Evaluation of Microbiological and Toxicological Quality (Heavy Metals) in Fresh Artisan Cheese Commercialized in Puebla City, Mexico

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Abstract—Artisanal fresh cheese made from unpasteurized milk and under uncontrolled production conditions represents an important focus for public health in developing countries. The present work was carried out with the purpose of evaluating the microbiological and toxicological quality (heavy metals) in fresh artisanal cheeses and marketed in the municipality of Puebla. The process was divided into two samplings covering the months of September, October and November of 2016, the second sampling was carried out in the months of February, March and June of 2017, processing a total of 89 samples from 10 markets located in the urban area of the municipality of Puebla. The microbial growth of mesophilic bacteria and total coliform bacteria was evaluated by the plate count technique, in accordance with the guidelines of Bacteriological Analytical Manual from the Food and Drug Administration. The results showed the following average counts for both samplings and all markets: for aerobic mesophyll bacteria 5.55 log CFU/g and for total coliforms of 3.80 log CFU/g. The quantification of heavy metals was carried out by the technique of atomic absorption spectrophotometry. The average results obtained were, for copper 1.81 mg/kg, chromium 0.06 mg/kg, lead 2.96 mg/kg, cadmium 0.13 mg/kg and for the element mercury was 0.03 μg/kg.

Index Terms—Fresh cheese, bacterial counts, heavy metals, atomic absorption spectroscopy, dairy products

I. INTRODUCTION

Cheese production in Mexico between 2008 and 2017 went from 150,000 tons in 2008 to 469,408 tons until May 2017 [1]. The production of cheese constitutes a substantial economic outlet for small and medium milk producers due to the low profitability of their activity, originated by the increase in the prices of inputs for production and the low margin of appropriation of the surplus in the agroindustrial chain. The artisanal cheese making, therefore, is very important, not only because it produces a product with recognized sensory properties, but also because of its capacity to generate and maintain rural employment for a large number of agents in the agribusiness chain of milk; This is: farmers, cheesemakers and merchants [2].

Presence of pathogenic microorganisms in cheese depends on the quality and thermal treatment of the milk, the sanitization procedures of the cheese factory, the quality of the crops, curd handling during processing, storage temperature, transport and distribution of the pieces and many other factors related to the process in general. The foregoing is important due to the high levels of humidity presented by fresh Mexican cheeses elaborated in an artisanal way, which are mostly commercialized at room temperature, which causes an development of ideal means for pathogenic microorganisms that causes infections and food poisonings [3].

On the other hand, it is well known that bovine milk is a food with high nutritional value and its composition varies depending on the breed, feeding, age, lactation period of the cow, time of year and milking system, among other factors. However, when it is produced under conditions of contamination it can be a reservoir of substances, compounds and/or elements such as heavy metals, which are highly toxic, are not

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biodegradable and consequently are considered as causing serious problems to public health, since they are carcinogenic and mutagenic cytotoxic [4].

In 2017, Castro-Gonzalez and collaborators reported that dairy cattle in the Alto Balsas sub-basin in Puebla and Tlaxcala consume a large amount of dry matter daily from alfalfa plants grown on agricultural soils contaminated with heavy metals, which they are of anthropogenic origin, alfalfa being considered as a plant tolerant to heavy metals, tends to bioaccumulate them in the edible parts and thereby cause contamination in animals that consume them, thereby affecting milk production putting at risk population's health. The main objective of this work was to determine the sanitary and toxicological quality of fresh cheeses marketed in Puebla city by accounting for aerobic mesophilic bacteria, total coliforms and the quantification of heavy metals such as Cd, Cr, Cu, Pb and Hg.

II. MATERIALS AND METHODS

A. Sample Collection

A total of 89 samples were collected in aleatory way from 10 markets established in Puebla city during two different periods of the year. The first one was covering September, October and November of 2016 and the second one in the months of February, March and June of 2017. Data of markets and their location are shown in

 TABLE I.
 LOCATION OF MARKETS IN PUEBLA CITY AND DATES OF SAMPLING COLLECTION.

