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Technical note

Characterization of the milk and artisanal cheese of the region of Ojos Negros, Baja California, Mexico

Laura E. Silva-Paz [a]

Gerardo E. Medina-Basulto [a]

Gilberto López-Valencia [a]*

Martin F. Montaño-Gómez [a]

Rafael Villa-Angulo [b]

José C. Herrera Ramírez [a]

Ana L. González-Silva [a]

Francisco Monge-Navarro [a]

Sergio A. Cueto-González [a]

Gerardo Felipe-García [a]

[a] Universidad Autónoma de Baja California. Instituto de Investigaciones en Ciencias Veterinarias. Fracc. Laguna Campestre carretera a San Felipe km 3.5, Mexicali, Baja California. México.

[b] Universidad Autónoma de Baja California. Instituto de Ingeniería. México.

*Corresponding author: gilbertolopez@uabc.edu.mx

Abstract:

The community of Ojos Negros is located in the municipality of Ensenada, Baja California, Mexico. Since 1930, local residents make a greatly appreciated handmade cheese in the region; however, the raw milk and cheese have never been analyzed for the microbiological quality and hygiene of the final product. The objective of this study was to evaluate the microbiological, physical and chemical quality of the raw milk used to produce cheese, and the artisanal cheese produced in the 22 individual production units. Samples of cheese and milk were collected from dairy production units in order to perform microbiological tests. There were physical and chemical determinations of protein, fat and lactose, using a LACTOSCAN-S analyzer. The results of milk analysis showed protein (33.11 g/L) and fat (39.89 g/L) contents within the parameters of the regulations. For the microbiological quality of milk, the results of the aerobic mesophilic count showed a 64 % compliance with the regulations; however, the same aerobic mesophilic count in the cheese samples resulted in only 4 % compliance. No *Salmonella* spp. or *Listeria monocytogenes* were detected in any of the tested milk or cheese samples. Good sanitation and manufacturing practices should be incorporated in order to enhance the sanitary quality and hygiene standards for the production of artisanal cheese in the community of Ojos Negros.

Key words: Ojos Negros, Artisanal cheese, Chemical composition, Microbiological quality.

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In Mexico the elaboration of artisanal cheese by medium-size and small producers is estimated at approximately 25 % of the total cheese produced per year[(1)]. The preparation and sale of artisanal cheeses constitutes one of the main sources of income for small-scale farmers despite the low profitability of their activity[(2,3)]. The so-called "pressed cheese of Ojos Negros" has been produced in the traditional way since 1930 in the Ojos Negros region of the municipality of Ensenada, Baja California, Mexico. Currently, the production of this cheese (produced from unpasteurized milk) reaches 30 t per month, providing sustenance for approximately 65 families[(4)]. However, the cheese producers face a new challenge because Mexican regulations stipulate that the milk used for cheese production must be pasteurized[(5)]. In addition, sanitary practices must be implemented to ensure a safe product that does not pose a risk to the consumer[(6)]. In 2010 the regional producers organized an association in order to seek technical assistance and succeeded in developing dairy production units; however, the microbiological

quality of the product is questionable, given the use of raw milk without pasteurization, coupled with the absence of an appropriate system of good manufacturing practices[7]. Therefore, the objective of this study was to perform a situational analysis of the microbiological and physicochemical quality of milk and cheese produced in the 22 cheese production units of that region.

The study was carried out in 22 production units (PU) of artisanal cheese located in the town of Ojos Negros, in the municipality of Ensenada, Baja California, Mexico (31°45' and 32°04' N and 116°06' and 116°27' W). All the PU participate in the tuberculosis and brucellosis control program. The production system is semi-extensive (free grazing and stabling) and has adequate infrastructure and equipment for producing milk and cheeses at a household level. On average, each herd produces 450 L of milk intended for the manufacture of artisanal cheese. The producers are in the initial phase of implementing a program of good manufacturing practices and hygiene.

The milk samples were collected from all the PUs on the same day according to the specifications of the NMX-F-718-COFOCA[8]. Out of each PU, 100 ml of milk were obtained from the tank in order to determine its sanitary quality; the cheeses were sampled according to the guidelines of the NOM-109-SSA[9]. A piece of whole cheese of approximately 2.5 kg, was collected only once from each PU. Samples of milk and cheese were transported in cooler at a temperature of between 7 and 10 °C to the Dairy Analysis Laboratory of Analysis of the Institute for Research on Veterinary Sciences (IICV) of the UABC for further processing.

