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Physicochemical and microbiological evaluation of traditional queso molido (ground cheese) during maturation

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ABSTRACT

Objective: To evaluate the changes in the main microorganisms groups and the physicochemical properties of Queso Molido (Ground Cheese) from the Central Mexican Highlands, during a 90 days maturation period.

Methodology: The fat, protein, moisture, ash and chloride contents, acidity and pH were analyzed. Microbiological analyzes of total coliforms, lactic acid bacteria, yeasts and *Staphylococcus* were also assessed. An ANOVA and a principal component analysis were performed to analyze the effect maturation had on the physicochemical and microbiological characteristics.

Results: Both the physicochemical and microbiological parameters showed significant differences ($p<0.05$) during the evaluated maturation period. The protein concentration increased, fat and acidity at the end of maturation, along with the absence of coliform bacteria, a decrease in lactic acid bacteria (LAB) and a high concentration of *Staphylococcus* and yeasts.

Limitations: It is necessary to evaluate these variables in other locations and similar production systems in other latitudes of the country.

Conclusion: The maturation of Queso Molido does not improve its microbiological quality.

Keywords: Main components, raw milk, fresh cheese, traditional.

INTRODUCTION

The onset of the Official Mexican Standard. Products and services. Milk, milk formula, combined dairy products and dairy derivatives. Sanitary provisions and specifications (NOM-243-SSA1-2010), prohibits cheese production from raw milk, specifically due to problems in their microbiological quality, which may present alterations for the consumer's health. Prates *et al*. (2017) indicate that fresh cheese consumption has been associated with foodborne disease outbreaks in different parts of the world, due to the potential survival of pathogenic bacteria in the unpasteurized milk cheeses. Because of that, there is an undeniable concern regarding the (artisan) production systems, that to date, use this method for the elaboration of their products, a situation shared both nationally and internationally (Alvarado *et al*., 2007; Ercan *et al*., 2014; Cámara *et al*., 2017).

The importance of artisan cheese production worldwide involves specific characteristics to each one depending on their region (Martins *et al*., 2015), its connection with its regional culture, territory, history and lifestyle (De la Rosa-Alcaraz *et al*., 2020), which distinguish them as a product with typical aromas and flavors, conferred by the

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milk's microorganisms (Ordiales *et al*., 2013). These peculiarities are affected by pasteurization and starter cultures. Furthermore, most producers do not have enough technology to improve the quality of their products. Despite this, the manufacture of these cheeses on a small scale has taken on greater importance, as they are highly accepted products by consumers, as well as an important economic source (Longaray *et al*., 2012).

The cheese maturation process is an alternative to address the health problems associated with this kind of fresh cheeses made from fresh unpasteurized milk. This process comprises a period in which the cheese remains stored at a certain temperature and relative humidity conditions. Depending on the cheese type, their final characteristics (physicochemical and microbiological) directly depend on the natural microflora of their milk, given that its bacterial population is high and due to the effect of the proteolysis and lipolysis associated with the maturation, that gives these cheeses their characteristic flavors and aromas (Sant'Anna *et al*., 2018).

Various studies have shown that after a maturation period of at least 60 days, this product is suitable for consumption (Mas *et al*., 2002). Other sources indicate that just two weeks of maturation is enough to achieve a safe cheese (Cámara *et al*., 2017; González *et al*., 2019), due to the effect lactic acid bacteria that prevail during maturity and that acts as a natural selector inhibiting pathogens (Caridi *et al*., 2003), coupled with pH decrease (Ercan *et al*., 2014).

In the Central Highlands of Mexico, the so-called queso Molido Cheese is produced with raw cow's milk, following a traditional process. This cheese is a fresh, soft paste, not pressed. Its presentation is in the form of a low cylinder, with a weight ranging between 200 g to about 1 kg (Cervantes *et al*., 2006).

To place fresh cheese in a market with greater added value, there is the proposal to pass it through a maturing process. Therefore, this study focused on evaluating the main microorganisms groups, as well as their physicochemical characteristics of queso Molido Cheese during a 90 days maturation period.