Market Name	Abb	LZC	SD	NPC
Melchor Ocampo	Melchor Ocampo "El Carmen" MO 72530	70520	06/09/2016	3
"El Carmen"		12530	24/02/2017	4
Venustiano Carranza	VC	72090	11/03/2016	3
			03/16/2017	3
Independencia	Independencia IN 72240	72240	26/10/2016	4
Independencia	110	/2240	22/01/2017	4
5 de Mayo	5M	72000	12/10/2016	5
			06/08/2017	5
Acocota	AC	72000	01/06/2017	4
			06/25/2017	4
Nicolás Bravo	NB	72000	03/01/2016	5
			02/16/2017	5
Emiliano Zanata	FZ 72470	10/23/2016	5	
Eumano Eupau		12410	03/14/2017	5
28 de Octubre	vo	72230	10/20/2016	7
			03/12/2017	7
Defensores de la DR Republica	72090	11/07/2016	5	
	2.0		05/19/2017	5
Xonacatenec	хо	72310	01/08/2017	3
ronacatepee			07/25/2017	3

LZC= Location, Zip Code SD= Sampling Date NPC=Number of pieces collected

B. Microbiological Analysis

Microbiological analysis: Preparation and dilution of samples for microbiological analysis was carried out following the protocol of ISO 6887-1983 Microbiology General Guidance. Samples were collected in sterile plastic bags (Nasco whirl-pack, USA) and brought in ice boxes. All samples were stored at -20 °C until analyzed. Account of aerobic mesophilic bacteria and total

coliform bacteria was performed following the conventional method of plate count according with the Bacteriological Analytical Manual from the Food and Drug Administration (USA).

C. Heavy Metals Determination

Quantification of heavy metals was performed in a Perkinelmer AAnalyst 200 spectrophotometer (PerkinElmer, USA) using hollow cathode lamps and certified standards obtained from Perkinelmer too for production of calibration curves. Previously the samples were digested following the Dry Ashing Method according to the guidelines of manufacturer as follows: It was placed 5 g of sample in a well-glazed porcelain dish. In a furnace they were brought to 550 °C, and maintained temperature for 4 hours. Samples were cooled, added 10 mL of 3N HCl and boiled for 10 minutes. Cooled, filtered into a 100 mL volumetric flask, and diluted to volume with deionized water. Table II shows the equipment conditions. Mercury was determined in the same equipment adding the accessory for generation of hydrides with a spectral bandwidth of the monochromator of 0.7 nm at a wavelength of 253.7 nm.

D. Data Analysis

Statistical differences were computed trough Minitab 16[®] Software. For calculation of mean values and standard deviation, Microsoft Excel[®] (2010) was used.

Metal	Wavelength (nm)	Slit Width (nm)	Calibration equation
Cd	228.80	2.7/1.35	LTZ
Cr	357.87	2.7/0.8	LTZ
Cu	324.75	2.7/0.8	LTZ
Pb	283.31	2.7/1.05	LTZ
Cd	228.80	2.7/1.35	LTZ
Cr	357.87	2.7/0.8	LTZ

 TABLE II.
 Atomic Absorption Equipment Conditions for CD, CR, CU and PB Quantification

LTZ= Linear Through Zero

III. RESULTS AND DISCUSSION

A. Aerobic Mesophilic Bacteria

Fig. 1 shows results for the both sampling periods. In the first one, the highest number of aerobic mesophilic microorganisms was found in Venustiano Carranza market with a value of 7.95 log CFU/g, in contrast the lowest value was presented in Independencia market with 3.95 log CFU/g. Average value of all markets for the first sample collection was 5.51 ± 1.17 log CFU/g. In the second sampling the greater number of microorganisms was in the Defensores de la República market with 6.47 log CFU/g and the lowest growth was found in the Melchor Ocampo market with 4.29 Log CFU/g. However, the average value of these microorganisms in all markets for this second sampling was 5.58 ± 0.6 log CFU/g which does not represent a significant difference (P> 0.05) between both sampling periods. Taking both samples into account, the average was $5.55 \pm 0.91 \log \text{CFU/g}$.

■ 1st. ■ 2nd.

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 Figure 1.
 Number of aerobic mesophilic bacteria in artisanal fresh cheese collected from the markets of the Municipality of Puebla during the months of September, October and November 2016 (1st) and February, March and June 2017 (2nd). Venustiano Carranza (VC), Nicol ás Bravo (NB), Melchor Ocampo (MO) May 5 (5M), October 28 (VO), Emiliano Zapata (EZ), Defenders of the Republic (DR), Independence (IN), Xonaca (XO) and Acocota (AC).

