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Original

Comparative study of the hygienic-sanitary, physicochemical, and microbiological quality of bovine milk in southeastern Mexico

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ABSTRACT

Objective. Evaluate the hygienic-sanitary, physicochemical and microbiological quality of bovine milk for sale, produced in a family stable and in three public markets in Tabasco state. **Materials and methods.** The hygienic-sanitary quality was determined according to general information of the farm, facilities, milking process, cleaning and disinfection. Physicochemical quality was determined by evaluating total proteins, casein, butyric fat, lactose, non-fatty solids and density. Microbiological quality was determined by titratable acidity, alcohol test, somatic cell content, oxide-reduction potential, foreign matter, bacterial inhibitors, and aerobic mesophilic bacteria. **Results.** The milk produced in the family stable was evaluated with the highest physicochemical and microbiological quality, in reference to the standards evaluated by the official Mexican regulations established in Mexico by the Council for the promotion of the quality of milk and its derivatives, A. C. Instead, milk in three public markets, it was considered as rejection, indicating possible contamination during milking processes, as well as handling and transport to public markets. **Conclusions.** Milk samples from public markets were considered not suitable for human consumption or for the production of by-products.

Keywords: Human nutrition; mastitis; mesophilic bacteria; milk quality; protein; somatic cells (*Source: DeCS*).

RESUMEN

Objetivo. Evaluar la calidad higiénico-sanitaria, fisicoquímica y microbiológica de leche cruda bovina destinada a la venta, producida en un establo familiar y en tres mercados públicos del estado de Tabasco. **Materiales y métodos.** La calidad higiénico-sanitaria se determinó de acuerdo con la información general del predio, instalaciones, proceso de ordeño, limpieza y desinfección. La calidad fisicoquímica se determinó evaluando proteínas totales, caseína, grasa butírica, lactosa, sólidos no

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grasos y densidad. La calidad microbiológica fue determinada mediante la acidez titulable, prueba de alcohol, contenido de células somáticas, potencial de óxido-reducción, materia extraña, inhibidores bacterianos y bacterias mesofílicas aerobias. **Resultados.** La leche producida en el establo familiar, fue evaluada con la máxima calidad fisicoquímica y microbiológica en referencia a los estándares que evalúa la normatividad oficial mexicana establecidas en México por el Consejo para el fomento de la calidad de la leche y sus derivados, A. C. En cambio, la leche en los tres mercados públicos se consideró de rechazo, indicando posible contaminación durante los procesos de ordeña, así como en el manejo y transporte hacia los mercados públicos. **Conclusiones.** Las muestras de leche provenientes de los mercados públicos se consideraron no aptas para consumo humano ni para elaboración de subproductos.

Palabras clave: Bacterias mesofílicas; calidad de leche; células somáticas; mastitis; nutrición humana; proteína (*Fuente: DeCS*).

INTRODUCTION

Milk from cows is of dietary importance because it is considered a complete food for human nutrition. It provides macronutrients such as carbohydrates, lipids, and proteins in addition micronutrients and minerals such as calcium, chlorine, copper, iodine, iron, magnesium, phosphorus, potassium, sodium, and zinc and vitamins such as vitamin A, B6, B12, C, D, and E, thiamine, riboflavin, and folate. The total solids composition of milk, mainly the protein and fat content, enables the production of additional dairy products such as cheese, cream, and butter through technological processes (1). The flavor of milk is mostly determined by the amino acids and short-chain fatty acids (2). Their composition can be influenced by various factors, such as cow breed, genetics, diet, environment, and management (3).

According to the Food and Agriculture Organization of the United Nations, global milk production in 2017 was estimated at more than 652 million tons (4). In 2019, the Agrifood and Fisheries Information Service reported a production of 12,380 tons for Mexico. In tropical countries, most milk comes from dual-purpose bovine production systems. Similarly, in Mexico, more than 30% of dairy production comes from tropical dual-purpose systems (5). In particular, in the tropical state of Tabasco, a production of 103,894 liters was recorded in 2019. Given the scale of milk production and consumption and the importance of milk for human nutrition, it is of interest to verify the quality of the milk produced and distributed in the markets of the state of Tabasco.

It is essential that the milk produced by both small-scale producers and industry comply with the established hygienic-sanitary, physicochemical, and microbiological standards (6). Also, the identification of contaminants, such as foreign matter, formaldehydes, oxidants, and chlorinated derivatives, among others, in raw bovine milk is a matter of importance for public health, especially considering that approximately 75% of produced milk is destined for mass human consumption (7).

