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ORIGINAL ARTICLE

Effect of Good Hygiene Practices Implementation in the Milk Sanitary Quality Used in the Cotija Cheese Elaboration

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ABSTRACT

In order to improve the milk sanitary quality used in the Cotija cheese elaboration, a Good Hygienic Practices (GHP) training program was implemented in 20 dairy farms in the municipality of Cotija, Michoacán-Mexico. Two samplings were made in each of the dairy farms, one before and the other after of the GHP implementation. The sampling points were: raw milk, milkers' hands, udders and utensils used in the milking process; in all of them, the Mesophilic Aerobic Bacteria (MAB) and Total Coliform Organisms (TCO) were determined. Pathogens such as Salmonella spp, E. coli and Staphylococcus aureus were also determined in raw milk, according the Norms. Water samples used in the cleaning process in each of the dairy farms were also taken, to which it were determined their fecal coliforms content, according the Norm. After GHP implementation, the average content of MAB and TCO in raw milk was reduced by 97.84% and 89.74%, respectively, and in both cases were below the maximum limits allowed by the Official Norms. The initial load of these microorganisms on the milker's hands was reduced by 97.9% and 95.7%, but only 85.75% and 83.33% of the samples had a content lower to the maximum limit accepted by the Norm. By other hand, in the cow udders the reduction was of 99.2% and 97.2%, and in the utensils used in the milking process, the reduction was of 93.3% and 87.69% respectively and their average quantities in all samples were lower than maximum limit allowing by the Official Norms. With the GHP implementation in the milking process, the presence of pathogenic microorganisms in milk also was reduced considerably, its presence only was detected in 4.17% of the analyzed samples. Finally FCO count in the water was reduced in 93.75% but it was still above the provisions of the Norm.

Keywords: Milk, Cotija cheese, Good Hygienic Practices, Sierra Jal-Mich.

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INTRODUTION

Cheese is a much-appreciated food by man because of its nutritional and sensory qualities, it has been made for centuries from milk from cows, sheep, goats and other ruminants [1]. The Cheese making in Mexico began with the arrival of the Spanish in the sixteenth century who brought various animals such as cows and goats, from which they obtained milk for the elaboration a wide cheeses variety [2]. Because of its strong historical roots and its own distinctive features, currently in Mexico various types of cheeses handmade are produced, which they are highly appreciated by the local population. Some of the most popular traditional cheeses in Mexico are Oaxaca cheese, Chihuahua cheese and Cotija cheese between others, the latter is known too as the "mexican parmesan cheese" [3].

The Cotija cheese was born because of the Spaniards settlement in the Cotija valley and surrounding, who in search of gold and clearances for their animals transformed this region into a livestock area. The Cotija cheese has been done by more than four centuries by the families living in the mountainous region between Jalisco and Michoacán states, where the cheese is a fundamental icon of cultural and territorial identity of its inhabitants. The Cotija cheese is a handmade product that it is obtained from raw milk of

creole or zebu cattle, which are fed under a system of free grazing. The height conditions, climate and soil, as well as relative humidity and temperature of the area, generate a typical vegetation of the place that coupled with the type of livestock, determine the milk quality and of course of the cheese quality that with it is elaborates [4].

The Cotija cheese is an uncooked cheese, ripened, with friable texture, strong flavor and salted, hard pasta, cylindrical in shape and large size, weighing approximately 20 kg (Figure 1), distinctive characteristics that make it unique in the world. It has a wide acceptance in the domestic market and consumption is increasing in the United States of America, where now a days live more than thirty millions of Mexican people. The Cotija cheese obtained the collective brand "Cotija Cheese Region of Origin" in 2005 and won the "Best foreign cheese" in the championship world of quality cheeses held in Cremona, Italy, in 2006 and has received awards too for its texture, taste and odor characteristics [5].

Since the Cotija cheese is made from unpasteurized raw milk, and in its elaboration process is not used any chemical preservatives that minimize or eliminate the bacterial load, its sanitary quality is determined by two main factors that are the sanitary quality of milk and the hygienic conditions of the process [5]. In order to produce milk with high bacteriological quality, it should pay careful attention to hygiene of all process operations, since milk is a real breeding ground for microorganisms and it is contaminated easily. The fresh milk from a healthy cow leaves the udder at a temperature of 37°C and is virtually free of bacteria, but must be cooled and protected from contamination as soon as it gets, since microorganisms able to impair its quality are everywhere. The cow udders, milkers' hands, air, water, feed, soil, utensils and milking equipment are the main sources of contamination of milk [6].