Cheese is a fermented food that during its preparation can normally reach microbial populations of the order 9 log CFU/g. However, foods that contain more than 6 log CFU/g may show decomposition detected by smell, taste or appearance [5]. In 2009, Romero-Castillo and collaborators reported accounts of 6.90 log CFU/g of aerobic mesophiles in cream cheese from Tonala Chiapas, on the other hand, Resendiz *et al.*, in 2010 reported 7.5 log CFU/g in fresh cheese prepared from of unpasteurized milk [6]. In Tuzuapan Puebla, S ánchez and collaborators in 2016 obtained an average growth of 9.26 log CFU/g in artisanal cheese in Estado de Mexico [7].

The results found in this work are below those obtained by these authors, although the NOM-243-SSA1-2010 a Mexican standard applied to chees manufacturing [8], does not mention the maximum permissible limit of aerobic mesophilic bacteria for the fresh cheese of artisan elaboration, the reported finding reveals the scarce hygienic treatment with which the product is elaborated can alter or modify the sensory characteristics of the product.

B. Total Coliform Bacteria

In Mexico the maximum permissible limit of coliforms is stablished by the Mexican standard NOM-243-SSA1-2010. This limit in dairy products must be ≤ 20 CFU/g or ml (≤ 1.30 log CFU/g). Fig. 2 shows that none market sampled comply with this limit. In the first sampling, an average value of 4.35 ± 1.64 log CFU/g was reached and during the second sample of 3.26 ± 1.62 log CFU/g, there being a significant difference (P <0.05) between both results. These results can be explained by the fact that, during the months of the second sampling, some retailers refrigerate their product due to an increase in the environmental temperature.

Taking both samples into account, the average of total coliforms was $3.80 \pm 1.59 \log \text{CFU/g}$, with a maximum value of 7.99 log CFU/g and a minimum of 1.38 log CFU/g. Gonz ález and Franco in 2015 reported 6.94 log CFU/g of coliforms in Oaxacan aro cheese [9], similar results were obtained by Romero-Castillo and collaborators in 2009 in Mexican tropical cheese from the Tonala region, Chiapas with 7.28 log CFU/g, as well as S ánchez J. and collaborators in 2016 report 9.27 log CFU/g in artisan cheese from the municipality of Zacazonapan, Estado de M éxico.

C. Cd

Cadmium is considered highly toxic in humans even in small quantities, which is why its finding in food becomes more important [10]. Fig. 3 shows the found values of this element in both sampling periods and the different markets in Puebla city. The highest values in the first sampling were the Nicolas Bravo and Independencia markets with 0.39 and 0.38 mg/Kg respectively while the lowest value was presented in the Venustiano Carranza market with 0.04 mg/Kg, the average value for this first period was 0.13 ± 0.14 mg/Kg. The sampling rate was 0.13 ± 0.14 mg/Kg and there were significant differences among markets (P <0.05).

For the second sampling, a maximum value of 0.55 mg/Kg was found in the Independencia market and a minimum of 0.03 mg/Kg in the 28 de Octubre market with an average value of 0.14 \pm 0.17 mg/Kg. No statistical difference was found between the two sampling periods (P> 0.05).The total average considering both samples and the total markets was 0.13 \pm 0.15 mg/kg.



Figure 2. Number of total coliform bacteria in artisanal fresh cheese collected from the markets of the Municipality of Puebla during the months of September, October and November 2016 (1st) and February, March and June 2017 (2nd). Venustiano Carranza (VC), Nicol & Bravo (NB), Melchor Ocampo (MO) May 5 (5M), October 28 (VO), Emiliano Zapata (EZ), Defenders of the Republic (DR), Independence (IN), Xonaca (XO) and Acocota (AC).

This value is higher than those reported by Meshref and collaborators in 2014 in Egyptian cheese with 0.051 mg/kg [11] and also by what is reported in white cheese collected in different supermarkets in the city of Ramallah in Palestine with content between 7.75 and 55.25 μ g/Kg [12]. Our results are similar to those

found by Vural and collaborators in 2017 who report values of 0.175 mg/kg in herbal cheese in Turkey [13] and with Anastasio and collaborators in 2006 who reported values of 0.13, 0.19 and 0.11 mg/kg in Riccota cheese, fresh and ripened respectively [14].

