Short communication

Presence of coliform bacteria, fecal coliforms, *Escherichia coli* and *Salmonella* on corn tortillas in central Mexico

Carlos A. Gómez-Aldapa, Esmeralda Rangel-Vargas, Andrés M. Cruz Gálvez, Alma D. Román-Gutiérrez, Javier Castro-Rosas*

Centro de Investigaciones Químicas. Instituto de Ciencias Básicas e Ingeniería, Universidad Autónoma del Estado de Hidalgo, Ciudad del Conocimiento, Carretera Pachuca-Tulancingo Km. 4.5, 42183 Mineral de la Reforma, Hidalgo, Mexico

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ABSTRACT

Corn tortillas are a primary foodstuff in Mexico with nearly 12 million tons produced and consumed annually. Federal health regulations control tortilla production and sale in Mexico, but most producers do not fully implement them. No data are currently available on microbiological quality or presence of pathogenic bacteria on tortillas produced in Mexico or other countries. Analyses of coliform bacteria (CB), fecal coliforms (FC), *Escherichia coli* and *Salmonella* frequency were done on corn tortillas. Tortilla samples were collected from small-scale producers called *tortillerías*. Two hundred samples (500 g) were acquired at *tortillerías* in five municipalities in Hidalgo State, Mexico. CB were detected on 70% of samples, FC on 40%, *E. coli* on 32% and *Salmonella* on 2%. In positive samples, CB concentration ranged from 1×10^1 to 1×10^6 CFU/g, and FC and *E. coli* concentrations from 3 to 1100 MPN/g. CB, FC and *E. coli* presence and concentration all correlated well with *Salmonella* presence in tortilla. The analysis of Kruskal−Wallis−ANOVA and median test of microbiological data showed that the microbiological quality of tortillas samples did not differ between the different *tortillerías*. In addition, CB, FC and *E. coli* all correlated well with *Salmonella* presence in tortilla.

This is the first report of microbiological quality of corn tortillas and *Salmonella* isolation from tortillas in Mexico. Corn tortillas could be a potential source of enteropathogenic bacteria as *Salmonella*.

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1. Introduction

Tortillas are flat discs made from corn (*Zea mays* L.) or wheat (*Triticum* spp.) and varying in dimensions from 12 to 18 cm and from one to 4 mm thick. Originally developed by ancient Meso-american civilizations, corn tortillas are still a major foodstuff for Mexicans, and tortillas and derivative products are a staple food in Mexico and Central America. Indeed, tortillas are consumed by approximately 95% of Mexicans, and annual production and consumption nears 12 million tons of corn tortillas. Tortillas and chips from them are also well established in markets in the Unites States, Asia and Europe (Cortés-Gémez, Martín-Martínez, Martínez-Bustos, & Vázquez Carrillo, 2005).

In Mexico, corn tortillas are produced and distributed commercially at four scales: micro (hand-made); small (mechanized); medium (e.g. supermarkets); and large (industrial). Small-scale producers, often consisting of no more than a single tortilla machine and known as *tortillerías*, account for 60% of tortilla production (Ayala-Rodríguez et al., 2009).

Corn tortillas are produced from either traditionally milled, wet, lime-soaked dough, or dehydrated corn flour. In traditional processing, the corn is first cooked in lime and water for 15−40 min, and the cooked grain steeped for 12−20 h. After steeping it is called *nixtamal*. The *nixtamal* is washed to separate it from the excess water, lime and pericarp fragments, and ground in a stone mill to produce a soft dough known as *masa*. The *masa* is shaped into small circular discs and cooked on a hot metal surface, resulting in tortillas. In *tortillerías*, production is semi-mechanized, with machinery type, cooking time and production rate controlled by federal regulations (Secretaria de Salud, 2009). This is a continuous process in which the *masa* is shaped into discs and heated on both sides. During cooking, tortillas reach a maximum temperature of approximately 80 °C for 20−40 s. After cooking, the tortillas move along a metal mesh conveyor belt to cool to a final temperature of approximately 50 °C. These are collected by a worker, usually with bare hands.

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* Corresponding author. Tel.: +52 771 717 2000x6501; fax: +52 771 717 2000x6502.

E-mail address: jcastro@uaeh.edu.mx (J. Castro-Rosas).

Federal regulations for tortilla manufacture include food sanitation and food safety rules to be met by tortilla producers throughout the elaboration process to prevent contamination by pathogenic microorganisms or reduce them to levels which pose no consumer health risk (Secretaria de Salud, 2002). Most tortillerías do not successfully apply all these regulations all the time (Anonymous, 2012; Castro-Rosas, Estrada-Hernández, & Gómez-Aldapa, 2008; López, 2012). For example, the person who collects the finished tortillas frequently also weighs them out, collects payment, cleans tortilla and masa debris from counters using a visibly dirty rag and may even have direct contact with fresh masa. Rarely if ever does the worker wash his hands or use gloves when changing activities. Multiple potential bacterial contamination sources, most of human origin, are also plainly visible during processing (Anonymous, 2012; Castro-Rosas et al., 2008; López, 2012).

