



Hygienic quality of the traditional red chorizo commercialized in the city of Toluca, State of Mexico



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Abstract:

The traditional *red chorizo* that is commercialized in Toluca is a sausage with great fame; however, little is known about its quality. The objective of this study was to determine the hygienic quality of traditional red chorizo in different points of sale. 75 samples of the four main markets of the city of Toluca and 10 specialized butcher shops were analyzed in three periods. The water activity (a_w), humidity, pH, acidity and nitrite content were determined based on the Mexican Official Standards. Aerobic mesophilic bacteria (AMB), lactic acid bacteria (LAB), total coliforms and fecal coliforms were counted; the presence of *Salmonella* spp. and *Escherichia coli* spp. was detected. The traditional red chorizo did not exhibit

significant differences ($P>0.05$) in most of the analyzed physicochemical and microbiological variables. It showed a a_w over 0.95; humidity 40 to 50 %; pH below 5 and nitrite content below the maximum limits (156 mg/kg) accepted for sausages according to official standards. The AMB count was between 7.3 to 7.8 Log₁₀ CFU/g and 7.8 to 8.1 Log₁₀ CFU/g for LAB; between 11.1 to 48.6 MPN/g for total coliforms, and between 4.9 to 9.7 MPN/g for fecal coliforms. *Salmonella* spp. and *E. coli* exhibited incidence rates in all markets, regardless of their nature, of 11.1 to 60 % and 16.7 to 43.3 %, respectively. The traditional red chorizo of Toluca has distinctive characteristics; yet, it is imperative to implement sanitary management programs during its production, storage and commercialization in order to guarantee a sausage that is not only typical of the region but also innocuous.

Key words: Safety, Traditional Mexican sausages, *Enterobacteriaceae*, Quality.

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The increasing worth and demand of traditional foods whose quality and reputation is associated to a territorial origin has prompted studies to characterize (typification) them and assess their quality. The characterization of traditional foods —especially of artisanal sausages— is a common practice in European countries like France⁽¹⁾, Germany⁽²⁾, Italy⁽³⁾, and Spain^(4,5). Particularly in Spain, studies on dry fermented sausages like the Pamplona chorizo^(4,5) and the Galician onion sausage⁽⁶⁾ are prominent. These have determined the color and the texture⁽⁴⁾, the volatile compounds⁽⁴⁾, and the biochemical changes during the ripening process^(6,7), as well as the effect of starter cultures on the volatile compound profile, the microbial count, and the physico-chemical and sensory parameters⁽⁸⁾.

In the case of Mexico, the techniques for the manufacture of sausages were brought by the Spaniards during the Conquest⁽⁹⁾. Later, the local ingenuity, creativity and biodiversity resulted in a great variety of sausages, the most prominent of which is chorizo, a sausage that became popular since the colonial times⁽¹⁰⁾.

Chorizo is a cured meat product made from a mixture of minced pork meat and pork fat with added salts such as sodium chloride, nitrites, nitrates, and other permitted food additives⁽¹¹⁾. Mexican chorizos are characterized for containing mixtures of chili peppers, and they are very important in Mexican gastronomy. Today, varieties of traditional chorizos are produced mainly in states like Hidalgo, Veracruz, Oaxaca, and the state of Mexico. However, despite their gastronomic and economic importance, few studies have been conducted on their physico-chemical⁽¹²⁾, sensory⁽¹³⁾ and microbiological⁽¹⁴⁾ characteristics.

In the Valley of Toluca, in the state of Mexico, the small-scale production of red and green chorizos, which are commercialized in the main permanent markets and street markets of the city, is deeply rooted⁽¹⁵⁾. The fame of Toluca as a chorizo-producing city is such that, for many, “Toluca” is synonymous with chorizo⁽¹⁶⁾. However, although the manufacture of these sausages has a great economic importance for the region⁽¹⁵⁾, only preliminary studies on the physico-chemical and morphological quality of traditional green chorizos exist⁽¹⁷⁾, while there are virtually no studies on the general quality —much less on the sanitary quality— of the traditional red chorizo.

Based on the above, and led by the awareness of the importance of assessing the microbiological quality of regional foods, the purpose of this study was to determine the sanitary quality of the traditional red chorizo commercialized in the main markets and butcher shops specialized in the sale of pork meat products in the city of Toluca.

Samples of traditional red chorizo were obtained from 25 points of sale, 15 of which were established shops in the main permanent markets of the city of Toluca, and 10 were butcher shops specialized in the production and sale of pork meat products. Three samplings were carried out: the first, in October-November, 2015; the second, in January-February, 2016, and the third, in March-April, 2016.