Microbial rules exist for assessing the level of hygiene that have been obtained, processed and stored the foods. The indicator microorganisms are used to evaluate the quality and safety of raw or processed foods, in addition to assess and verify the effectiveness of the systems to reduce health risks such as the Good Manufacturing Practices (GMP) and the Good Hygienic Practices (GHP) [7]. Among bacteriological tests commonly used for the determination of indicator microorganisms are taken: the determination of Mesophilic Aerobic Bacteria (MAB) and the determination of Total Coliform Organisms (TCO) [8].

One of the main reasons of because the Cotija cheese has not had the desired commercial peak, is due to little or null application of good hygiene practices in both, the milking process as in the elaboration process, maturation, storage, transport and handling of cheese Cotija. Therefore, the objective of this research was to improve the microbiological quality of the milk used in the elaboration of the Cotija cheese, through training and implementation of Good Hygienic Practice in the milking process.

MATERIALS AND METHODS

Investigation Strategy

Twenty dairy farms located in the Cotija municipality from Michoacan state, Mexico, were selected for this study. Between July and November 2012, monthly sampling were conducted during milking of cattle herds. The sample points were the cow udders, milkers' hands, the utensils and equipment used for milking and milk obtained. Samples of the water used for cleaning were also taken. On each occasion, three samples of each of the selected points in each of the dairy farms included in the study were taken.

A training program in Good Hygiene Practices was implemented to all staff working in the various participating dairy farms from January to June 2013. During the training, emphasis was placed on the importance of microbiological quality of milk, the quality of water used, and the hygiene of all actors involved in the milking process, as are facilities, staff, udder cows, utensils and equipment. The staff was trained also in procedures for washing, cleaning and disinfection of all stakeholders in the process of milking. After the training and implementation of Good Hygienic Practices in the milking process, during the months of July to November 2013 again the samples were taken in the same previously selected sampling points.

Sampling

The milk and water samples were collected in sterile glass bottles of 100 mL. The flotation technique with a sterile swab moistened with peptone broth 0.1% was used in sampling the cow udders, milkers' hands, the utensils and equipment used during milking process. This technique consists of passing the swabs over an area of 25 cm^2 bounded by a template [9]. All samples were placed in a portable cooler at 4°C and transported to the CIIDIR's laboratory , where they were analyzed in a period no longer than 2 hours after being obtained.

Milk percentage chemical analysis

The moisture content, ash, fat, protein and total carbohydrates in the different milk samples was determined according to the Official Methods of Analysis [10].

Microbiological analysis

The quantification of Mesophilic Aerobic Bacteria (MAB) in both milk and in the sampled surfaces was carried out according to the methodology proposed in NOM-092-SSA1-1994 [11], while quantifying the Total Coliform Organisms (TCO) was based in the methodology proposed in the NOM-113-SSA1-1994 [12]. In water, total coliforms organisms and *Escherichia coli* were determined according to the technique of the most probable number (NOM-112-SSA1-1994) [13]. In milk, *Salmonella spp* was also determined according to the methodology proposed in NOM-114-SSA1-1994 [14], *Staphylococcus aureus* was determined according with NOM-115-SSA1-1994 [15], and the detection and quantification of *Escherichia coli* was determined by the MUG + fluorescence method.

Statistical Analysis

For the variables MAB and TCO, was used an experimental design of paired samples, where the results before and after the application of Good Hygienic Practice in the milking process was compared using student t test at a significance level of 0.05 [16]. In the statistical analysis the package Statgraphics plus version 5 was used. For the frequency variables *Escherichia coli, Staphylococcus aureus* and *Salmonella spp*, descriptive statistics were used.

RESULTS AND DISCUSSION

Milk percentage chemical analysis

The large scatter in the results obtained on the milk composition (Table 1) is attributable to factors such as different animal's breeds, the feeding and the stage of lactation, the animal's age, zootechnical factors, climatic factors, and physiological factors, between others [6]. Fats and proteins are the most valuable components of milk in the cheese making process [17], its content on a dry basis was 31.93 ± 1.48 % and to 27.19 ± 1.33 %, respectively.