One of the main factors influencing the quality of milk during production is hygiene. Adequate hygiene practices must be employed throughout the production process during the pre-milking, milking, and post-milking stages. They must be followed by personnel, during animal handling and use of equipment, and throughout the facilities in order to reduce the risk of contamination with pathogenic microorganisms (8). One of the causes of microbiological contamination that affects the composition and physicochemical characteristics of milk is mastitis, which is often accompanied by an increase in the somatic cell (SC) count. With an increase in the SC count, the composition of milk, enzymatic activity, coagulation time, productivity, and quality of derivatives are negatively impacted. High bacterial and SC levels can have a significant effect even in pasteurized milk and cheese products due to the reduction in lactose, casein, fat, and protein contents, which shortens the life of products and reduces consumer acceptance (1). Also, milk storage and transportation to point of sale are factors in the post-milking stage that can affect the quality of milk for direct consumption or production of derivatives given the potential points of contamination or lack of adequate refrigeration to prevent microbial growth (8).

The objective of the present study was to evaluate the hygienic-sanitary, physicochemical, and microbiological quality of raw cow's milk at the point of production and in several markets where it is distributed for sale in the state of Tabasco, Mexico.

MATERIALS AND METHODS

Geographical location of the production units. The study was carried out in four establishments in the state of Tabasco: a family farm (FF) and three public markets where raw cow's milk is sold for human consumption. The FF was located in the municipality of Cunduacán, Tabasco, Mexico (longitude −93.180556 and latitude 18.146111), at an altitude of 20 meters above sea level. The region has a hot-humid climate, rainfall in the summer, and an average temperature and annual precipitation of 28.7°C and 1940.6 mm, respectively.

The three public markets were located in Villahermosa, Tabasco, Mexico: the Pino Suárez market (longitude −93.3833 and latitude 18.3542), Tianguis Campesino market (longitude −92.9182424 and latitude 17.998573), and Tamulté market (longitude −92.9596527.597 and latitude 17.9713493) (PM, CM and TM, respectively).

Sampling characteristics. The milk sampling comprised a period of 28 days during the *norte* season (December–January), a period of strong northerly winds blowing from the Gulf of Mexico. Samples were collected in sterile phenol-free glass bottles three times a week and were transported to the Bromatology Laboratory of the Academic Division of Health Sciences (ADHS) of the Autonomous Juárez University of Tabasco (Universidad Juárez Autónoma de Tabasco [UJAT]) in a cooler with refrigerant maintained at a temperature of 4°C.

Sampling on the family farm. Sampling was carried out in a semi-intensive system with an average production of 8.6 ± 1.8 kg of milk/cow/day. Thirty-five cows were sampled. The milking is mechanical and performed once a day in the morning (04:00–06:30 h); three workers participate in this activity. The sampling was carried out once the milking was finished. The genotypes were crosses of Holstein × Gyr and Brown Swiss × Gyr. The milking system was integrated with a cooling tank with a storage capacity of 1000 L that maintains milk at 4°C. The FF directly sells raw milk to the public and to artisanal cheese producers.

Sampling in the markets. Sampling was carried out in the public markets PM, CM, and TM where raw cow's milk is sold for human consumption. It was performed simultaneously with the sampling of the FF.

Chemical reagents. Sodium hydroxide, lactose monohydrate, formaldehyde, phosphoric acid, anhydrous sodium sulfate, benzalkonium chloride, xylene, ethanol, yeast extract agar, chromotropic acid disodium salt, hydrochloric acid, starch, glycerin, potassium iodide, methylene blue, acetic acid, phosphate buffer (PBS) adjusted to pH 7.4, methyl red, sulfuric acid, zinc, zinc acetate dihydrate, boric acid, benzoic acid, petroleum ether, copper sulfate in solution, potassium sulfate, potassium, sodium tartrate, sodium thiosulfate, and chloroform were purchased from Merck® Millipore (Germany).

Hygienic-sanitary quality. To evaluate the hygienic-sanitary quality of the milk samples from the FF, a previously described questionnaire was applied (9). It solicits general information about the farm's characteristics, including its facilities and different aspects of the milking, cleaning, and disinfection processes. In public markets, data were acquired through a survey applied to vendors regarding the sanitary specifications of the product and its origin.

The evaluation of the physicochemical and microbiological quality of milk was carried out in accordance with the standards established in Mexico by the Council for the Promotion of the Quality of Milk and its Derivatives (COFOCALEC), which are based on the internationally established methods of the AOAC. The evaluated parameters are described at following.

Physicochemical quality. All determinations were made in triplicate, including total proteins, casein, butterfat, lactose, non-fat solids, and density (6). The utilized methods are described below.