Physico-chemical analyses were conducted. Water activity (a_w) was determined with the method exposed by Decagon⁽¹⁸⁾. The pH and total titrable acidity were determined with the methods reported by Guerrero *et al.*⁽¹⁹⁾ The moisture content was estimated through oven-drying⁽²⁰⁾. The nitrite content was determined using the Griess method⁽¹¹⁾.

Microbial counts were carried out using dilutions to 10^{-7} . Aerial mesophilic bacteria were determined in PCA agar (B. D. Bioxon, Mexico City, Mexico), incubated at 35 ± 1 °C during 48 h⁽²¹⁾. The most probable number (MPN) technique was utilized for determining the total and fecal coliforms according to the norm NOM-112-SSA1⁽²²⁾. The presence of *Escherichia coli* was determined based on those tubes that tested positive for coliforms with the MPN technique on EMB agar plates (B. D. Bioxon, Mexico City, Mexico) at 35 ± 1 °C during 24 h. Gram staining was then applied to suspicious colonies.

The IMViC biochemical test (B. D. Dixon, Mexico City, Mexico) was applied to strains with a morphology of Gram-negative bacilli. *Salmonella spp.* was determined using the methodology exposed in the norm NOM-114-SSA1-1994⁽²³⁾; RVS broth tubes (B. D. Difco, USA) and tetrathionate broth (B. D. Bioxon, Mexico City, Mexico) were utilized as means of enrichment; HE agar (B. D. Difco, USA), SB agar (B.D. Bioxon, Mexico City, Mexico) and SS agar (B. D. Bioxon, Mexico City, Mexico) were utilized as selective means. Suspicious colonies were transferred to TSI biochemical identification media (B. D. Bioxon, Mexico City, Mexico) and LIA (B. D. Bioxon, Mexico City, Mexico). Lactic acid bacteria

were determined through inoculation using the double layer method⁽²⁴⁾ in MRS agar at 35 °C during 48 h.

All the samples were analyzed in triplicate, and the data obtained were subjected to a variance analysis (ANOVA) and to Tukey's mean comparison test, using the Statgraphics Centurion XVI software, version 16.1.03, Warrenton, Virginia.

It is essential to produce foods of good sanitary quality, as the microorganisms present in these may cause not only decay, but also diseases that may put the consumers' health at risk⁽²⁵⁾.

A large amount of traditional foods are produced today in Mexico in an artisanal manner; however, they frequently exhibit deficiencies in their quality, especially in their sanitary quality⁽²⁶⁾. The studies conducted in the Aro cheese that are marked in the municipality of Oaxaca⁽²⁷⁾, the cheeses of Zacazonapan, in the State of Mexico⁽²⁸⁾, in chorizos commercialized in Hidalgo⁽¹³⁾, and in green chorizo commercialized in Toluca⁽²⁹⁾.

It is important to clarify that the groups of establishments where the samples analyzed in this study were obtained have certain specific characteristics (Table 1). The specialized butcher shops (B) are established shops equipped with refrigerated counters and specific storage areas; similar characteristic may be seen in the shops of market M_d; they both cater to a medium-high sector of the population. Instead, the establishments in markets M_a, M_b and M_c are characterized by their limited spaces, without specific areas for production and storage and without proper hygiene; the shops of market M_b target the middle-low sector and wholesale stores.

Mexican traditional chorizos have certain unique characteristics; first, they are fresh, not ripe; they are lightly acidified, and they are cooked before they are consumed. Particularly the traditional red chorizos of Toluca are characterized by containing mixes of different chili peppers (*Capsicum annum*), including *puya*, *guajillo*, and *ancho*, among others; furthermore, depending on the variety, they include dried fruits such as pine nuts (*Jatropha curcas*), raisins (*Vitis vinifera*), almonds (*Prunus dulcis*), and pecans (*Carya illinoensis*), among others⁽³⁰⁾. Other distinctive aspects of the product are its format (*i.e.* the length and diameter), and the physico-chemical parameters (*e.g.* a_w , pH, acidity) determined in this study.

Table 1: Characteristics of the groups of establishments where red chorizo is commercialized in the city of Toluca

Establishment group	Degree of technification	Type of storage	Commercial sector
M _a (n=15)	Artesanal	Intemperie	Middle-low
M _b (n=15)	Artesanal	Intemperie	Middle-low
M _c (n=6)	Artesanal	Intemperie	Middle-low
M _d (n=9)	Artesanal	Refrigeration	Middle-high
B (n=30)	Semi-industrial	Refrigeration	Middle-high

M= markets; B= specialized butcher shops.