Bacteriological milk analysis

The MAB burden found in the different analyzed milk samples before the GHP implementing are shown in Figure 2. The average content of Mesophilic Aerobic Bacteria was 5.520 ± 0.945 log CFU/mL. The content of MAB in 77% of samples was less than the maximum limit allowed by the Mexican Official Norm NMX-F-700-COFOCALEC-2004 [18], which establishes a maximum burden of MAB of 10^6 CFU/mL. Therefore, the remaining 23% of the samples exceeded this limit imposed by this norm.

As an indicator of the hygiene with the milking process is performed, the MAB count is a factor taken into account in the milk price. Some buyers set better prices e incentives for milk that has load low of MAB [19]. On the other hand, the high counts of MAB in raw products do not necessarily indicate a risk to consumer health; however, when no information about the conditions of sanitation and control on production, a MAB count may constitute a valuable reference on the microbiological quality of food [17, 20].

The TCO content in the milk samples analyzed before the GHP implementation was widely dispersed (Figure 3). The average content was 5.02 ± 1.10 log CFU/mL and the dispersion was greater than in the MAB content. It is noteworthy that in Mexico there is not a reference value which can set a limit for this microorganism's indicator group. After the GHP implementation, the TCO content in the milk samples decreased to 4.03 ± 0.37 log CFU/mL only.

The MAB and TCO contents in the different sampling points, which were obtained before and after implementation of the program of GHP in the milking process, are shown in Table 2. Different letters in the same sampling point and the same microorganism type, indicate statistically significant difference ($p<0.05$). In all cases, there was a highly significant difference ($p<0.05$) between the results of the MAB and TCO content obtained before with the obtained after the GHP implementing in all the sampled points in the milking process.

After the GHP implementation in the milking process, the MAB content in raw milk was reduced by 97.84 ± 0.14%, while the TCO content decreased by 89.74 ± 3.13%. In the United States of America, the maximum acceptable content of MAB in raw milk is 4 log CFU/mL, unlike the maximum limit set by the Mexican Official Norm NMX-F-700-COFOCALEC-2004 [18], which establishes a maximum of 6 log CFU/mL [19]. The MAB content in 80% of milk samples analyzed after GHP implementation in the milking process was less to 4 log CFU/mL. These results are consistent with those reported by other researchers [21], who agree that the implementation of GHP in dairy farms significantly reduces the bacterial load of milk obtained.

Furthermore, the MAB as TCO content in 100% of samples taken from the milker's hands before implementing the GHP in the milking process was higher than that established by the Mexican Official Norm NOM-093-SSA1-1994 [22], which establishes a maximum 3 X 10^3 UFC/cm^2 for both types of microorganisms. After of GHP implementation, the load of MAB and TCO in the milker's hands was

reduced by 97.9% and 95.7%, but only 85.75% and 83.33% of the samples had a content of MAB and TCO lower to the maximum limit accepted by the Norm respectively.

The high content of MAB and TCO in the cow udders before GHP implementation, are indicative of both the udder and cows teats were not washed and disinfected before the milking process. After GHP implementation, the MAB and TCO content in cow udders decreased by 2.06 and 1.56 log units, respectively. That is, the burden of MAB and TCO in the cow udders fell by 99.2% and 97.2%, respectively. These results reflect the importance of a training continuous of the milkers, on cleaning, washing and disinfection of the cow udders and teats, as well as the cleaning and sealing of the teats of milked cows.

The high bacterial load of both types of indicator microorganisms on the surfaces of the different utensils that are used in the milking process, reflect the lack of cleaning and disinfection of these utensils. Before the GHP implementation, the found quantities of both types of microorganisms, MAB and TCO exceeded the maximum limits permitted by the norms, which are 4x102 and 2x102 CFU/cm2 respectively. With the GHP implementation, counts of MAB and TCO decreased in 93.3% and 87.69%, respectively. With the GHP implementation of, the contents of MAB and TCO on the surfaces of the utensils used in the milking process were less than those established by the Norm in all samples. This means that through of cleaning and sanitization of the milking equipment and utensils, it is possible decrease the bacterial load in them, which would lead to better sanitary quality of milk obtained.