Total proteins. The samples were digested in a Kjeldahl Labconco® apparatus (USA) at 410 to 430 °C. After digestion, they were cooled to room temperature under laboratory conditions (24°C) and were distilled until obtaining a volume of 150 mL. The distillate was titrated with HCl 0.1 N using Wesslow's indicator. The percentage of protein was expressed as g/L.

Casein. The casein was precipitated by placing distilled water in the samples and adding acetic acid solution until reaching a pH of 4.6 according to a pH meter (SM-3BW®, Science Med, Finland). Then, the total proteins were quantified, and the results were expressed in g/L.

Butterfat. The samples were dehydrated in a drying oven (ED-23®, Binder, Germany). Then, they were placed in a fat extractor (GL-45®, Labconco, USA) under continuous extraction. The results were expressed in g/L.

Lactose. The percentage of direct reducers in lactose was determined using a solution of potassium ferrocyanide and zinc acetate. The results were expressed in g/L.

Non-fat solids. Once the total solids and fat content of the milk was determined, the non-fat solids content, composed of lactose, proteins, and mineral salts, was calculated by subtraction. The results were expressed in g/L.

Density. The samples were heated to 40°C in a water bath (DSB-1000D®, Digisystem Lab Instruments, Taiwan) for 5 min and then brought to a temperature of 20°C. A Quevenne thermo-lactodensimeter (71384000®, Nahita, Japan) was used to measure the density, and the results were expressed in g/mL.

Microbiological quality. The following determinations were made in triplicate: titratable acidity, thermostability (via an alcohol test), SC content, oxidation-reduction potential (reductase test), foreign matter, bacterial inhibitors, and total mesophilic bacteria count (6). The utilized methods are described below.

Titratable acidity. Alkalimetric titration with 0.1 N sodium hydroxide using a phenolphthalein solution (1% v/v, in ethanol) as an indicator was performed to measure titratable acidity until a pH of 8.3 was reached according to a pH meter (23-F®, HANNA, Germany). The results were expressed in g/L.

Thermostability (alcohol test). Two mL of 72% ethyl alcohol was added to the milk sample; then, it was shaken and observed to verify the formation of lumps or clots.

Somatic cell content. A smear was prepared on a slide, the SC were stained and observed under the microscope (BX41®, Olympus, Japan) at 400X. The number of SCs was determined by direct counting in a Neubauer chamber using the modified Newman-Lampert stain. The results were expressed as the number of SCs/mL.

Rust-reducing potential (reductase). Milk samples were placed in a tube, and methylene blue was added. They were mixed and then incubated at 37°C in a water bath (DSB1000D®, DS Lab Digital Taiwan), and the time of disappearance of the blue color was recorded. The results were expressed according to the estimated number of bacteria per mL.

Presence of foreign matter. Using a filtration system, the entire samples were processed with the help of a vacuum pump (FE-1500L®, Felisa, Mexico). Foreign material was searched for under a binocular microscope (BX-41®, Olympus, Japan).

Bacterial inhibitors. The presence/absence of formaldehydes, oxidants, quaternary ammonium salts, and chlorinated derivatives was evaluated.

Aerobic mesophilic bacteria. Serial dilutions (10:1, 10:2, 10:3, and 10:4) of the milk samples were made and seeded in duplicate with the spatulation technique on standard count agar (BD, Bioxon) inside a biological safety hood (Class II A/B3, Forma Scientific, USA). They were then incubated in a bacteriological oven (EC-41®, Ríos Rocha, Mexico) at a temperature of 35 ± 2°C for 48 ± 2 h. The bacteria count was performed using a colony counter (CM1-300®, Figursa, Mexico), and the number of colonies was expressed as colony forming units (CFUs) per mL (CFUs/mL).

Analysis of the results. A completely randomized design was used, the treatments were the different sampling sites, and the response variables were those describing the hygienic-sanitary, physicochemical, and microbiological quality of the milk samples. Data were analyzed by a one-way ANOVA test using the Statgraphics version 16 package. The comparison of means was carried out using a Tukey test; the results were considered significant at $p<0.05$ (95% confidence).

RESULTS

The evaluation of the hygienic-sanitary quality included the pre-milking, milking, and post-milking stages in addition to the cleaning, disinfection, and management of the facilities. The FF complied with 85% of the standards evaluated by the questionnaire. However, several aspects of the management of the facilities were not compliant: For example, the walls were not painted a light color. Chickens were present inside the milking room. The water was not purified using an established concentration of chlorine (2 mg/L). And, there were no records of annual physicochemical sampling or biannual microbiological sampling.