In order to understand the presence of indicator microorganisms, first it was necessary to carry out the physico-chemical determinations shown in Table 2. In general, the a_w of the chorizo commercialized in the main groups of establishments analyzed herein had a mean value above 0.95, showing a significant difference ($P<0.05$) between market M_a and the specialized butcher shops (B). These values are consistent because they relate to a fresh sausage; furthermore, they are slightly higher than those reported for the chorizos of the state of Hidalgo (0.93-0.95)⁽¹³⁾ and the green chorizo commercialized in Toluca (0.94-0.96)⁽¹⁷⁾. Doubtless, such high a_w values were a factor that influenced the development of bacteria, but not of fungi or yeasts. As for the moisture content, the product exhibited percentages ranging between 41.8 and 50.1%; Furthermore, those establishment groups where chorizos are kept in refrigeration (M_d and B) exhibited a higher moisture percentage. These values were higher than those reported for chorizos of the state of Hidalgo (36 a 43 %)⁽¹³⁾. The high a_w values in a food item suggest a larger amount of water available for the development of chemical-enzyme reactions, as well as a greater microbial growth. On the other hand, the moisture content refers to the total water content, without specifying the portion of water that is associated to other molecules.

Table 2: Physico-chemical parameters of the traditional red chorizo commercialized in different groups of establishments in the city of Toluca

Establishment group	a_w	Moisture (%)	pH	Acidity (% lactic acid)	Nitrites (mg/kg)
M _a (n=15)	0.963±0.003 ^a	47.056±1.714 ^b	4.572±0.085 ^a	0.022±0.002 ^a	0.267±0.332 ^a
M _b (n=15)	0.959±0.003 ^{ab}	41.803±1.714 ^a	4.470±0.085 ^a	0.021±0.002 ^a	0.667±0.332 ^{ab}
M _c (n=6)	0.969±0.005 ^{ab}	46.515±2.710 ^{ab}	4.728±0.134 ^{ab}	0.022±0.003 ^{ab}	2.600±0.525 ^c
M _d (n=9)	0.964±0.004 ^{ab}	50.101±2.213 ^b	4.588±0.110 ^{ab}	0.028±0.002 ^{bc}	0.573±0.428 ^{ab}
B (n=30)	0.970±0.002 ^b	49.762±1.212 ^b	4.785±0.060 ^b	0.030±0.002 ^c	1.145±0.428 ^b

M= markets; B= specialized butcher shops.

a,b,c: Different superscripts in the same column indicate significant differences between the establishments ($P<0.05$).

The pH values were below 4.8, and there was no significant difference ($P>0.05$) between the chorizos from the various markets. The chorizo samples from specialized butcher shops (B) had significantly higher pH values than those from the markets M_a and M_b. A similar behavior was observed in regard to acidity, where the specialized butcher shops group exhibited significant differences ($P<0.05$) with respect to markets M_a, M_b and M_c. Studies conducted on Pamplona chorizo and Galician onion sausages have reported pH values of 4.4 and 4.8, respectively^(4,6); these values are understandable, given the ripening process, which lasts approximately 50 days. In the case of chorizos sold in Tulancingo, Mexico, pH values of 4.41 to 5.15 have been reported⁽³¹⁾, while green chorizos sold in Toluca have exhibited pH values between 4.49 and 5.64⁽¹⁷⁾. The fact that the traditional chorizos commercialized in Mexico, particularly in Toluca, exhibit pH values close to those of the ripened Spanish chorizos may be due to the combined effect of spontaneous fermentation during the airing process, with the consequent development of lactic bacteria and the production of organic acids, and the acidic nature of an important amount of ingredients such as vinegar, red wine, white wine, and dry red chili peppers, e.g. *guajillo*, *puya*, *ancho*, *chipotle*, and *chile de árbol* (*Capsicum annum* L.) reported in their formulation^(14,30).

As for nitrites, they are added with antimicrobial and antioxidant purposes, as well as to fix certain organoleptic characteristics of certain meat products and contribute to their development⁽³²⁾. In this study, chorizos of all the analyzed establishment groups exhibited nitrite values below 0.27 mg/kg; this value is far below the maximum limits (156 mg/kg) permitted by the Mexican official norms for sausages⁽¹¹⁾. These results agree with those documented by traditional producers, who claim that they do not add nitrites to their formulations⁽³⁰⁾. The marginal presence of nitrites may be ascribed to the biological oxidation of amines, or to the anaerobic reduction of the nitrate naturally present in the vegetables used to produce them, rather than to the intentional addition of nitrites for product conservation purposes. In certain locations, chorizos are usually manufactured without added nitrites, and this is a provision that must be met by such products as the Cantimpalos chorizo, which has a designation of origin⁽³³⁾.

