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Microbial quality and prevalence of foodborne pathogens of cheeses commercialized at different retail points in Mexico

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Abstract

Microbial quality and the prevalence of foodborne pathogens (*E. coli* 0157:H7, *Salmonella* spp., *Listeria monocytogenes* and *Campylobacter jejuni*) of cheeses sold at three retail points (supermarkets [SM], street markets [ST], and convenience grocery stores [CGS]) in Chihuahua, Mexico, were evaluated (n=90). The most commonly found cheeses in the retail points were Chihuahua (60%) and Ranchero (28.8%). According to Mexican standards, three SM, two CGS, and none of the ST cheeses complied with the regulations. Two cheeses (cheddar and Ranchero) from SM (2.22%) were positive for *L. monocytogenes*. Eight cheeses (8.88%) were positive for *Salmonella* spp. (5 from ST and 3 from CGS). *E.coli* 0157:H7 and *Campylobacter jejuni* were negative in all samples. In the Ranchero cheese, total coliforms (TC), faecal coliforms (FC), and yeast and moulds (Y&M) showed no significant differences ($P > 0.05$) among retail points. Nevertheless, in Chihuahua cheese, the numbers of total coliforms, faecal coliforms, and yeast and moulds were statistically different ($P < 0.05$) among retail points. PSM Chihuahua cheese had the lowest numbers of total (0.99 ± 1.0 Log_{10}CFU/g) and faecal coliforms (166 ± 154 Log_{10}CFU/g). CGS cheeses had the lowest counts in terms of yeast and moulds (3.1 ± 2.2 Log_{10}CFU/g). The results revealed that most cheeses, regardless of the retail point, did not conform to Mexican standards. The number of total and faecal coliforms indicates either flaws during the production and commercialization processes or the ill-handling of raw materials.

Keywords: Mexican cheese; foodborne pathogens; microbial quality.

Practical Applications: The assessment of the microbial quality of cheeses from different retail points in México describes the potential risk that the cheeses might represent for the consumer. The results must also be considered when issuing public policies that aim to protect the health of the population.

1 Introduction

Cheese manufacturing and trade are a major industry worldwide (Organisation for Economic Co-operation and Development, 2016) and of great importance in Mexico mainly due to cheese's ease of fabrication and acceptance among consumers. This dairy sector has presented an increase in demand due to the recognition that some cheeses receive as functional foods, especially regarding the contribution of prebiotics and probiotics (Dantas et al., 2016; Belsito et al., 2017). They also present components such as peptides, polysaccharides, fatty acids, organic acids, gamma aminobutyric acid (GABA), and conjugated linolenic acid (CLA), all recognized as bioactive components that exert positive effects on consumer health (Santiago-López et al., 2017).

The dairy industry in Mexico is the third most important agricultural economic activities, which accounts for more than half of the national production. Chihuahua is the Mexican state with the highest production of milk and cheeses (mainly Chihuahua cheese), some of which are still produced using traditional techniques and still prevail in the manufacturing of unpasteurised cheeses at the small and medium scale (Servicio de Información Agroalimentaria y Pesquera, 2016). As almost all genuine Mexican cheeses are made from raw milk, innocuousness cannot be fully ensured, which might be risky for consumers (Villegas & Cervantes, 2011). It is so that the adoption of pasteurizing the milk with which the cheeses are made has not been successful among the producers of artisan cheeses and farms that produce small quantities of cheese; this due to the demand of cheeses made with raw milk by consumers (Johnson, 2017). As cheese does not regularly undergo further cooking before consumption, it might be considered as a ready-to-eat (RTE) food. As such, any improper manufacturing practices and handling during production, distribution, and sale pose an important health risk for consumers (Reij & Den Aantrekker, 2004). Producing cheese with raw milk is not permitted under Mexican health regulations (México, 1996). However, in the US, federal regulations permit the manufacture and sale of cheese using raw milk, but only if the cheeses have been aged at least 60 days at a temperature higher than 1.7 °C (CFR 21, Part 133) (FDA, 2017a).