MATERIAL AND METHODS

Experiment location

The experimental study took place in the Central Highlands of Mexico, located between the coordinates 20° 06'' north and 99° 50'' west. The altitude is 2400 m above sea level and a maximum temperature of 24.3 °C, a minimum of 2.3 °C and a mean of 13.2 °C, semi-cold, subhumid climate, with rains in summer, its average annual rainfall is 699.6 mm (INAFED, 2015).

Cheese samples

A batch of 12 pieces of Queso Molido (ground cheese) of an approximate weight of 250 g each was evaluated. The samples were refrigerated at 4 °C, and wrapped in poly-paper, maintaining the same conditions for all cheeses. Four maturation periods were evaluated: 1) 1 day, 30 days, 60 days and 90 days after been elaborated. Of each period, three cheese pieces were evaluated. The samples were transported at 4 °C and kept refrigerated for later analysis, according to the aging period.

Physicochemical analysis

The fat (933.05), protein (991.2), moisture (926.08) and ash (935.42) were determined following the official AOAC methods (1990). The acidity was estimated using 0.1 M NaOH/phenophthalein as an indicator; pH using an Orion 520A potentiometer (Montes de Oca-Flores *et al*., 2009) and chlorides with the Volhard method (NMX-F-360-1981). All analyzes were done by triplicate.

Microbiological analysis

At each stage, 10 g cheese was aseptically sampled from the center of the sample and placed in 90 mL of peptone solution (AOAC 966.23). Total coliforms were assessed following the NOM-113-SSA1-1994; lactic acid bacteria following Lancelle and Vasek (2002); yeasts following the AOAC 997.02; staphylococcus following the NOM-115-SSA1-1994. Microbial counts were performed in a colony counter and calculations were performed to obtain colony-forming units per gram (CFU/g).

Statistical analysis

The microbiological counts were transformed to $\log_{10}$ cfu/g. An ANOVA by completely randomized blocks was performed to evaluate the differences in the physicochemical and microbiological parameters. The existing differences were calculated using the Tukey test ($p \leq 0.05$), using the statistical package Statgraphics 18.

RESULTS AND DISCUSSION

Physicochemical properties during Queso Molido maturation

There were significant differences ($p \leq 0.05$) in all the

physicochemical properties of Queso Molido during the maturation period (Table 1).

Table 1. Physicochemical composition during the maturation period of Queso Molido.

	Maturation of the cheese (days)				EEM
	1	30	60	90	
Fat (%)	20,55^{a}	22,0^{ab}	22,9^{ab}	25,41^{b}	1,76
Protein (%)	18,53^{a}	18,93^{a}	18,94^{a}	21,75^{b}	0,27
Moisture (%)	49,97^{bc}	50,35^{c}	48,71^{b}	46,62^{a}	0,6
Ash (%)	1,98^{c}	1,87^{b}	1,9^{bc}	1,62^{a}	0,03
Acidity (^{o}D)	21,44^{a}	26,11^{b}	21,88^{a}	25,56^{b}	1,11
pH	4,67^{a}	4,6^{a}	5,34^{b}	5,1^{b}	0,14
Chlorides (%)	3,4^{b}	1,67^{a}	1,95^{a}	1,72^{a}	0,15

SEM=Standard error of the mean. a, b, c=Different letters in the same column indicate statistical differences (p≤0.05).

The percentages of fat and protein behaved in a similar way during the first three maturation periods (1 to 60 days), the last period with the highest percentage. Brandielli *et al*. (2019), in a cheese from pasteurized cow's milk, during its maturation stages (0, 60 and 120 days) reported differences in the protein and fat percentages, with the highest concentration at the end of the period (24.09 and 28.1 %, respectively).

The moisture percentage behaved inversely decreasing at the end of maturation period (49.97-46.62%), a fact that promotes an protein and fat content increase.

In determining the milk acidity, various methods are used, which give different interpretations. For example, pH measures actual or acquired acidity and titratable acidity measures both natural and actual or acquired acidity. The latter, measured through titration with a NaOH solution, which according to its concentration allows to express the acidity under different scales regard to the NaOH concentration: in Dornic degrees (°D) if the concentration of NaOH is 0.11 N, Thörner degrees (°Th) if the solution concentration is 0.1 N, Sohxlet-Henkel degrees (°SH) if the solution concentration is 0.25 N (Pulgar, 1988).