Gran et al., (2001) [23] determined the content of MAB on utensils that are used during the process of milking dairy cattle on small farms in Zimbabwe, Africa; 83% of the analyzed samples had a load of MAB > 300 CFU/20 cm^2. They also found that in 28% of the milk samples sent to the industry, they arrive with a load of MAB > 5 log CFU/mL. They conclude that the high content of MAB in milk upon arrival in the industry, is due to unhygienic of the utensils used in the process of milking, and the increase in bacterial load during the journey from farm to industry to the industry due to the lack of cooling of the milk immediately after milking and during the journey to the industry. By the other hand, Murphy and Boor (2000)[8], reported that the main sources of contamination of the milk with the cheese is made are: dirty or sick animals, dirty equipment, contaminated water and polluted environment.

Determination of fecal coliforms organisms in water

Before the implementation of GHP, the content of Fecal Coliform Organisms (FCO) in the water used for washing the cow udders, milker's hands, equipment and utensils, was FCO = 800 ± 50 CFU/mL, which is indicative that this water is not potable, as established by the Mexican Official standard (NOM-127-SSA1-1994) [24]. This Norm states that water used in the milking process should be free of FCO. After GHP application in the milking process, FCO count in the water was 50 ± 15 CFU/mL, still well above the provisions of the Norm.

On the other hand, the World Health Organization [25] states that the E. coli test must be negative in 100 mL samples of water that it is used in the milking process, because coliforms and E. coli are often used as indicator microorganisms and their presence implies the risk that other microorganisms enteric pathogens are also present [26].

Determination of pathogens microorganisms in milk

Before GHP implementation, the presence of *Salmonella* spp was positive in 91.25% of the milk samples, while the presence of *E. coli* and *S. aureus* were positive in 95.83% of the milk samples analyzed. After GHP implementation, the presence of *Salmonella spp* was positive in only 8.75% of milk samples tested, whereas both *E. coli* and *S. aureus* were positive in 4.17% of milk samples. It is known that the presence of *Salmonella spp*, *Escherichia coli* and *Staphylococcus aureus* in milk is mainly due to the lack of hygiene in the milking process [27].

The presence of pathogenic microorganisms in the milk with the Cotija cheese is made represents a high risk to the consumers health, especially if in its production process the milk is not subjected to any treatment either thermal or chemical as use of preservatives, through which the pathogenic microorganisms are removed. Chombo (2003) notes that the maturation process of Cotija cheese is sufficient for complete elimination of pathogenic microorganisms that may be present in the milk that the product is made [28].

Both milk and water are considered among the main responsible foods for diseases transmission, which are generally known as Foodborne Diseases [29, 30]. In spite of the efforts of organizations who care about the population health and the consumer protection, these diseases continue to rise at national and international levels [31].

In general it can be said that in the area of the Jal-Mich saw, as a result of inadequate infrastructure, lack of electricity and drinking water, and lack of worker training in systems to reduce health risks as are the GHP, milk obtained in the different dairy farms in the area, does not meets the quality and safety requirements established by the Mexican Official Standard that applies for this purpose.

Figure 1. Cotija cheese pieces

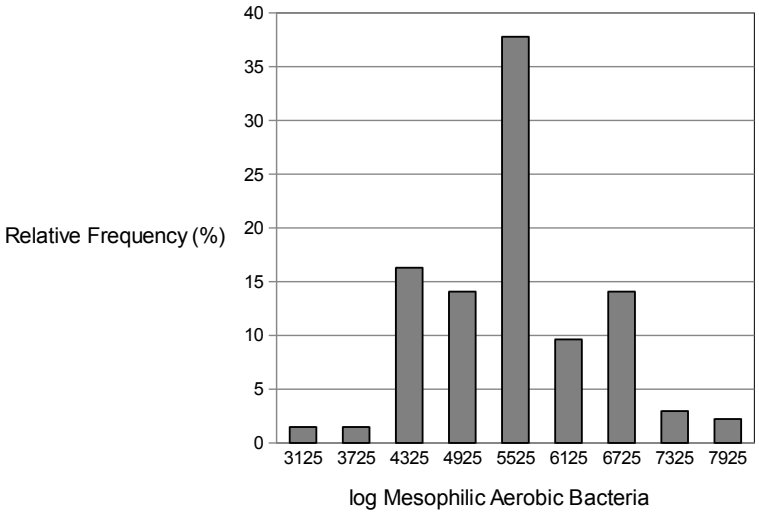

Figure 2: Mesophilic Aerobic Bacteria content in raw milk obtained from different dairy farms before implementing the Good Hygienic Practice