The microbiological analyses of the milk and cheese were performed according to the procedure described in Appendices B10, B16, B17 of the NOM-243-SSA1[5], requiring, for milk and cheese, 1 ml and 10 g of sample dissolved in 9 ml and 90 ml, respectively, of 1% buffered peptone water (Difco, New Jersey). The colony forming units (CFU) of each sample were counted by performing five dilutions placing 1 ml on plates in duplicate for aerobic mesophilic bacteria in Standard Count Agar (MCD Lab, Tlalnepantla, Mexico); plates with 25 to 250 colonies were selected for counting. In order to determine the presence of fecal coliform bacteria, those plates with a register of 30 to 300 colonies were selected, and 1 ml of each dilution was planted and analyzed after five days in order to count the fungi and yeasts. In order to perform the analysis for *Salmonella*, 10 ml of milk and 10 g of cheese were pre-enriched in 90 ml of peptone water (Difco, New Jersey) and after 24 h of incubation at 35 ± 2 °C, they were enriched in tetrathionate broth (Difco, New Jersey) and Rappapot Vassilidis base (Difco, New Jersey); the sample was subsequently enriched through planting in XLD agar (Difco, New Jersey), Hecktona (Difco, New Jersey) and Bright Green media (Difco, New Jersey), identifying the bacterium by means of TSI (BD Bioxon, Cuautitlan, State of Mexico), LIA (BD Bioxon, Cuautitlan, State of Mexico), Urea (Difco, New Jersey), and RMVP biochemical tests (Difco, New Jersey).

For the analysis of *Listeria monocyogenes*, a 25 g sample of cheese was homogenized in 225 ml of UVM broth (Difco, New Jersey), incubated at 30 °C for 24 h, and then enriched in 10 ml of Fraser broth (Difco, New Jersey) for 24 h at 35 ± 2 °C. Plantings were made in Oxford agar plates (Difco, New Jersey); colonies with brown pigmentation with a halo were isolated for purification and identification in Brain Heart Infusion Agar (Difco, New Jersey), using Gram positive coccobacilli chain morphology, positive motility at 20-25 °C, negative oxidase test, and positive catalase and API-Listeria tests (bioMerieux, St. Louis, MO), respectively, for confirmation, according to the respective procedures of appendices B13 and B12 of the NOM-243-SSA[5].

The acidity in the milk was determined according to the procedure described in the NOM-155-SCFI[10], and the pH was evaluated with a potentiometer (Hanna Instruments, Carrolton TX). The physicochemical determination of the percentage of protein, fat, and lactose in the milk was performed using the LACTOSCAN-S analyzer (Milk Analyzer LTD model LS 90, Bulgaria).

The mean ± standard error was calculated for each one of the analyzed variables. Student's t-test was used to detect differences ($P<0.05$) between the averages of each parameter, compared to the desirable parameters of the NOM standards that apply. The results of the physicochemical and nutritional quality of milk used in the elaboration of artisanal cheese are presented in Table 1. No significant differences were observed ($P<0.05$) between the averages of protein detected for each class (A, B, and C) with respect to a reference parameter that indicates the NMX-F-700-COFOCALEC normativity[11]. With regard to the fat, the class exhibited no difference ($P>0.05$) between the desirable parameter of the NOM and the average value. However, when comparing the average for class C against the desirable parameter, significant differences ($P<0.05$) were identified. In this study, the figures for A protein classes (32.98 g/L) and fat (35.62 g/L) were better than those reported by Bernal[12] in small herds of the State of Mexico, and Oliszewski[13] in dairy herds of the rural region of the Las Trancas Basin in Argentina, with lower amounts of protein (30.55 g/L) and fat (34.0 g/L); however, both figures are considered to be indicative of a good quality milk. A possible explanation for these variations could be the nutritional management of the cows with respect to the rationing of nutrients, free-range grazing or genetic factors in the breeds of animals, as pointed out by De la Cruz[14].