The determination of indicator microorganisms in foods makes it possible to detect poor management practices or contamination; in most cases, a high number of indicator microorganisms may increase the risk of occurrence of pathogenic microorganisms⁽³⁴⁾. Particularly in the case of the traditional red chorizo commercialized in Toluca, values of 7.39 to 7.85 log₁₀ CFU/g were registered for aerobic mesophilic bacteria (Table 3). No significant differences were found ($P>0.05$) between the different groups of establishments. The presence of aerobic mesophiles usually indicates the degree of contamination of a sample. However, this does not apply to fermented foods, like the chorizo type analyzed here, since by nature a high bacterial multiplication is desirable for fermentation. The presence of aerobic mesophilic bacteria and lactic acid bacteria in sausages like the traditional Galician chorizo has been observed to increase during the fermentation process, reaching values of up to 8.55 log₁₀ CFU/g and 8.1 log₁₀ CFU/g, respectively, after 30 d of ripening⁽⁷⁾.

Table 3: Mean values and standard deviation in the counts of various microbial groups present in the traditional red chorizo commercialized in Toluca

Establishment group	Aerobic mesophilic bacteria (Log ₁₀ UFC/g)	Lactic acid bacteria (Log ₁₀ UFC/g)	Total coliforms (NMP/g)	Fecal coliforms (NMP/g)
M _a (n=15)	7.76±0.48 ^a	7.90±0.36 ^a	48.56±57.29 ^b	7.85±6.11 ^a
M _b (n=15)	7.85±0.56 ^a	7.87±0.46 ^a	19.61±16.73 ^a	9.67±4.67 ^a
M _c (n=6)	7.60±0.44 ^a	7.84±0.47 ^a	14.33±10.87 ^a	5.73±3.44 ^a
M _d (n=9)	7.39±0.49 ^a	8.07±0.63 ^a	11.07± 8.49 ^a	4.87±3.38 ^a
B (n=30)	7.80±0.49 ^a	8.01±0.47 ^a	34.06± 56.30 ^b	7.36±5.59 ^a

M= markets; B= specialized butcher shops.

MPN= minimum permitted number.

^{ab} Different superscripts in the same column indicate significant differences between markets ($P<0.05$).

In the case of fresh sausages, studies conducted on chorizos from the state of Hidalgo, Mexico, have documented a count of aerobic mesophilic bacteria ranging between 7.17 and 8.73 log₁₀ CFU/g⁽¹³⁾, while values above 8.0 log₁₀ CFU/g have been reported in chorizos from Mexico City⁽³⁵⁾. These differences may be due to the nature of the ingredients and production processes.

As for the coliforms (Table 3), their presence indicates the efficiency of the food sanitization and disinfection processes⁽³⁴⁾. Values for total coliforms ranging between 11.07 and 48.56 MPN/g were found, with significant differences ($P<0.05$) between the groups of establishments M_a and B with respect to the rest of the establishment groups. Fecal coliforms exhibited values between 4.87 and 48.56 MPN/g, without significant differences ($P>0.05$),

regardless of the group of establishments. This may be due, among other reasons, to poor quality of the raw materials utilized, to failures in the cold chain during the manufacture and commercialization process, to a lack of specific storage spaces for the raw materials and/or to the inexistence of a cleaning plan for the packs or containers⁽³¹⁾.

The Mexican Official Norm for processed meat products (NOM-213-SSA1-2002)⁽¹¹⁾ does not indicate the maximum permitted number of fecal coliforms allowed in raw products like chorizo ; it only establishes that the limit must be <3 MPN/g for cooked products. However, since chorizo is an essential ingredient in Toluca gastronomy, it is very important to apply a correct degree of cooking in which the minimum temperature at the thermal core of this meat product is 74 °C, according to the norm NOM-251-SSA1-2009⁽³⁶⁾. This will allow avoiding the incidence of food-borne infections that may put at risk the health of local consumers and of those tourists who in the course of their visit to the city of Toluca wish to taste this famous sausage, especially when it is added to traditional fast foods, such as *tacos*, *tortas*, *sopes*, and others.

