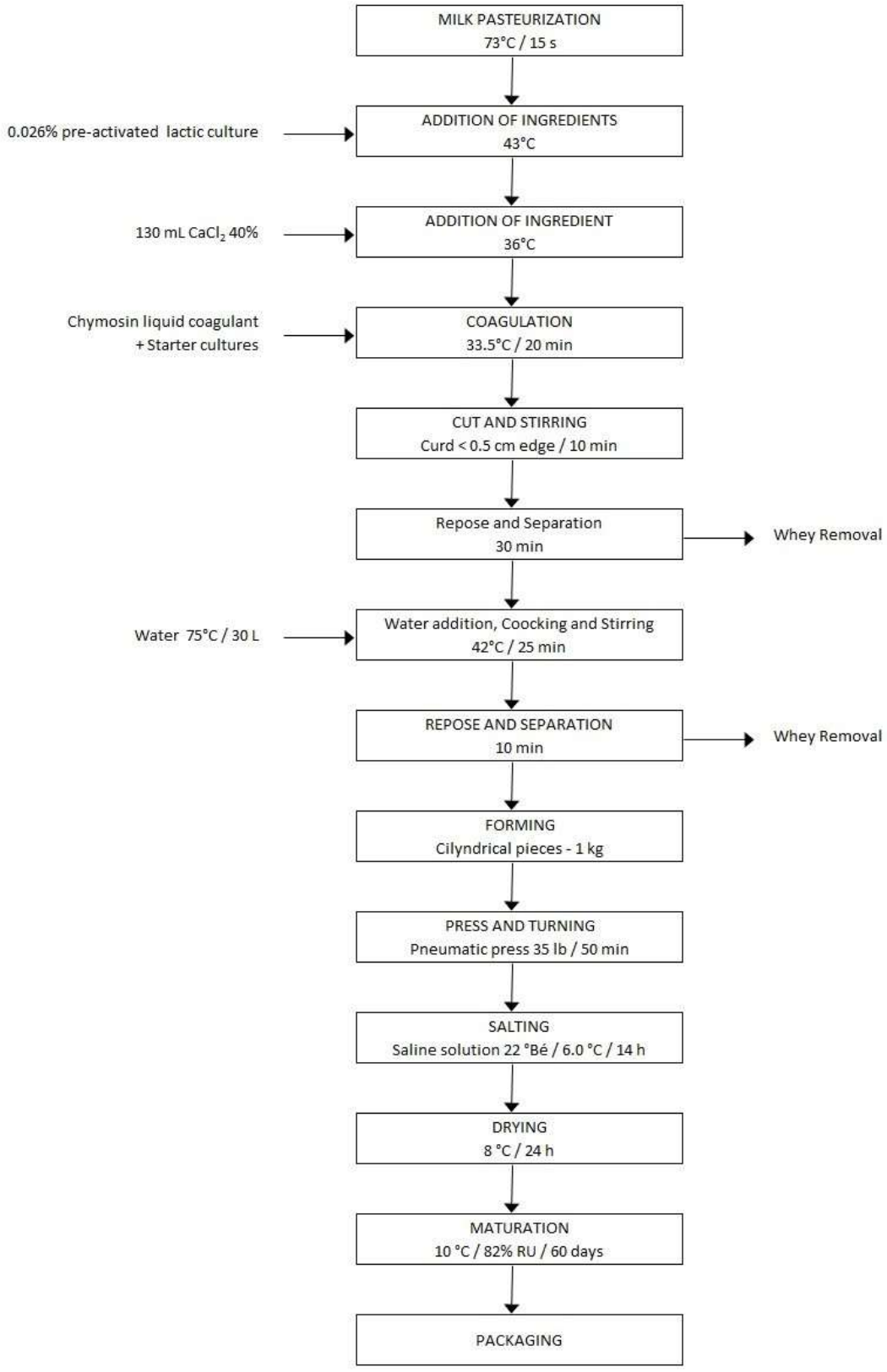
2.2 Cheese Production

The production process is represented in Figure 1. Each production batch utilized 300 L of whole milk (3.5% fat) pasteurized at 73 °C for 15 seconds. Pasteurized milk was transferred to the manufacturing tank for addition of 0.026% of pre-activated lactic yeast (at 43 °C); 130 mL of 40% calcium chloride (at 36 °C) and liquid chymosin coagulant (at 33.5 °C) were added).