The acidity concentration increased at the end of maturation (21.44-25.56 °D), although it presented its maximum value on day 30 (26.11 °D), observing an inconsistent behavior. Dervisoglu and Aydemir (2007) reported a progressive increase in acidity for each of the stages, until the end of maturation (120 days) and as a consequence a pH decrease due to the production of lactic acid by the effect of lactic bacteria (Dalla *et al*., 2008). The opposite was obtained in this research with a pH increase (4.67 to 5.1). Mas *et al*. (2002) and Temizkan *et al*. (2014) reported increased pH values at the end of the maturation. Kongo *et al*. (2009) indicate that this behavior may be related to secondary proteolytic activity by bacteria and yeasts and as a consequence of the accumulation of free fatty amino acids and ammonia.

There was a decrease in the percentage of chlorides at the end of the maturation (3.4-1.72), a situation that may be related to ash decrease. On the contrary, Çetinkaya and Fatih (2019) reported an increase in chlorides at the end of it. Lima *et al*. (2008) compared the salt and moisture content and/or water activity in the maturation process, mentioning that there is an inverse relationship between these, a behavior not observed in this research.

The decrease in the ash percentage (1.99-1.62) does not coincide with that reported by Arenas *et al*. (2015) and Cakır and Cakmakc (2018) who found progressive increases until the end of it, due to the effect of decreased humidity.

Table 2. Microbiological composition during the maturation period of Queso Molido (log_{10})

	Maturation of the cheese (days)				EEM
	1	30	60	90	
CT	6,2^{c}	4,19^{b}	5,85^{c}	0,0^{a}	0,25
BAL	9,59^{c}	7,69^{a}	8,31^{b}	7,66^{a}	0,09
Lev.	9,59^{d}	7,66^{a}	8,80^{c}	8,53^{b}	0,09
St.	7,14^{c}	5,96^{a}	6,50^{b}	7,44^{c}	0,19

SEM=Standard error from the mean.
a-d=Different letters indicate statistical differences (p<0.05).
TC=Total Coliforms.
LAB=Lactic Acid Bacteria.
St.=*Staphylococcus*.

Microbiological properties during the maturation of Queso Molido

The microbiological properties are shown in Table 2. All the parameters evaluated in the maturation process showed significant differences (p≤0.05). High values were observed at the beginning regard total coliforms, yeasts and *Staphylococcus*, a factor

that could be related to mishandling and the conditions during the transport of the raw materials.

Staphylococcus and yeast colony-forming units decrease by the end of the maturation process (7.44 and 8.53, respectively), although this decrease cannot be considered sufficient compared to a product for direct human consumption. The maximum permissible limits by Mexican regulations are 100 cfu/g and 500 cfu/g respectively. In our study, the greatest decrease occurred at 30 years of maturation, possibly due to a greater increase in acidity during the same stage (26.11 °D) and at the lowest pH (4.6), increasing again during the last two periods.

There were no total coliform counts in the last maturation period (90 days). Ceylan *et al*. (2007) reported a decrease at day 30 and 90 of maturation (<1 cfu/g), in a cheese made with unpasteurized milk, while Dolci *et al*. (2010), obtained 1.4 cfu/g at day 90 and <5.0 cfu/g up to the day 150 of maturation.

There was a decrease in ALB at the end of the maturation period (9.6-7.67 log). Similar behaviors were reported by Cakır and Cakmakc (2018) in relation to bacteria of the *Lactobacillus* and *Lactococcus* genera, due to the increase in salt concentrations and acidic during maturing, in addition to low pH (4.04-4.29) (Bontinis *et al*., 2008). For this research, there was only an acidity increase (21.4-25.5), not the case of the salt percentage (3.4-1.7).

CONCLUSIONS

Although by the end of the maturation period the complete decrease of coliform bacteria is observed, it is not due to the effect of the physicochemical properties. The presence of *Staphylococcus* and yeasts at the end of maturation in cheeses indicate that they are not suitable for consumption, since they represent a health risk to consumers. The above indicates that the 90 days of maturation period is not enough time for the product to be considered suitable for consumption. Although at the end of the maturation period they showed a slight decrease in pathogenic bacteria, it is important to improve the transport conditions of the raw materials, in addition to better control of its storage conditions.

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