The presence of lactic acid bacteria (LAB) is inherent to raw-cured fermented sausages because they are added intentionally during the manufacture process or because they develop during the ripening process. LAB counts in the order of 8.0 a 8-5 log₁₀ CFU/g and 8.6 log₁₀ CFU/g, respectively, have been reported in the traditional chorizos made with pork of the Chato Murciano breed or in ripened Galician chorizos^(7,37), depending on the ripening time. In contrast, lower counts, of 7.4 to 9.0 log₁₀ CFU/g, have been reported in the fresh and slightly acidified chorizos of the state of Hidalgo⁽¹³⁾. LAB counts between 7.84 and 8.07 log₁₀ CFU/g were found, without significant differences ($P>0.05$) between the studied establishment groups. The presence of LAB favors spontaneous fermentation during the airing or drying process (1-3 d)⁽³¹⁾, and it could be partly responsible for both the acidity and the stability of the product.

Salmonella is one of the main pathogenic bacteria, and its simple presence, even in low numbers, entails a significant health hazard⁽³⁸⁾. According to the norm NOM-213-SSA1-2002⁽¹¹⁾, the presence of *Salmonella* spp. is not allowed in cooked, cured, marinated, brined, or raw meat products like chorizo; it should be “absent” from 25 g of product. Nevertheless, according to the literature^(38,39,40), its presence has been reported in samples of fresh chorizos of certain localities of Mexico. This is ascribed, among other causes, to poor hygienic handling of the product, to the lack of an ongoing control of the temperature along the supply chain of the sausages (cold chain) from its production to its distribution, storage, and exhibition at the point of sale, commercialization and consumption. This is a consequence of the lack of investment in adequate equipment and infrastructure. Another aspect that might also compromise the innocuousness of the product and favor the presence of *Salmonella* in it is the use of natural guts for stuffing⁽³⁸⁾.

The presence of *Salmonella* in the traditional red chorizo analyzed here was positive in the product from markets as well as from certain specialized butcher shops (Table 4). The highest percentage of incidence (60 %) was observed in the market M_b, where 15 samples tested positive. The group of establishments within the market M_b caters to a middle-low sector, and has a great affluence due to its closeness to the Central Bus Station of Toluca. On the other hand, the shops in market M_d were the ones with the lowest percentage (11%) of samples that tested positive for *Salmonella* (9 samples); this market targets a middle-high sector and has security facilities and hygiene. Given the infrastructure of specialized butcher shops, we expected to find in them a lower incidence of *Salmonella*; however, this was not the case. The presence of *Salmonella* spp. in this type of establishments may have been due to cross-contamination favored by the handling of other meat products (for example, pork brawn, fresh meat, chili pork meat, among others), which are often commercialized and displayed together with chorizo.

Table 4: Percentage of samples of traditional red chorizo of Toluca that tested positive for *Salmonella* spp. and *Escherichia coli* by establishment group

Establishment group	<i>Salmonella</i> spp.	<i>E. coli</i>
M _a (n=15)	20.0	40.0
M _b (n=15)	60.0	40.0
M _c (n=6)	33.3	16.7
M _d (n=9)	11.1	22.2
B (n=30)	30.0	43.33

M= markets; B= specialized butcher shops.

Based on the above, the red chorizo commercialized in Toluca may represent a latent risk factor for the health of the consumers; hence the need to implement control and monitoring of the temperatures, as well as an adequate hygienic handling throughout the supply chain, in order to eliminate the possibility of development of pathogenic microorganisms. Furthermore, it is advisable to raise the awareness of the consumers and of those in charge of food and drink establishments regarding the appropriate handling and cooking of the product, and to remind them that the product must be cooked at a temperature above 74 °C (at its core), during an adequate number of hours, in order to eliminate the potential risk of food-borne infections due to the development of *Salmonella* and other pathogenic microorganisms in the product.

On the other hand, *E. coli* is generally a harmless bacterium; however, pathogenic strains can exchange genes and generate disease-causing variations⁽³⁴⁾. Samples in all the groups of

establishments tested positive for *E. coli*. The group of establishments with the highest incidence was that of specialized butcher shops (B), with 43.33% (30 positive samples) (Table 4). Oddly enough, the group of establishments with the lowest percentage of positive tests was M_c, which caters to the middle-low sector of the population, and where with 17 % of the samples (6 samples) tested positive.

Although the traditional red chorizo commercialized in Toluca is manufactured by different producers from their own recipes, the samples exhibited high similarities in terms of their physico-chemical and even biological characteristics, especially in terms of the number of LAB.

The fact of having found samples of traditional red chorizo that tested positive for *Salmonella* spp. and *E. coli* represents a call to attention for both producers and vendors, and at the same time suggests the need to implement programs to allow the selection of higher-quality raw materials, the improvement of the hygienic handling of the product, and the maintenance of the cold chain. This may contribute to maintain or improve the reputation and increase the sales of this very traditional and iconic sausage beyond a local level, leading to a better life quality for those who for centuries, from one generation to the next, have preserved the *know how* in relation to its manufacture.

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