In the markets, the owners of the establishments where milk was sold were asked questions about the origin of the milk. None of them had knowledge of the hygienic-sanitary measures followed during the milk collection process nor during its transport to the point of sale. In all cases, the milk was transported without being placed in a cooling tank. It is possible that the milk was previously refrigerated or delivered the same day as milking, although this was not verified.

Based on the Mexican regulations established by the Council for the Promotion of the Quality of Milk and its Derivatives, the physicochemical quality of milk according to the content of butterfat and total proteins can be categorized as class A, B, or C, with class A corresponding with the highest quality. According to the content of casein, lactose, non-fat solids, and density, a sample is accepted or rejected.

Milk from the FF was classified as class A based on its butterfat and total protein content and was "accepted" based on its casein, lactose, and non-fat solids contents and density. However, the samples obtained from the three public markets (CM, PM, and TM) were "rejected" (Table 1).

The milk samples from TM were classified as class B due to the butterfat content. The samples from CM were classified as class C due to the total protein content. The lowest values ($p<0.05$) for butterfat and non-fat solids were observed in the TM samples, and the lowest values ($p<0.05$) of total proteins, casein, and lactose in the CM samples.

Table 1. Evaluation of the physicochemical quality of milk samples from a family farm and three public markets in the state of Tabasco.

Est	Physicochemical parameters of milk					
	BF g/L	**TP g/L**	**Cas g/L**	**Lac g/L**	**NFS g/L**	**Den g/mL**
FF	34.10 ± 0.75 Class A	31.70 ± 0.48 Class A	31.70 ± 0.48 Accepted	45.04 ± 0.06 Accepted	83.70 ± 0.82 Accepted	1.0334 ± 0.01 Accepted
CM	32.46 ± 1.86 Class A	29.27 ± 0.36* Class C	23.01 ± 3.28* Rejected	35.39 ± 4.56* Rejected	80.20 ± 1.31 Rejected	1.0334 ± 0.01 Accepted
PM	32.77 ± 2.98 Class A	30.04 ± 0.42 Class B	23.79 ± 1.50 Rejected	39.88 ± 1.69 Rejected	80.40 ± 2.94 Rejected	1.0302 ± 0.02 Accepted
TM	31.55 ± 0.68 * Class B	31.17 ± 0.16 Class A	23.75 ± 1.46 Rejected	42.06 ± 1.49 Rejected	79.80 ± 2.98* Rejected	1.0311 ± 0.01 Accepted

Est: establishment, BF: butterfat, TP: total proteins, Cas: casein, Lac: lactose, NFS: non-fatty solids, Den: density, FF: family farm, CM: Campesino Market, PM: Pino Suárez Market, TM: Tamulté Market. *Indicates significant statistical difference ($p<0.05$).

Based on the standards of the Council for the Promotion of the Quality of Milk and its Derivatives, the microbiological quality of milk samples can be classified as "approved" or "not approved" according to the titratable acidity test, alcohol test, and presence of foreign matter and bacterial inhibitors. According to the number of aerobic mesophilic bacteria (AMB) and the SC count, the samples can be classified as class 1, 2, 3, or 4, with class 1 corresponding with the highest quality. Finally, according to the reductase test, the samples can be classified as "good," "fair," or "poor."

The samples from the FF had the best microbiological quality. They passed the tests for titratable acidity, alcohol, and presence of foreign matter and bacterial inhibitors. According to the AMB and SC count, they were classified as class 1, and according to the reductase evaluation, as "good." However, the milk samples evaluated in the three markets did not pass the tests for titratable acidity, alcohol, and presence of foreign matter and bacterial inhibitors. According to the reductase test, the quality was poor and, according to the SC count, the samples were in class 2 (Table 2).

Table 2. Evaluation of the microbiological quality of milk samples from a family farm and three public markets in the state of Tabasco.

Est	Microbiological parameters of milk						
	TA g/L	**AT**	**FM**	**BI**	**AMB CFU/mL**	**SC SC/mL**	**RE Bacteria/mL**
FF	1.45 ± 0.05 Approved	Negative Approved	Free Approved	Negative Approved	20500 ± 6476.45 Class 1	28985 ± 2568.18 Class 1	5 h: 100000 at 200000 Good
CM	1.80 ± 0.8 * Not Approved	Positive Not Approved	Presence Not Approved	Positive Not Approved	106.287 ± 5.308 * Class 2	77.345 ± 2.938 Class 2	< 2 h: 2 at 10 million Bad
PM	1.78 ± 0.2 Not Approved	Positive Not Approved	Presence Not Approved	Positive Not Approved	104.928 ± 5.564 Class 2	79.528 ± 2.793 * Class 2	< 2 h: 2 at 10 million Bad
TM	1.76 ± 2.4 Not Approved	Positive Not Approved	Presence Not Approved	Positive Not Approved	104.007 ± 5.390 Class 2	78.610 ± 1.340 Class 2	< 2 h: 2 at 10 million Bad