Table 1: Physicochemical quality and nutritional status of milk used in the elaboration of artisanal cheese in 22 PUs in the region of Ojos Negros, Mexico

* Indicator	Reference Values** g/L	N (%)	Mean (SD)
Protein, g/L	Class A desirable ≥ 31a	14 (63)	32.98 [b] (0.287)
	Class B minimum 30 -30.9	6 (27)	30.46 [a] (0.116)
	Class C minimum 28 to 28.9	2 (10)	28.10 [b] (0.100)
Fat, g/L	Class A desirable ≥ 32to	10 (46)	35.62 [a] (3.949)
	Class B minimum 31	1 (4)	31.50 [a]
	Class C minimum 30	11 (50)	27.01 [b] (1.138)
Lactose, g/L	Desirable 47.5 To	15 (73)	47.74[a] (0.495)
	Not desirable < 44	7 (27)	42.80[b] (0.728)
Lactic acid, g/L	Not desirable ≥ 1.9	15 (68)	2.438[b] (0.076)
	Desirable 1.3 - 1.8 to	4 (18)	1.720 [b] (0.080)
	Not desirable ≤ 1.29	3 (14)	1.153 [b] (0.016)
pH	Not desirable ≥ 6.9	4 (18)	6.95 [b] (0.016)
	Desirable 6.5-6.8 to	15 (68)	6.70 [a] (0.045)
	Not desirable ≤ 6.4	3 (14)	6.33 [a] (0.066)

SD= standard deviation.

* NMX-F-700-COFOCALEC-2012.

[ab] Mean values of each variable with different letters differ significantly (P<0.05) from the parameters.

With regard to lactose, 73 % of the PUs exhibited a desirable level without differences (P>0.05) with respect to the desirable parameter of the NOM. 27 % of the PUs that were identified as having low levels of lactose; a possible explanation for this is that they were also identified with cases of mastitis, as this disease is known to lead to a reduction in the secretion of lactose in milk[15,16]. As for lactic acid, only 18 % of the PU were found to have acceptable levels of compliance with the norm. In addition, 68 % of the PU exceeded the parameter of the norm (P<0.05). One possible explanation is that bacterial populations degrade lactose with storage time, progressively developing acidity in milk[17,18,19]. On the other hand, 68 % of the PU had a desirable average pH, while 18 % of the PU exhibited an average pH > 6.95 (P<0.05) This can be partly explained because the milk is kept in storage at room temperature (between 15 to 32 °C) for more than 6 h; this leads to an increase of lactic microorganisms and, above all, of coliforms and, therefore, to increased acidity[19,20]. The mean titratable acidity (1,720 g/L) and pH (6.70) observed in this study exhibited similar values to those reported for the Argentina region by Oliszewski[13], with an acidity of 1,726 g/L and a pH of 6.75.

Table 2 shows the microbiological quality of milk used in the elaboration of artisanal cheese. 64 % of the PUs obtained desirable (50 %) to acceptable (14 %) mean values with respect to the NMX-F-700-COFOCALEC[11] for aerobic mesophiles. These figures are similar to those reported by Oliszewski (4.94 log CFU/ml)[13]. With regard to the averages of the undesirable classes,[3,4] compared with the reference parameter, a significant difference ($P<0.05$) was detected. Similar results were obtained by Perkins[21] and De la Cruz[14], who pointed out that mesophilic counts of > 6 log are indicative of health issues in the herd. One possible explanation is that the herds do not have a secondary preventive medicine program to identify health issues, including subclinical mastitis, a situation that is reflected in high mesophilic levels.

Table 2: Microbiological quality of milk used in the preparation of cheese at 22 artisanal PU in the region of Ojos Negros, Mexico

Indicator	Reference values (log_{10} CFU/ ml^{-1})	N (%)	Mean (SD)
*Aerobic mesophilic count /ml			
Class 1	≤100,000 UFC/ml (≤5.0) [a]	11 (50) Desirable	1.67 [b] (0.189)
Class 2	101,000-300.000 (5.1 – 5.47)	3 (14) Acceptable	5.11 [a] (0.110)
Class 3	≥301,000 – 599,000 (≥5.48 – 5.77)	1 (4) Not desirable	5.60 [a]
Class 4	≥600,000 – 1,200,000 (≥5.78)	7 (32) Not desirable	7.53 [b] (0.348)
**Coliforms (CFU/ml)	≤10 UFC/ml (≤ 1) [a]	ND Desirable	
	<11-100 UFC/ml (1.04-2.0)	1 (5) Not desirable	2.00 [b]
	≥101 UFC/ml (≥2.1)	21 (95) Not desirable	5.589 [b] (0.384)
[3]*Salmonella spp* 25g	Absence	22 (100)	
[3]*Listeria monocytogenes* 25g	Absence	22 (100)	

SD= standard deviation.

*NMX-F-700-COFOCALEC-2012.