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Contamination of the cheeses can be of chemical, physical or biological origin, nevertheless the origin or the cause of the last two have been the most studied. The most reported physical hazards in dairy products are: foreing objects, insects, hair, plastics, metal and fabric (Aguiar et al., 2018). Most common biological hazards found in cheeses are *Salmonella* spp., *Listeria monocytogenes*, *E.coli* 0157:H7 and *S.aureus* (Saltijeral et al., 1999; Torres-Vitela et al., 2012; Cody et al., 1999; Gould et al., 2014; Torres-Vitela et al., 2012; Le et al., 2014). However, the survival of pathogens present in cheese, as a result of contamination during processing, maturation or packaging, depends on various factors such as water activity, antagonism with other microorganisms and pH history since its preparation (Johnson, 2017)

Foodborne diseases are a major public health problem worldwide. However, as Mexico lacks an effective surveillance program in terms of foodborne outbreaks, few cheese-borne outbreaks have been reported. A retrospective study of foodborne outbreaks in Mexico found that 29% of outbreaks were associated with cheese consumption (Parrilla-Cerillo et al., 1993), but these numbers might be higher. In the US, seven cheese-borne outbreaks were reported from 2010 to 2017, six due to *Listeria monocytogenes* and one to *E.coli* 0157:H7 (Center for Disease Control and Prevention, 2017). Cheese consumption has historically been implicated in important outbreaks; the predominant organisms included *Salmonella*, *L. monocytogenes*, *E. coli*, and *S. aureus* (Little et al., 2008; Guzman-Hernandez et al., 2016; Alcázar Montañez et al., 2006). The identification of foodborne pathogens in foods can provide insights into the safety of retail foods and, therefore, the potential risk to consumers' health.

The state of Chihuahua is located along the border with Texas in the US, where the introduction of cheese is allowed by US regulations. In this sense, three different sale points can be differentiated: supermarkets (SM), public street markets (ST) (*tianguis*), and convenience grocery stores (CGS) (*tienda de abarrotes*).

Public street markets are installed in open, free-access areas within a city and change locations regularly. Most do not follow health and safety regulations, and occasionally local authorities supervise them; hence, the wholesomeness of the products might not be guaranteed. A convenience grocery store is a small store located within a neighbourhood and offers an assortment of RTE foods. As in ST, some RTE products might pose a risk for the consumer, as they are manufactured in small family businesses without sanitary licenses. On the other hand, supermarkets are establishments constantly monitored by the proper authority. Hence, the aims of this study were 1) to evaluate the overall microbiological quality of cheeses sold in three retail points (supermarkets, street markets, and convenience grocery stores) and 2) to identify the prevalence of *E.coli* 0157:H7, *Salmonella* spp., *Listeria monocytogenes*, and *Campylobacter jejuni*. It is hypothesized that cheeses sold in SM would have better microbiological quality than those from ST and CGS.

2 Materials and methods

2.1 Sample collection

Ninety samples of cheeses were purchased from three types of establishments (30 from each retail point: street market (ST), supermarket (SM), and convenience grocery store(CGS)) located in the city of Chihuahua, México. All samples were transported in iceboxes to the laboratory, where they are stored at 5 °C-7 °C and processed the next day.

2.2 Cheese characteristics

Five different types of cheeses were analysed. Fresh cheeses included 1) Panela, 2) Ranchero, and 3) mozzarella (fresh cheese from cooked pasta). Mature cheeses included 4) Chihuahua (might be sold without aging) and 5) Gouda.

2.3 Microbiological analysis

Ten grams from each cheese were aseptically homogenized in 90 mL of phosphate buffer and mixed with a Stomacher (Lab Blender, London, UK) at a maximum speed for 2 min. The homogenized sample was serially diluted (1:10) in a sterile phosphate buffer according to the Official Mexican Standards (NOM-243-SSA1-2010) (México, 2010).