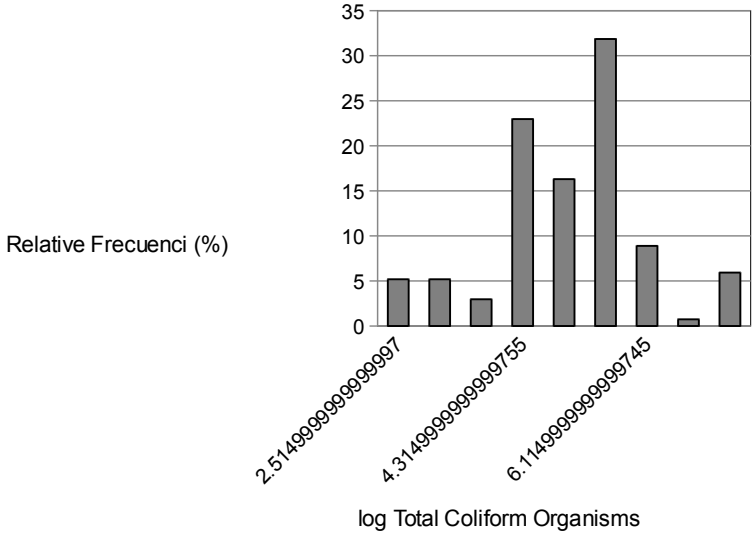

Figure 3: Total Coliform Organisms content in raw milk obtained from different dairy farms before implementing the Good Hygienic Practices

Table 1: Composition of the milk with the Cotija cheese is made

Constituent	Content	
	% Wet basis	% Dry basis
Moisture	86.5 ± 0.95	-------
Total solids	13.5 ± 0.35	100.00
Fats	4.31 ± 0.20	31.93 ± 1.48
Proteins	3.67 ± 0.18	27.19 ± 1.33
Total carbohydrates	4.75 ± 0.15	35.19 ± 1.11
Ashes	0.77 ± 0.10	5.70 ± 0.74
Nonfat solids	9.19 ± 0.15	68.07 ± 1.48

Table 2: Content of MAB and TCO at the different sampling points in the milking process before and after implementing Good Hygiene Practices

Sampling point	M A B		T C O	
	Before GHP	After GHP	Before GHP	After GHP
Raw milk (log CFU/mL)	5.52 ± 0.95^{a}	3.85 ± 0.32^{b}	5.02 ± 1.10^{a}	4.03 ± 0.37^{b}
Milker's hands (log CFU/cm^2)	4.78 ± 0.34^{a}	3.11 ± 0.27^{b}	4.07 ± 0.32^{a}	2.87 ± 0.25^{b}
Caw udders (log CFU/cm^2)	5.29 ± 0.88^{a}	3.23 ± 0.25^{b}	4.78 ± 0.81^{a}	3.22 ± 0.28^{b}
Utensils (CFU/cm^2)	1804 ± 120^{a}	286 ± 36^{b}	1283 ± 125^{a}	158 ± 14^{b}

CONCLUTIONS

Because milk is not subjected to any heat treatment that ensure the elimination of pathogens during the Cotija cheese elaboration, *Salmonella spp, Escherichia coli* and *Staphylococcus aureus* in milk, represent a high risk to the consumers health, whether if milk is consumed raw or as Cotija cheese.

Staff training and implementation of Good Hygienic Practice in the milking process, contributes to the production of milk with better sanitary quality, but has been insufficient to comply with regulations and ensure product safety.

Until there is not availability of potable water for personal hygiene and for washing udders, equipment and utensils used in the process of milking, it will be very difficult for the milk produced meets the quality and safety parameters that set the Norms.

It is necessary that the Federal, State and Municipal government will improve the infrastructure, provide water and electricity to all human settlements found in the Sierra Jal-Mich and thus minimize the factors that impact the quality milk with the traditional Cotija cheese is made.

Research is needed to assess the impact of the milk pasteurization on functional properties and organoleptic characteristics and the sanitary quality of Cotija cheese.

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