Enzymatic coagulation was promoted with the use of blend of Lactic acid bacteria (FD-DVS RSF-736, Chr. Hansen®) composed of *Lactobacillus helveticus* strains, *Lactococcus lactis* subsp. cremoris, *Lactococcus lactis* subsp. lactis and *Streptococcus thermophilus*. Starter culture dilution and pre-activation were performed by diluting a freeze-dried 500 U starter culture envelope in one liter of UHT milk aseptically at 37 °C. After homogeneous mixing, the preparation was stored in freezer at -15 °C (for 48 hours) in sterile 80 mL plastic bottles for use at the time of manufacture.

Milk coagulation was performed for approximately 20 minutes, cut with metal wires, mixing for 10 minutes, repose for 30 minutes and separation of 35 L of whey.

Figure 1 - Cheese Production Flowchart.



Source: Authors.

After this process, the mixture was stirred with 30 L of water at 75 ± 1°C. The product was heated to 42 ± 1°C and remained at this temperature under agitation for 25 min, followed by repose of 10 minutes. The whey was removed and cheese was molded into cylindrical pieces of 1 kg and submitted to pneumatic press at 35 lb for 50 min. The cheese pieces were unmolded and immersed in saline solution (22° Bé) at 6 °C for 14 hours and dried in chamber at 8 ± 1 ºC for 24 hours. After this process, the cheese pieces were stored in a maturation chamber up to 60 days at 10 ± 2 ºC with relative humidity of 82 ± 2%. During this period, the pieces were turned over every two days.

2.3 Gathering and Sampling

Three batches of cheese were produced, following the same manufacturing process. However, in the second and third batch, cheese samples were collected only soon after manufacturing (time 0), because at this time lactose content showed values under the detection limits for both methods.

The cheeses were evaluated during maturation, and in each period (0, 7, 15, 30 and 60 days), three pieces of cheese were randomly collected and vacuum sealed in plastic polyethylene packages and storage in Ultrafreezer at -50 °C (Coldlab® CL 166-50H).

2.4 Physical-chemical analysis of cheeses

The pH was determined using portable pH meter (Caplab® 200p, São Paulo, Brazil). The acidity (expressed as lactic acid) was evaluated by titration (Brasil, 2018)

Determination of water activity was performed at 25 °C using Labmaster Novasina® equipment (AG CH 8853, Lachen, Switzerland). Moisture and fixed mineral residue (ash) were analyzed using gravimetric method. Humidity was determined at 105 °C with circulation and air renewal (Solab®, SL 102, Piracicaba, São Paulo) and ash in muffle at 550 ° C (Zezimaq®, Contagem, Minas Gerais) (Brasil, 2018). Chloride content analysis was performed using argentometric method, titration with 0.1 N Silver Nitrate solution (Brasil, 2018).

Lactose quantification was performed by two methods: quantitative analysis of lactose reducing glycerides, using titration, which results in less accurate results, in accordance with IN No. 68/2006 (Brasil, 2018) and ion chromatography with eletrochemical detection (IC-EC), capable to determine low concentrations of lactose with high selectivity, required by

recent update in Brazilian legislation for zero-lactose products (AOAC 982.14 mod.) (AOAC, 2005).

2.5 Data treatment

The results were submitted to Analysis of Variance (ANOVA) and Tukey's test for comparison between means, using Sisvar® 5.6 software. All analyzes were performed in triplicate and the results were expressed as mean ± standard deviation.

3. Results and Discussion

3.1 Raw Material Characterization

The average quality parameters of the refrigerated raw milk used for cheese production are shown in Table 1. The results revealed that the raw material was in compliance with the quality standards required for refrigerated raw milk (Brasil, 2018), for all parameters evaluated.

Table 1 – Quality parameters of refrigerated raw milk used for the manufacture of cheeses.

Quality Parameters	**Results***	**Requirements according to IN 76/2018****
Acidity gramas de ácido lático/100 mL	0.15 ± 0.06	0.14 – 0.18
Density at 15 °C (g/mL)	1.032 ± 0.20	1.028 – 1.034
Cryoscopy (°Horvet)	- 0.540 ± 2.65	- 0.530 to - 0.550
Fat (g/100g)	3.55 ± 0.02	Min. 3.0
Total Solids (g/100g)	12.05 ± 0.06	Min. 11.4
Defatted Dry Extract (g/100g)	8.50 ± 0.04	Min. 8.4
Protein (g/100g)	3.03 ± 0.01	Min. 2.9
Lactose (g/100g)	4.55 ± 0.02	Min. 4.3
Somatic Cell Count (x 1000 cel/mL)	168 ± 24.62	Max. 500
Standard Plate Count (x 1000 CFU/mL)	101 ± 43.55	Max. 300
Antibiotic residue	Negative	-
Search for acid neutralizers	Absent	Absent

*Results expressed as mean ± standard deviation of the analysis performed in triplicate (n=9). **Brasil (2018).