Each dilution was plated on specific media and incubated aerobically as follows. Plate count agar (PCA; CM0325, Oxoid, Basingstoke, UK) was incubated at 30 °C for aerobic mesophilic microorganisms. Baird-Parker agar (BP; 276840, BD Bioxon, Heidelberg, Germany) was supplemented with egg yolk tellurite (BD™ D212357) at 37 °C for *Staphylococcus aureus*. Potato dextrose agar (PDA; 213300, BD Bioxon, Heidelberg, Germany) was acidified with 10% tartaric acid (T400 DL-tartaric, Merck, Saint Louis, MO, US) at 25 °C for moulds-yeasts. Violet red bile agar (VRB; CM0107, Oxoid, Basingstoke, UK) was incubated at 32 °C for total coliforms. Media were inoculated by spreading 100 µL of the diluted sample onto the agar, except for VRB, which was inoculated using the pour plate method (1000 µL). Media were incubated for the following times: VRB and BP 24 h, PCA 72 h, and PDA 120 days (Renye et al., 2008). Numbers of colony-forming units (CFU) were counted on the plates, with numbers ranging between 10 and 200 CFU.

Faecal coliforms (FC) were determined using Mexican Official Standard Guidelines NOM-243-SSA1-2010 (México, 2010) and the Food and Drug Administration (2017b) (FDA, most probable number (MPN)). Therefore, one millilitre of each dilution was inoculated into lactose broth (211835, BD Bioxon, Heidelberg, Germany) in fermentation tubes in triplicate. After 48 h of incubation at 37 °C, a loopful from positive cultures (determined by turbidity and gas production) was transferred to tubes containing *Escherichia coli* broth and incubated at 44.5 ± 0.02 °C for 48 h. Tubes with turbidity (growth) and gas production were considered positive for FC.

2.4 Molecular detection of Salmonella spp., E. coli O157:H7, Staphylococcus aureus, Listeria monocytogenes, and Campylobacter jejuni

DNA was obtained from 25 g of enriched cheese samples using the PrepSEQ™ Rapid Spin Sample Preparation Kit (4407760, Applied Biosystems, Foster City, US). Following the manufacturer's instructions, 750 µL of the enriched samples were used to isolate the DNA; eluted DNA samples were stored at -20 °C until further use.

Specific primers and sensitive probes were used for the detection of foodborne pathogens using real-time PCR. Detection kits (Applied Biosystems, Foster City, US) for each pathogen—MicroSEQ® *Salmonella* spp. (4403930), MicroSEQ® *E. coli* O157:H7 (4427409), TaqMan® *Staphylococcus aureus* (4368606), TaqMan® *Listeria monocytogenes* (4366102), and TaqMan® *Campylobacter jejuni* (4366570)—were used by setting up reactions with 18 L of DNA obtained from each cheese samples. The reactions were performed in duplicate; two positive internal controls and negative controls were added. The real-time PCR was performed on the 7500 Fast (Applied Biosystems) using 7500 Software v2.0

2.5 Statistical analysis

Microbial (Log_{10}CFU/g) data were recorded as mean ± standard deviation and statistically analysed using a one-way analysis of variance (ANOVA) followed by Tukey's comparison test to stablish the significance of mean differences at the 5% level of significance ($P < 0.05$) using SAS software (version 9.3, SAS Institute Inc., Carry, NC, US) (Statistical Analysis System, 2006).

3 Results

The most commonly found cheeses in the retail points were Chihuahua (60%) and Ranchero (28.8%). Results from each cheese were compared with Mexican regulations (Table 1), and the characteristics of the 90 samples are described in Table 2. None of the cheeses from ST met the requirements established by the Mexican standards, and only three SM and two CGS were in accordance with the regulations (México, 1996). Ranchero cheeses were not elaborated with pasteurized milk and did not meet the standards. Of the 54 Chihuahua cheeses, three were made with unpasteurized milk and four fully complied with regulations. Similarly, none of the Panela cheeses met the microbiological standards, and 3 out of 4 (75%) from ST were made from unpasteurized milk.