Est: establishment, TA: titratable acidity, AT: alcohol test, FM: foreign matter, BI: bacterial inhibitors, AMB: aerobic mesophilic bacteria, CFU: colony forming units, SC: somatic cells, RE: reductase, FF: family farm, CM: Campesino Market, PM: Pino Suárez Market, TM: Tamulté Market. *Indicates significant statistical difference ($p<0.05$).

DISCUSSION

Cow's milk is an essential food for human nutrition. It is also processed to obtain a variety of dairy products important for the human diet, such as cheese, given its high fat and protein content (3). It is important that the milk used to manufacture other dairy products is of high quality, as the quality and acceptance of products can be severely affected by inadequate hygiene practices during the milk production process, including the pre-milking, milking, and post-milking stages.

The nutritional quality of the milk samples from the FF was within the range of values considered acceptable for raw cow's milk according to the standards established for Mexico by the Council for the Promotion of the Quality of Milk and its Derivatives. These samples were also categorized as class A because of their protein and casein content (≥ 31 g/L). Another study previously found similar protein values (32.94 g/L) in bovine milk samples taken during the same months as those analyzed herein. To the contrary, in the milk samples from the public markets, the casein values did not reach the minimum required value. This could be attributed to a higher frequency of subclinical mastitis as well as poor hygiene during milking (10). Also, the decrease in casein values could correspond with an increase in serum proteins as a result of the high count of SCs, which can generate proteolytic enzymes that cause a decrease in the casein content (11).

The AMB (> 10000 CFU/mL) and SC counts are measures of the hygiene conditions during milk production. They can affect the quality and acceptance of dairy products by consumers (12). The bacterial count of all (100%) of the samples from the public markets indicated poor microbiological quality: The limits for CFUs and SCs were exceeded in all cases according to the standards established by the Council for the Promotion of the Quality of Milk and its Derivatives. The latter organization establishes the limits of total AMB at ≤ 100000 CFU/mL and of SCs at ≤ 400000 SC/mL. Counts below these limits indicate that the product is not acceptable for human consumption.

On the other hand, the milk samples evaluated in the FF had very low AMB and SC counts, even lower than those reported in a region south of Mexico City during the same months (10). They were, however, similar to those reported by another study in the state of Morelos (12). Given these findings, it is necessary to emphasize the importance of adequate facilities, hygiene practices, and milking management at the point of production but also the importance of knowing the origin of the milk at the point of sale.

Among the factors that cause high SC and AMB counts are the absence of sanitary measures in the milking routine, equipment malfunction, poor handling of disinfectants and sealants, non-identification of infectious agents and, finally, ineffective control measures and treatments. These multifactorial causes can be controlled within family farms when there are clear procedures, staff are properly trained, and processes are strictly monitored (9).

There is additional evidence that SC counts may rise during the northerly winds season or be correlated with the presence of *E. coli*, which can be attributed to the inadequate cleaning of the udders and milking equipment (13). However, during this season, another report found ideal SC counts in milk samples from stables with adequate hygiene management during the milking process and at vendors' establishments, even during winter and the northerly winds season (14).

Another pathogenic microorganism of interest present in milk and dairy products due to poor hygiene and milking management is *Staphylococcus aureus*, which causes bovine mastitis and results in high costs for the dairy industry (15). Although the treatment of choice for this condition is antibiotics (mainly penicillins and cephalosporins, among others), at present, there is a trend toward a more preventive approach based on the implementation of adequate hygiene practices during the milking process (16). However, cows with subclinical mastitis are considered a reservoir of this bacterium and could contaminate dairy products throughout the entire production chain (10).

In conclusion, with respect to the standards established for Mexico by the Council for the Promotion of the Quality of Milk and its Derivatives, the physicochemical and microbiological characteristics of the milk produced by the family farm in southeastern Mexican southeast received the highest quality classification. The milk samples evaluated in the three public markets were of lower physicochemical quality and did not pass the standards for microbiological quality, indicating that this milk is not suitable for human consumption or for the elaboration of other dairy products.

Future studies should examine the quality of raw cow's milk during different seasons of the year and in different production systems.

Conflict of interests

The authors of this work declare that there is no conflict of interest.

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