**NOM-243-SSA1-2010. Test methods: Total coliforms <10 CFU (≤1 log10 CFU ml-[1]).

ND= not detected.

[ab] Mean values of each variable with different letters differ significantly ($P<0.05$) from the parameters.

In addition, 100 % (22) of the PU had an average of undesirable coliforms (P<0.05) above the norm (NOM-243-SSA1)[5]. However, the results are lower than those reported by Oliszewski[13] with 5.64 log CFU/ml. The increase in mesophiles and fecal coliform bacteria does not necessarily indicate a direct fecal contamination in milk, but it denotes specific deficiencies in hygiene and milking routines without good management practices during the procurement and storage of milk[14,22,23]. Another important aspect was that none of the PU identified *Salmonella* spp. or *Listeria* spp., which is a desirable result, since these bacteria represent a risk to the consumers' health[24] .

The microbiological quality of the cheese produced in the 22 PU is shown in Table 3 where we can see that only 18 % of the PUs exhibited similar counts (P>0.05) to the desirable parameter of aerobic mesophiles. The rest of the PU exhibited undesirable averages (P<0.05) of mesophiles above the norm (NOM-243-SSA1)[5]. With regard to the coliforms, 100 % of the PU exhibited average figures above the parameter set by the regulations (P<0.05). The high incidence of coliforms and mesophiles in the cheeses of the PU indicates deficiencies in hygiene and manufacturing practices. One possible explanation is that the cheese is made with milk stored at an average temperature of 20 °C. Several studies show that the conservation temperature is an important factor that may damage its stability by increasing the number of altering microorganisms; thus, unless kept under control, these microorganisms may have a direct effect on the quality and useful life of the cheese[19,23,25,26]. It is important to note that values above 6 $\log_{10} \geq$ mesophiles and $\geq$5 $\log^{10}$ coliforms were also reported in other studies[27-30]. Torres[31] mentions that the increase of aerobic mesophiles is considered a normal process in the first 30 d of elaboration of the cheese, because there are chemical reactions that accompany the multiplication of microorganisms during the coagulation and drainage of the serum, due to the presence of lactic acid bacteria (LAB). Pathogenic bacteria such as *Salmonella* spp and *Listeria monocytogenes* were not detected in pressed cheese in compliance with the Mexican regulations.

Table 3: Microbiological quality of artisanal cheese of 22 PU in the region of Ojos Negros, Mexico

Indicator	Reference values[2] (log_{10} CFU/ g^{-1})	N (%)	Mean (SD)
Aerobic mesophilic count	≤100,000 UFC/ml (≤5) [a]	4 (18) Desirable	4.65 [a] (0.472)
	101,000-2,430,000 (5.1 – 6.4)	3 (14) Not desirable	6.43 [b] (0.115)
	>3,120,000 (≥6.5)	15 (68) Not desirable	7.26 [b] (0.420)
Coliforms	<100 UFC (≤ 2.0) [a]	ND Desirable	--
	990 – ≥184,000 (3 – ≥5)	22 (100) Not desirable	5.20 [b] (0.182)
Salmonella spp 25 g	Absence	22(100) Desirable	
Listeria monocytogenes 25 g	Absence	22 (100) Desirable	
*Molds and yeasts	500 UFC/g (2.7) [a]	1 (4) Desirable	2.70 [a]
	>500 UFC/g (>2.71)	21 (96) Not desirable	4.57 [b] (0.230)

SD= standard deviation.

*NOM-243- SSA1-2010 total coliforms <100 UFC (≤ 2.0 Log10 CFU ml^{-1})

[ab] Mean values of each variable with different letters differ significantly (P<0.05) from the parameters.

Conclusions and implications

The results show that certain parameters of physicochemical quality, such as protein, fat and lactose, outweigh the desirable parameters established by the norms. Another important aspect was that none of the PUs identified *Salmonella* spp. or *Listeria* spp.—a desirable result, since these bacteria represent a risk to consumers' health. Certain indicators of the sanitary quality of milk and cheese are above the parameters established by the regulations. Therefore, it is imperative that these PUs continue working on the implementation of a preventive medicine program that addresses the cases of infectious mastitis, and, above all, that they continue to train producers to improve the microbiological quality of milk and cheese and thus reduce those

indicators that are outside the norm. The artisanal production of the Ojos Negros cheese has a remarkable cultural, gastronomic and economic relevance in the region; however, its processes will have to be improved in order to comply with the requirements established by the current legislation and thereby prevent public health issues that may affect the marketing of their products.

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