Table 3 describes the microbiological characteristics of Panela cheeses. None of them met the microbiological standards

Table 1. Mexican standard overview of microbiological criteria for cheeses (México, 1996).

MicroorganiPSMos	Maximum limit		
	Fresh	Matured	Processed
Faecal coliforms (MPN/g)	100	50	---
Stapylococcus aureus (CFU/g)	1000	100	Less than 100
Moulds and yeasts (CFU/g)	500	500	100
Salmonella in 25g	Absent	Absent	Absent
Listeria monocytogenes in 25g	Negative	Negative	Negative

MPN/g = Most probable number per gram; CFU/g = Colony-forming units per gram.

Table 2. Characteristics of cheeses sold at different retail points in Chihuahua, Mexico.

Retail point	Cheese type	Number of samples (%)	Number of samples within microbiology standards*		Number of phosphatase positive samples (%)
			Satisfactory	Unsatisfactory	
Public Street markets	Panela	4 (13.3)	0	4	3 (75)
	Chihuahua	11 (36.7)	0	11	0 (0)
	Ranchero	15 (50.0)	0	15	14 (93.3)
Convenience grocery stores	Chihuahua	23 (76.7)	2	21	3 (15.0)
	Ranchero	7 (23.3)	0	7	5 (71.42)
Supermarkets	Panela	1 (3.3)	0	1	0 (0)
	Chihuahua	20 (66.7)	1	19	0 (0)
	Rachero	4 (13.3)	0	4	4 (100.0)
	Gouda	3 (10.0)	1	2	0 (0)
	Mozarella	2 (6.7)	1	1	0 (0)

*According to Mexican regulations (México, 1996).

Table 3. Microbial quality of Panela cheese sold at different retail points in Chihuahua, Mexico.

Retail point	Sample/ Phosphatase test	Faecal coliforms (MPN/g)	Total coliforms (Log CFU/g)	M&Y (Log CFU/g)	*S.aureus*	*Salmonella* spp.	*Listeria monocytogenes*
ST	1T/-	1600	4.42	3.72	<10	-	-
ST	20T/+	1600	3.70	4.95	<10	+	-
ST	23T/+	1600	3.58	4.75	<100	-	-
ST	28T/+	1600	4.43	ND	<10	+	-
SM	21S/-	1600	3.29	5.16	< 10	-	-

M&Y, moulds and yeasts; ST, Public street market; SM, supermarket; ND, not determined; MPN/g = Most probable number per gram; CFU/g = Colony-forming units per gram.

for FC and M&Y, and two samples from ST were positive for *Salmonella* spp.

Microbial characteristics of Ranchero cheeses are described in Table 4. One sample (17T) met the microbiological standards for FC, but not the standards for Y&M. Four out of 26 samples (15.38%) were positive for *Salmonella* spp.: three from ST and one from a CGS. One sample from a SM was positive for *Listeria monocytogenes*.

Microbial quality from Chihuahua cheese is shown in Table 5. Three out of 54 samples (5.55%) met the regulations: two from CGS (11A and 13A) and one from SM (24S). Two samples (3.70%) from CGS were positive for *Salmonella* spp. One sample from SM was positive for *Listeria monocytogenes*.

There were no significant differences ($P > 0.05$) when comparing the numbers of TC, FC, and Y&M for Ranchero cheese among retail points (Table 6). Cheese from SM was expected to have lower counts as retail points are usually the most regulated by the National Health Department. However, these samples had the highest numbers. Nevertheless, in Chihuahua cheese, the microbial numbers of total coliforms, faecal coliforms, and yeast and moulds (Table 6) were statistically different ($P < 0.05$) among retail points. PSM Chihuahua cheese had the lowest numbers of total coliforms (0.99 ± 1.0 Log_{10}CFU/g) and faecal coliforms (166 ± 154 Log_{10}CFU/g), and CGS cheese had the lowest counts in terms of yeast and moulds (3.1 ± 2.2 Log_{10}CFU/g).