D. Cr

Concentration of this element for both samplings in different markets and both sampling periods can be observed in Fig. 4. For the firstone, an average concentration of 0.07 \pm 0.03 mg/Kg was found with a maximum value of 0.12 mg/Kg in the 5 de Mayo market and a minimum of 0.03 mg/Kg in the 28 de Octubre market, with a significant difference among markets (P>0.05). For the second sampling, the maximum was found again in the May 5 market with 0.10 mg/Kg and the minimum in the Xonaca market with 0.03 mg/Kg. The average value for the second sampling was 0.06 \pm 0.03 mg/Kg. Taking into account both periods and all the markets, there is an average value of 0.06 \pm 0.03 mg/Kg and no significant difference was found between the two sampling periods (P >0.05). This value is lower compared to studies in Ricotta cheese, 0.32 mg/Kg, fresh, 0.46 mg/Kg and mature 0.47 mg/kg in Italy reported by Anastasio et al in 2006 and higher compared to fresh cow cheese collected southeast of Anatoli, Turkey with a concentration of 3.5 mg/kg mentioned by Vural et al in 2007, but they are within the range published by Mendil in 2006 who reports values between 0.02 and 0.62 mg/Kg in nine cheese varieties also in Turkey [15]. In Mexico in 2001 Grijalva-Haro and collaborators reported a Cr content of 0.4051 mg/Kg in fresh cheese [16]. Our findings are slightly higher than those reported by Castro- Gonzalez in 2018 who obtained concentrations of 0.01 and 0.02 mg/kg for Oaxaca and Ranchero cheese respectively in Puebla, Mexico. It is estimated that the total body content of an adult chromium ranges from 4 to 6 mg. The usual intake of chromium varies between 25 and 35 mg/day, that recommended for the American and Canadian population is 0.035 mg/kg-day for men and 0.025 mg/kg-day for women [17]

E. Cu

As is shown in Fig. 5 in the first sampling period the market with the highest copper concentration was the Nicol ás Bravo market with 5.17 mg/kg, and on the other side, the Emiliano Zapata market found the lowest value with 0.16 mg/kg. . The average value among markets during this first sampling for this element was 1.99 \pm 1.48 mg/Kg and there was a significant difference (P> 0.05) among markets. In the second sampling period the highest value was also presented in the Nicolás Bravo market with a concentration of 3.30 mg/kg, while the Acocota market had the lowest concentration with 0.17 mg/kg. The average value for this second sampling was 1.64 ± 0.92 mg/Kg, showing significant differences among markets (P<0.05) but not between one sampling and another (P>0.05). The average value during the entire sampling period and taking into account all markets was 1.81 ± 1.21 mg/kg. This great

variability between one market and another can be explained by the difference between processing protocols among producers, who in many cases they continue using copper utensils in their processes, although some others have already abandoned this practice.

According with Reilley, in adults the total body copper content ranges from 50 to 120 mg. Copper is widely distributed in foods, including animal products except cow's milk, which is a poor source of copper, contains 0.015 to 0.18 mg/L. The FAO/WHO committee set a provisional value of maximum daily allowable intake of 0.5 mg/kg of body weight. On the other hand, the Recommended Dietary Allowance (RDA) proposed by Reilly too, establishes that this should not be higher than 0.9 mg/Kg-day for men and women. Comparing our results with other works it can be mentioned that ours is below that found in herb cheese from southeast Anatolia, Turkey that was 2.65 mg/kg, according to Vural in 2007, however, our values are above of those reported by Durali in 2006 who report a Cu content of 0.10 to 0.17 mg/Kg in different types of cheese. On the other hand, Aly and collaborators in 2018 report an average of 0.47 mg/Kg in white cheese made in Jeddah Saudi Arabia and in Ramallah Palestine Swaileh and collaborators in 2012 report concentrations ranging from 0.61 to 1.23 mg/Kg also in white cheese. In Puebla, Mexico, Castro-Gonzalez in 2018 reported values of 0.02 mg/kg in Oaxaca and Ranchero cheese [18], which is well below what was reported by the other authors and what was found by us.



Figure 3. Concentration of Cd in artisanal fresh cheese collected from the markets of Puebla city during the months of September, October and November 2016 (1st.) and February, March and June 2017 (2nd). Nicol ás Bravo (NB), Venustiano Carranza (VC), Melchor Ocampo (MO) 5 de Mayo (5M), 28 de Octubre (VO), Emiliano Zapata

(EZ), Defensores de la Republica (DR), Independencia (IN), Xonaca (XO) and Acocota (AC).

F. Pb

Lead is capable of causing a toxic effect through food, it can come from various sources of contamination; pipes or water distribution networks, environmental contamination, food packaging, industries, manufacturing tools and even bioaccumulate in alfalfa and later on to go to cow's milk, according to Castro-Gonzalez in 2018. NOM-243-SSA1-2010 stablishes that the maximum allowable limit for fresh cheese should not exceed 0.5 mg/kg, on the other hand, the Codex Alimentarius stablishes that the content of lead in milk must be less than or equal to 0.02 mg/kg [19]. Fig. 6 shows the results found for both sampling periods and all markets. It can be seen that with the exception of the Venustiano Carranza, Melchor Ocampo and 28 de Octubre markets the rest is above 0.5 mg/Kg.