Source: Authors.

3.2 Evaluation of fresh cheeses (0 days of maturation)

The results of the physical-chemical analysis performed on the three batches of cheeses of fresh washed mass, soon after the manufacture, are shown in Table 2.

Table 2 – Physical-chemical parameters in cheeses of washed mass soon after manufacture (0 days of maturation).

Parameters**	Batch 1*	Batch 2*	Batch 3*
Lactose (Reducing carbohydrate in lactose) (g/100g)	< 0.28	< 0.28	< 0.28
Lactose (IC-EC) (g/100g)	< 0.005	< 0.005	< 0.005
Acidity in lactic acid (g/100g)	0.14 ±0.03^{b}	0.19 ± 0.01^{a}	0.18 ± 0.01^{a}
pH	5.64 ± 0.02^{b}	6.25 ± 0.30^{a}	6.48 ± 0.17^{a}
Water Activity	0.929 ± 0.00^{a}	0.931 ± 0.01^{a}	0.925 ± 0.00^{a}
Umidity (g/100g)	39.43 ± 0.87^{ab}	38.68 ± 0.50^{b}	39.73 ± 0.84^{a}
Fixed Mineral Residue (g/100g)	4.50 ± 0.07^{a}	3.91 ± 0.07^{b}	4.13 ± 0.54^{ab}
Chlorides (g/100g)	1.81 ± 0.03^{a}	1.04 ± 0.40^{b}	1.57 ± 0.38^{a}

* Results expressed as mean ± standard deviation of the analysis performed in real triplicate (n=9). **Different lowercase letters on the same line indicate significant difference between the cheeses (Tukey Test, p <0.05). Source: Authors.

In the three different batches, the lactose presented contents below 0.28 g/100 g and 0.005 g/100 g, which correspond to the detection limits of the methods used. Initially, the samples were evaluated by the lactose reducing glyceride method and after obtaining these results were also analyzed by electrochemical detection ion chromatography (IC-EC).

The average acidity of fresh cheese was 0.17 g/100 g lactic acid and pH 6.12. The cheeses were classified as medium humidity (between 36.0 and 45.9%) according to Portaria n^{o} 146 (Brasil, 1996), with average water activity of 0.928. The average ash content was 4.18 g/100 g and chlorides 1.47 g/100 g.

Considering the use of a pilot scale volume of milk (300 liters) for each batch, it can be said that the values obtained (Table 2) are within the expected range for variations in cheese production.