Importantly, none of the samples was positive for *E.coli* 0157:H7 or *Campylobacter jejuni* using real-time PCR. These types of rapid

Table 4. Microbial quality of Ranchero cheese sold at different retail points in Chihuahua, Mexico.

Retail point	Sample/ Phosphatase test	Faecal coliforms (MPN/g)	Total coliforms (Log CFU/g)	M&Y (Log CFU/g)	*S. aureus*	*Salmonella* spp.	*Listeria monocytogenes*
ST	3T/+	1600	4.44	3.50	<100	-	-
ST	4T/+	1600	4.76	3.48	<100	-	-
ST	5T/+	1600	3.68	ND	<100	-	-
ST	6T/-	1600	2.53	ND	<100	-	-
ST	7T/+	1600	4.79	ND	<100	-	-
ST	9T/+	1600	3.03	4.99	<100	-	-
ST	10T/+	1600	4.70	5.09	<100	+	-
ST	11T/+	1600	3.74	5.23	<100	-	-
ST	13T/+	1600	3.48	5.06	<100	+	-
ST	15T/+	1600	2.79	3.91	<10	-	-
ST	17T/+	79	0.34	2.84	<100	-	-
ST	21T/+	1600	3.69	5.50	<10	+	-
ST	25T/+	1600	3.37	4.12	<10	-	-
ST	26T/+	1600	4.32	5.57	<10	-	-
ST	30T/+	1600	3.25	ND	<100	-	-
CGS	2A/+	1600	2.54	2.99	<10	-	-
CGS	6A/-	1600	3.21	ND	<100	-	-
CGS	7A/+	1600	3.98	ND	<100	-	-
CGS	15A/-	1600	2.5	<10	<100	-	-
CGS	18A/+	240	2.42	4.11	<10	-	-
CGS	19A/+	1600	4.93	2.44	<10	-	-
CGS	28A/+	1600	3.55	4.95	<100	+	-
SM	12S/+	1600	4.60	5.05	<100	-	-
SM	13S/+	1600	2.38	5.32	<10	-	-
SM	29S/+	1600	3.46	3.82	<10	-	-
SM	30S/+	1600	3.90	5.63	<10	-	+

M&Y, moulds and yeasts; ST, Public street market; CGS, Convenience grocery store; SM, supermarket; ND, not determined; MPN/g = Most probable number per gram; CFU/g = Colony-forming units per gram.

Table 5. Microbial quality of Chihuahua cheese sold at different retail points in Chihuahua, Mexico.

Retail point	Sample/ Phosphatase test	Faecal coliforms (MPN/g)	Total coliforms (Log CFU/g)	M&Y (Log CFU/g)	*S.aureus*	*Salmonella* spp.	*Listeria monocytogenes*
ST	2T/-	1600	2.13	2.45	<10	-	-
ST	8T/-	1600	2.48	ND	<100	-	-
ST	12T/-	1600	3.60	4.36	<100	-	-
ST	14T/-	1600	1.94	3.15	<100	-	-
ST	16T/-	920	3.83	5.21	<100	-	-
ST	18T/-	920	1.89	3.76	<10	-	-

M&Y, moulds and yeasts; ST, Public street market; CGS, Convenience grocery store; SM, supermarket; ND, not determined; MPN/g = Most probable number per gram; CFU/g = Colony-forming units per gram.

Table 5. Continued...