Figure 4. Concentration of Cr in artisanal fresh cheese collected from the markets of Puebla city during the months of September, October and November 2016 (1st.) and February, March and June 2017 (2nd). Nicol ás Bravo (NB), Venustiano Carranza (VC), Melchor Ocampo (MO) 5 de Mayo (5M), 28 de Octubre (VO), Emiliano Zapata (EZ), Defensores de la Republica (DR), Independencia (IN), Xonaca (XO) and Acocota (AC).



Figure 5. Concentration of Cu in artisanal fresh cheese collected from the markets of Puebla city during the months of September,
October and November 2016 (1st.) and February, March and June 2017 (2nd). Nicol ás Bravo (NB), Venustiano Carranza (VC), Melchor
Ocampo (MO) 5 de Mayo (5M), 28 de Octubre (VO), Emiliano Zapata (EZ), Defensores de la Republica (DR), Independencia (IN), Xonaca (XO) and Acocota (AC).

The maximum value for both periods is reached by the Xonaca market with 9.28 and 8.29 mg/kg for the first and second periods, respectively. The lowest values are located in the Venustiano Carranza market with 0.19 and 0.10 mg/Kg for the first and second sampling periods.

The total average covering the two periods and all the markets was 2.96 ± 2.77 mg/Kg. Other works show that these results are superior, for example, to those reported by Meshref and collaborators in 2014 in

Kareish cheese produced in Egypt who report a concentration of 0.43 mg/kg, on the other hand, Anastasio and collaborators in 2006 analyzed cheese fresh, mature and Ricotta finding concentrations of this element with values of 0.47, 0.58 and 0.391 mg/kg respectively. Aly and collaborators in 2010 reported an average concentration of this element of 0.47 mg/Kg in white cheese marketed in Jeddah Saudi Arabia [20]. In Puebla, Mexico Castro-Gonz alez and collaborators in 2018 reported values of 0.05 mg/kg of Pb in Oaxaca cheese and 0.11 mg/kg for Ranchero cheese.

The same working group in 2017 previously reported high levels of health risk in people who consume food irrigated with water from the Alto Balsas River mainly in the municipalities of Tlaxcala and Puebla since they found high levels of heavy metals, specially of Pb that tends to bioaccumulate in alfalfa and later passes to the milk when the cows are fed.

G. Hg

Hg was only found during the first sampling period in the Defensores de la República market at a concentration of $0.03 \pm 0.002 \,\mu\text{g/Kg}$, which is above that reported by Anastasio and collaborators in 2006 in Ricotta cheese, fresh and mature who report values of 0.0017, 0.008 and $0.0056 \,\text{mg/Kg}$ respectively.

Figure 6. Concentration of Pb in artisanal fresh cheese collected from the markets of Puebla city during the months of September, October and November 2016 (1st.) and February, March and June 2017

(2nd). Nicol ás Bravo (NB), Venustiano Carranza (VC), Melchor Ocampo (MO) 5 de Mayo (5M), 28 de Octubre (VO), Emiliano Zapata (EZ), Defensores de la Republica (DR), Independencia (IN), Xonaca (XO) and Acocota (AC).

IV. CONCLUSIONS

The values found in this work for aerobic mesophilic bacteria were below the values reported by other authors, however, they are sufficient to consider that the hygienic handling of cheese pieces is deficient, which is related to the concentration of total coliforms that, in all markets and sampling periods, was above the value allowed by the official Mexican standard NOM-243-SSA1-2010 and overall international standards.

As for heavy metals, the content of Cr and Cd were within the limits stipulated by international norms and regulations as well as that reported by other authors. However, Cu and especially Pb resulted in values well above those recommended as safe and innocuous for human consumption. The Hg was only found in a single market and during a single sampling period so a deeper monitoring is suggested to corroborate or discard this result. The statistical analyzes showed that there are differences between the concentrations of heavy metals from one market to another, a fact that can be explained, because the retailers buy from producers from different places some near the municipality of Puebla and some others far from it. With the exception of the concentration of total coliform bacteria, the rest of the parameters measured in this study did not show significant differences between one sampling period and another.

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