3.3 Cheeses analysis during maturation

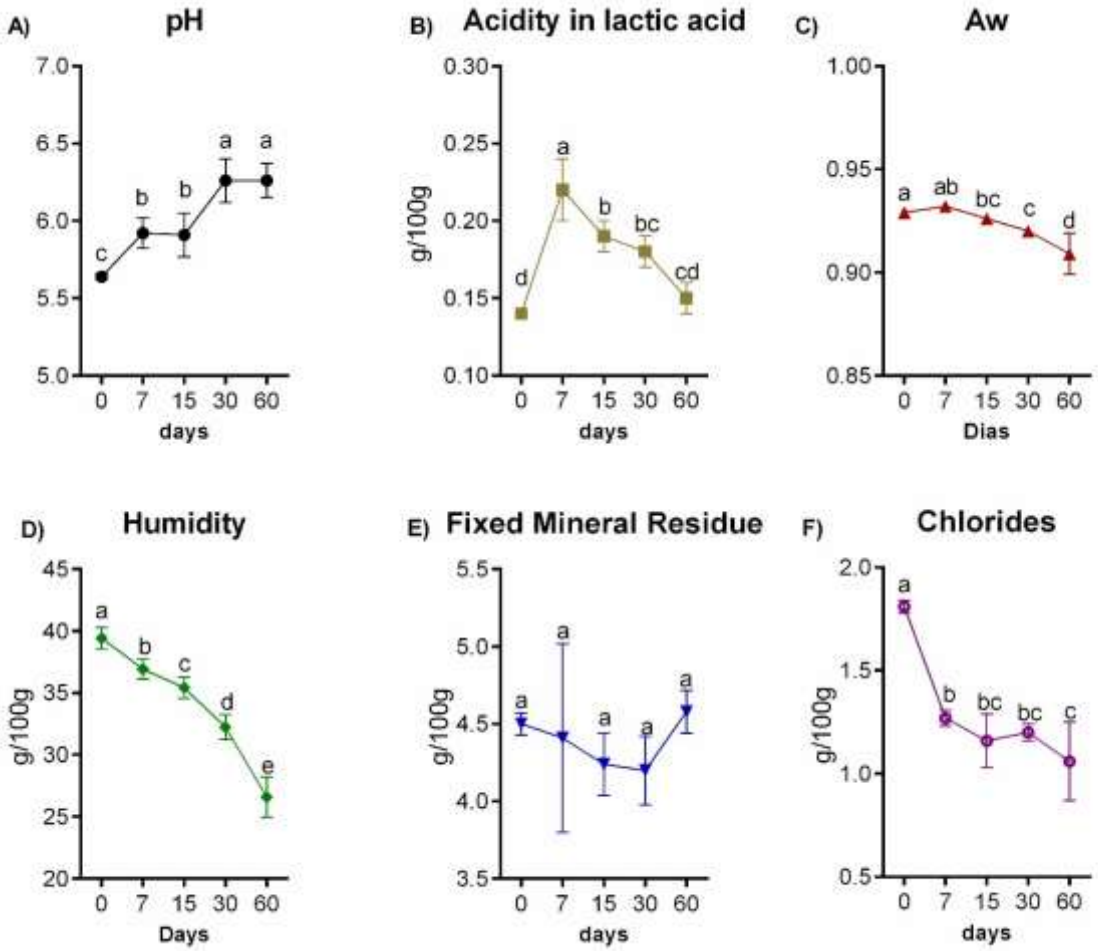
The results of the physical-chemical analysis of cheeses during the maturation period are presented in Table 3 and Figure 2 A-F.

Table 3 - Physical-chemical parameters in cheeses of washed mass during maturation.

Parameters	**Maturation period (days)***	
	0	**7**
Lactose (Reducing carbohydrate in lactose) (g/100g)	< 0.28	< 0.28
Lactose (IC-EC) (g/100g)	< 0.005	< 0.005

* Results expressed as mean ± standard deviation of the analysis performed in real triplicate (n=9).
Source: Authors.

Figure 2 - Physical-chemical parameters in cheeses of washed mass during maturation. Different letters indicate significant difference during mass maturation time (Tukey Test, p <0.05).



Source: Authors.

Regarding the quantification of lactose (Table 3), the results were <0.28 g/100 g (IN 68/2006) and <0.005 g / 100g (AOAC 982.14 mod) (AOAC, 2005) also for fresh cheese with 0 days of maturation. At 7 days of maturation, the lactose determination was done again in order to confirm the contents obtained previously and the same results were observed. Thus, it was not necessary to determine lactose amount in later periods of maturation. Therefore, cheeses produced can be classified as "Zero Lactose" according to current legislation, since the product contains lactose less than 100 mg/100 g (Brasil, 2017)

Factors that may be related to the low lactose content observed in these cheeses include the type of starter culture used, the curd cut and the whey removal procedure (whey removal and rinsing of the curd with hot water) (Moynihan et al., 2016). Regarding the type

of starter culture, possibly the reduced lactose content observed is due to the presence of the thermophilic lactic acid bacteria *Streptococcus thermophilus* in the inoculum used in this study, since this microorganism has the characteristic of being an excellent lactose fermenter (Beux, Todescatto, Marchi, & Pereira, 2020).

With respect to the curd cut, the reduced grain size (<0.5 cm of edge) of the cheeses provides higher heating of the mass, and thus elevated syneresis, due to grain contraction and whey elimination, with consequent decrease in the content of lactose in the grain, since lactose is soluble in the aqueous portion (Verruck, Dantas, & Prudencio, 2019).

The whey removal contributed to the decrease of lactose content. Studies have shown that curd washing significantly reduces the levels of residual lactose and lactic acid, a typical behavior due to the solubility of lactose in water (Hou, Hannon, McSweeney, Beresford, & Guinee, 2012). Fox et al. (2000) reported that replacing 35-45% whey after the curd was cut by an equal volume of warm water, the lactose content in Cheddar cheese was reduced to values of about 0.03% to 0.25% lactose.