Retail point	Sample/ Phosphatase test	Faecal coliforms (MPN/g)	Total coliforms (Log CFU/g)	M&Y (Log CFU/g)	*S.aureus*	*Salmonella* spp.	*Listeria monocytogenes*
ST	19T/-	49	<10	4.23	<100	-	-
ST	22T/-	1600	1.23	5.14	<10	-	-
ST	24T/-	540	1.38	2.92	<10	-	-
ST	27T/-	1600	2.30	4.37	<100	-	-
ST	29T/-	1600	3.38	5.20	<100	-	-
CGS	1A/-	920	1.00	2.82	<100	-	-
CGS	3A/-	79	<10	<10	<100	-	-
CGS	4A/-	1600	1.09	<10	<100	-	-
CGS	5A/-	1600	2.45	ND	<100	-	-
CGS	8A/-	240	1.03	ND	<100	-	-
CGS	9A/-	79	0.86	1.90	<10	-	-
CGS	10A/-	1600	2.70	0.62	<100	-	-
CGS	11A/-	3	1.45	1.38	<10	-	-
CGS	12A/-	1600	0.77	2.81	<100	-	-
CGS	13A/-	34	0.65	1.13	<10	-	-
CGS	14A/+	1600	1.53	0.15	<100	-	-
CGS	16A/-	79	1.55	2.12	<100	+	-
CGS	17A/-	3	3.14	<10	<10	+	-
CGS	20A/-	1600	4.63	0.86	<100	-	-
CGS	21A/-	1600	2.87	4.95	<100	-	-
CGS	22A/-	1600	2.62	4.77	<10	-	-
CGS	23A/-	1600	2.73	ND	<10	-	-
CGS	24A/-	1600	1.13	4.36	<10	-	-
CGS	25A/+	130	1.00	4.41	<100	-	-
CGS	26A/-	240	<10	5.03	<10	-	-
CGS	27A/-	1600	4.39	4.68	<100	-	-
CGS	29A/-	1600	2.80	ND	<100	-	-
CGS	30A/+	1600	4.30	4.84	<100	-	-
SM	3S/-	1600	1.13	0.65	<100	-	-
SM	4S/-	3	<10	3.70	<10	-	-
SM	6S/-	1600	2.95	4.67	<100	-	-
SM	7S/-	49	<10	4.20	<100	-	-
SM	10S/-	240	<10	4.45	<10	-	-
SM	11S/-	23	<10	4.20	<10	-	-
SM	14S/-	540	1.23	5.28	<10	-	-
SM	15S/-	1600	1.27	4.42	<10	-	-
SM	16S/-	1600	1.69	4.23	<10	-	-
SM	17S/-	240	1.17	2.92	<100	-	-
SM	18S/-	240	<10	5.35	<10	-	-
SM	19S/-	26	<10	2.87	<10	-	-
SM	20S/-	2	<10	5.13	<100	-	-
SM	22S/-	1600	2.64	3.59	<10	-	-
SM	23S/-	920	2.23	5.3	<10	-	-
SM	24S/-	2	<10	1.69	<100	-	-
SM	25S/-	2	<10	3.29	<10	-	-
SM	26S/-	2	1.20	4.20	<10	-	-
SM	27S/-	1600	2.41	4.24	<10	-	-
SM	28S/-	130	1.85	2.14	<100	-	+

M&Y, moulds and yeasts; ST, Public street market; CGS, Convenience grocery store; SM, supermarket; ND, not determined; MPN/g = Most probable number per gram; CFU/g = Colony-forming units per gram.

Table 6. Microbial quality of Ranchero and Chihuahua cheeses (means ± S.D.) sold at different retail points.

Cheese type	Retail point	Total coliforms (Log CFU/g)	Faecal coliforms (MPN/g)	Y&M (Log_{10}CFU/g)
Ranchero n=26	ST	3.5 ± 1.10 [a]	1499 ± 393 [a]	4.9 ± 1.10 [a]
	CGS	3.3 ± 0.93 [a]	1406 ± 514 [a]	3.8 ± 2.20 [a]
	SM	3.6 ± 0.93 [a]	1600 ± 0.0 [a]	5.0 ± 0.79 [a]
Chihuahua n=54	ST	2.2 ± 1.10 [a]	1239 ±550 [a]	4.3 ± 1.10 [a]
	CGS	1.9 ±1.40 [a]	983 ± 740 [a]	3.1 ± 2.20 [b]
	SM	0.99 ± 1.00 [b]	166 ±154 [b]	3.8 ± 1.30 [a]

Y&M = Yeasts and moulds; ST, Public street market; CGS, Convenience grocery store; SM, supermarket; MPN/g = Most probable number per gram; CFU/g = Colony-forming units per gram; [a,b,c]Different letters in the same column indicate significant differences ($p < 0.05$).

test systems are only used as screening techniques. Hence, negative results are accepted as such, but positive results require further confirmation using appropriate official methods (Feng, 2017).