As a cooked product, tortillas are generally consumed within 2 h of purchase. They are not considered a high-risk food nor are they associated with microbial disease outbreaks. Nonetheless, the presence of pathogenic bacteria is to be expected given the wide use of improper production and handling practices. In addition, because they are normally purchased while still hot, most consumers do not reheat them before eating them, further increasing the risk of illness if the tortillas are contaminated with a bacterial pathogen. No data are currently available on microbiological quality and safety of corn tortillas produced in tortillerias in Mexico or any other country. The present study objective was to measure CB, FC and Escherichiacoli and Salmonella frequency on corn tortillas.

2. Materials and methods

2.1. Tortilla samples

A total of 200 corn tortilla samples (500 g each) were purchased from 100 tortillerias in five municipalities in Hidalgo State, Mexico: Pachuca (35 tortillerias); Mineral de la Reforma (35 tortillerias); Tula (10 tortillerias); Tezontepec (10 tortillerias); and Tepeji del Rio (10 tortillerias). Two tortillas samples were picked out from each

tortillería in different days. Upon purchase, all samples were wrapped in paper by tortillería personnel, as is common practice. Average tortilla temperature at moment of purchase was 40 °C. After purchase, the tortilla samples were placed in a sterilized plastic bag, stored in a cooler with frozen gel packs for transport to the laboratory and analyzed no more than 4 h after purchase.

2.2. Microbiological analyses

Sub-samples (100 g) of each sample were randomly taken from each tortilla sample and placed into a sterile plastic bag; lactose broth (LB) was added in order to have a final dilution of $1:10$ (10^{-1}). The bags were pummeled in a stomacher for 1 min, and sample dilutions were prepared for microorganism counts carried out using peptone diluent (0.1%). Samples were analyzed to detect the presence of coliform bacteria (CB) following the method in the Bacteriological Analytical Manual (FDA, 2010), and fecal coliforms (FC) and E. coli as we previously described (Castro-Rosas et al., 2012). The most probable number (MPN) of FC and E. coli was calculated following the MNP method in the Bacteriological Analytical Manual (FDA, 2010). Salmonella was tested from tortillas sub-samples in LB as we previously described (Castro-Rosas et al., 2011).

2.3. Statistical analysis

Kruskal–Wallis–ANOVA and median test was used to determine significance differences ($p < 0.05$) between median counts of indicator microorganisms in tortillas samples obtained from different tortillerias of municipalities. The Pearson correlation coefficient was used to compare the relationship between the presence of CB, FC, E. coli, and Salmonella. A p value < 0.05 was considered significant. All statistical analyses were run with the Statistical 8 program (StatSoft, Inc., Tulsa, version 8).

3. Results and discussion

Overall, the analyzed tortilla samples had poor microbiological quality (Table 1). Among the 200 samples, CB were detected in

Table 1
Coliform bacteria (CB), thermotolerant coliform (FC) and E. coli frequencies and concentrations, and Salmonella frequency on corn tortilla samples[a] from five municipalities in Hidalgo state, Mexico.

Municipality (no. of samples)	Microorganisms	Range			Number of positive samples (%)
		Minimum	Median	Maximum	
Pachuca (70)	CB (CFU/g)	<10	1.5×10^2	4.6×10^4	48 (68.6)
	FC (MPN/g)	<3	<3	1100	28 (40)
	E. coli (MPN/g)	<3	<3	460	22 (31.4)
	Salmonella	Nd[b]	Nd	Nd	2 (2.8)
Mineral de la Reforma (70)	CB (CFU/g)	<10	2.0×10^2	2.7×10^4	52 (74.3)
	FC (MPN/g)	<3	<3	1100	28 (40)
	E. coli (MPN/g)	<3	<3	460	22 (31.4)
	Salmonella	Nd	Nd	Nd	1 (1.4)
Tula (20)	CB (CFU/g)	<10	1.7×10^2	2.8×10^4	14 (70)
	FC (MPN/g)	<3	1.5	1100	10 (50)
	E. coli (MPN/g)	<3	<3	460	6 (30)
	Salmonella	Nd	Nd	Nd	1 (5)
Tezontepec (20)	CB (CFU/g)	<10	1.6×10^2	3.0×10^4	14 (70)
	FC (MPN/g)	<3	<3	460	8 (40)
	E. coli (MPN/g)	<3	<3	210	6 (30)
	Salmonella	Nd	Nd	Nd	0 (0)
Tepeji (20)	CB (CFU/g)	<10	1.8×10^2	1.2×10^4	12 (60)
	FC (MPN/g)	<3	4.6	1100	10 (50)
	E. coli (MPN/g)	<3	1.5	290	6 (30)
	Salmonella	Nd	Nd	Nd	0 (0)

[a] $n = 200$. Minimum, median and maximum values are in CFU per g for coliform bacteria and in most probable number (MPN) per g for fecal coliforms and E. coli.
[b] Not determined.