According to Gille et al. (2018), the matured cheeses are naturally lactose-free. This fact contradicts the commercial appeal made by the dairy industry, highlighting the low lactose content on the label of matured cheeses as a differential point of other matured cheeses. In this study, even cheeses not yet matured (fresh), presented reduced lactose content due to their manufacturing process.

The process of washing of coagulated product (after whey draining) with water can reduce the residual lactose content. Several parameters influence this reduction such as: (i) particle size and contact area, (ii) washing method, (iii) Coagulated mass and water temperature, (iv) composition and structure of mass particles and (v) degree of agitation (Moynihan et al., 2016).

At 7 days of maturation, was observed an increase in acidity and a later decline in these values, consistent with the behavior of the pH of the cheeses. This behavior is observed due to the metabolization of residual traces of lactose present in cheeses, causing a slight decline in pH, by the formation of lactic acid. After, in consequence of the proteolysis resulting from biochemical reactions during maturation, the pH increased again. This increase in pH is common in many varieties of cheese, probably due to proteolysis and degradation of amino acids and consequent (Fox et al., 2000).

The pH values (Figure 2A) and acidity (Figure 2B) differed from the study by Dickel et al. (2016), which reported pH from 5.62 and 5.87 and acidity expressed in lactic acid between 0.28% and 0.43% in Colonial cheeses. Sousa et al. (2014) obtained pH of cheeses

between 5.68 and 5.18, values below the results found in this study. The lower acidity and higher pH observed can be related to curd washing, in which the partial removal of fermentable sugars by lactic bacteria occurs, resulting in reduced titratable acidity and lower decrease of pH of the product during maturation. As observed in the present study, Fox et al. (2000) and Hou et al. (2012), noted that curd washing and maturation time influence the pH of the cheeses. The curd washing procedure significantly increases the pH of cheeses, a fact related to the low levels of lactose and, consequently, lactic acid in the cheeses.

Regarding the water activity parameter (Figure 2C), results showed a significant difference in the final maturation periods, with a gradual loss of free water as a function of maturation of the cheeses. Similar results were described by Brandielli et al. (2019) showing a significant decrease in water activity over the maturation period.

During maturation period, cheeses tend to lose moisture by evaporation, contributing to the reduction of water activity during maturation. The proteins in the cheese are hydrated, and this bond with water makes the water unavailable for bacterial growth. In the process of proteolysis during maturation, with formation of peptides and amino acids and lipolysis, forming glycerol and fatty acids there is a reduction in the availability of water, because the water molecules are added in the hydrolysis bonds. Also, salt concentration and organic acids (lactate, acetate and propionate) are dissolved in aqueous portion, decreasing its vapor pressure (Fox et al., 2000).

As for moisture (Figure 2D), there was a significant difference between all maturation periods. At 0 days of maturation, the cheeses presented 39.28 ± 0.38 g/100 g of moisture, being classified according to the Cheese Technical Regulation (Brasil, 1996), as medium moisture cheeses, characterized as semi-hard cheeses. Same behavior was observed by Brandielli et al. (2019). At 15 days of maturation, it can be observed that the cheeses presented 35.42 ± 0.86 g/100 g of moisture, being classified from this period as cheeses of low humidity, and it can also be named as hard cheese (Brasil, 1996)

No significant change in ash content (Figure 2E) was observed during the different maturation periods. Regarding the quantification of chlorides, the values found in the cheeses were 1.06 ± 0.1 to 1.47 ± 0.18 g/100 g, where traditional values in fresh cheeses normaly vary from 1.4 to 1.6% and Dickel et al. (2016) reported averages of 1.62% to 1.81% for Colonial cheeses. These values are related to addition of calcium chloride for mass coagulation and sodium choride addition during salting. Probably a lower content of salt was added, in accordance with the current trend of sodium content reduction in food products.

4. Final Considerations

The production of cheese using washing step procedure resulted in low lactose content soon after production, before the beginning of the maturation period. The starter culture utilized and small grain size contributed for the low lactose content of the cheeses. These results present a technical alternative to the dairy industry, which can use these processes to produce naturally lactose-free cheeses with no need for intentional enzymatic hydrolysis (addition of lactase).

Acknowledgements

To the Nato Bom Cheese Industry, for the necessary support and to the UFFS (Federal University of Fronteira Sul) and UFTPR (Federal Technological University of Paraná) for the aid and support.

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