4 Discussion

In this study, five out of 90 (5.5%) samples were in accordance with the regulations (México, 1996), and a third (32.2%) were produced using unpasteurized milk, which is a known source of pathogens (Kousta et al., 2010). There is a benefit to using pasteurized milk, and this is because the cheeses have greater consistency in quality. However, pasteurization does not guarantee that the cheeses are free of pathogens, although it could be guaranteed if contamination of the cheeses after pasteurization is avoided (Johnson, 2017).

SM was expected to comply with Mexican official standards because they are closely monitored; however, there were no differences among retail points. The results might be normal for the country, as similar data have been reported for unpasteurized fresh cheese in a southeast Mexico state (Guzman-Hernandez et al., 2016).

One third of the samples were produced with unpasteurized milk, and most were positive for FC and did not met official regulations (México, 1996). As previously stated, US federal regulations permit the manufacture and sale of cheese made with raw milk, but only if they have been aged at least 60 days at a temperature higher than 1.7°C (CFR 21, Part 133) (Food and Drug Administration, 2017a). This regulation implies that pathogens, if present in the raw milk, would not survive the process (including aging). Furthermore, unpasteurized cheese aged for at least 60 days has rarely been associated with foodborne diseases. However, several studies have suggested that some pathogens in raw milk could survive cheese manufacturing and aging (Bachmann & Spahr, 1995; Leyer & Johnson, 1992), as shown in the outbreaks linked to the consumption of unpasteurized cheeses in the US (Center for Disease Control and Prevention, 2010, 2013, 2014a, b, 2015).

In this study, two cheeses (cheddar and Ranchero) from PSM (2.22%) were positive for *L. monocytogenes* while 8 cheeses (8.88%; 5 from PSM and 3 from CGS) were positive for *Salmonella* spp. According to Mexican regulations (México, 1996), *Salmonella* spp. and *L. monocytogenes* present in 25 g of cheese is not acceptable in fresh, matured, and processed cheese (México, 1996). Despite the deficient epidemiological surveillance in Mexico, the presence of *L. monocytogenes* in different types of fresh cheese have been reported in previous studies (Saltijeral et al., 1999; Torres-Vitela et al., 2012; Moreno-Enriquez et al., 2007; Soto Beltran et al., 2015; Rosas-Barbosa et al., 2014). Cases of pathogens in aged cheeses, such as Chihuahua, have not been reported (Saltijeral et al., 1999; Alcázar Montañez et al., 2006); however, in this study one pasteurized SM Chihuahua cheese was positive for *L. monocytogenes*. Studies have suggested that contamination from *L. monocytogenes* occurs predominantly post-processing (Moreno-Enriquez et al., 2007; Rosas-Barbosa et al., 2014; Kabuki et al., 2004) because this pathogen has been be isolated in cheese factories from floors, drains, containers (Kabuki et al., 2004), and staff (Kousta et al., 2010; O'Brien et al., 2009). In Mexico, studies have shown that *L. monocytogenes* is found throughout the cheese-making process, plant environment, milk, and retail points, suggesting that surfaces in contact with food would be the most common source of contamination, although it is more common than raw or inadequate pasteurized milk (Moreno-Enriquez et al., 2007; Rosas-Barbosa et al., 2014; Kousta et al., 2010; O'Brien et al., 2009).

Salmonella spp. was present in 8 cheese samples (8.88%). Five were fresh cheeses made with raw milk acquired at ST; the remaining three samples were acquired at CGS, two Chihuahua cheeses made with pasteurized milk and one Ranchero cheese made from raw milk. The pasteurization of milk is one of the most effective measures to prevent microbial contamination. It has been widely reported that the consumption of raw milk cheeses is microbiologically unsafe (Gould et al., 2014; Alcázar-Montañez et al., 2006; MacDonald et al., 2005; Torres-Vitela et al., 2012; Villar et al., 1999). Meanwhile, the presence of *Salmonella* in cheese is a result of insufficient pasteurization (D'Aoust, Warburton & Sewell, 1985).

Mexican fresh cheeses have had a high incidence of *Salmonella* spp. and, therefore, foodborne outbreaks (Cody et al., 1999; Gould et al., 2014; Torres-Vitela et al., 2012). However, the statistics of foodborne outbreaks, due to the consumption of cheese, in Mexico would be higher than those reported since the health department does not oblige physicians to report cases of this type of illnesses they treat. Obtaining this data then represents a challenge for those interested. In Brazil, the website *Reclame Aquí* was successfully created, this page being a communication channel between the industry and the final consumer, and is currently the 33 most visited website in Brazil; in a span of 4 years they received 515 quality complaints of dairy products. This page has been used successfully to investigate the incidence of physical contamination in dairy products (Aguiar et al., 2018). The opinion of consumers is important because it is an indicator of the hygienic and sanitary quality of dairy products, which affects the credibility of the food industry.

Some of the cheeses that were found in the sampled points, mainly rancheros cheeses, come from artisan cheese producers. In Mexico, to our knowledge, there is no research pertaining to the perceptions and knowledge that artisan cheese producers have about the sanitary rules of food management. This is important because even when artisan cheese producers recognize biological hazards (*L. monocytogenes*, *E. coli*, *Salmonella* spp., and *S. aureus*), the potential sources of these and the impact they have on health of the consumer and in the business. They also recognize that there are various barriers to compliance with sanitary regulations, such as the documentation required by various plans (for example, HACCP) and the need for additional resources to be in compliance with them (Le et al., 2014). However, it has been found that the implementation of good manufacturing practices, by itself, helps increase the percentage of compliance with regulations (Costa Dias et al., 2012). In a mozzarella cheese processing plant, where 60% of the non-conformities were due to non-compliance in relation to personnel hygiene, the implementation of good manufacturing practices that included: GMP training (personnel hygiene, chemical handling for sanitization) and the use of posters highlighting

hygienic practices during the process, such as hand washing, proper use of sanitary and hygiene of uniforms, reduced this percentage of non-compliance by half (Costa Dias et al., 2012). Likewise, factors that have been associated with the presence of *S.aureus*, *E.coli*, *L.monocytogenes* and *Salmonella* spp., are those related to the handling or manipulation, type of milk (pasteurized or raw) and the knowledge they present. employees about various aspects of cheese making such as microbiology of cheeses, hygiene practices and cleaning programs (Carrascosa et al., 2016). This is important because most of the medium and small producers of cheese in Mexico do not have financing for the implementation of food safety programs, which are costly; however, most could access training in good manufacturing practices.

5 Conclusion

The results reveal that most cheeses, regardless of the retail point, did not conform to Mexican standards. Surprisingly, although supermarkets follow strict hygienic codes and regulations, Ranchero cheeses from these retail points were not microbiologically different from those from street markets and convenience grocery stores. The high numbers of total and faecal coliforms show the contamination of the product, either by the raw materials or by flaws in the production or commercialization process before reaching the consumer. The high count of mould and yeast shows poor sanitary conditions of the production practices. The presence of *S. aureus* represents a potential risk to the health of the consumed. Most dairies should implement and maintain a food safety plan that contains a risk analysis, preventive controls, monitoring procedures and a plan of corrective actions, this must also contain a training program on good manufacturing practices for their employees. Thus, one of the main challenges of the cheese industry is to ensure the training of personnel. Likewise is important to assess the cheese chain process thoroughly, from the milk to the plate.

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