

# Microbiological quality of ready-to-eat street-vended flavored waters and fruits salads in Reynosa, Tamaulipas, Mexico

Calidad microbiológica de aguas y ensaladas de frutas “listas para comer” vendidas en la ciudad de Reynosa, Tamaulipas, México

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## ABSTRACT

The aim of this study was to evaluate the microbiological quality of flavored waters and fruit salads by determining the presence of aerobic mesophilic, fecal coliform and pathogen organisms, such as *Salmonella* spp., *Shigella* spp. and *Staphylococcus aureus*. The presence of microorganisms was determined based on the Mexican Official Standards for each microorganism and reported protocols. We performed a microbiological analysis of 48 samples of flavored water sold in some ice cream stores, and 20 samples of fruit salad from the supermarket. Aerobic mesophiles were detected in 82.3% of all samples, while fecal coliforms and *S. aureus* were detected in 35.2% of them. *Salmonella* spp. was isolated in 16.1% of the samples; *Klebsiella* spp. in 33.8% of them, while *Shigella* spp. only in 2.9% of the total. The results presented in this study reveal the need for preventive measures, with a special focus on hygienic food handling and processing practices, which could help preventing a great number of foodborne diseases.

## RESUMEN

El logro de este trabajo fue evaluar la calidad microbiológica de aguas y ensaladas de frutas, se determinó la presencia de mesófilos aerobios, coliformes fecales patógenos como *Salmonella* ssp., *Shigella* ssp. y *Staphylococcus aureus*. La presencia de microorganismos fue determinada basándose en normas oficiales y protocolos establecidos. Analizamos 48 muestras de aguas de frutas provenientes de neverías y 20 muestras de ensaladas provenientes de súper mercados. Los aerobios mesófilos fueron detectados en el 82.3% de las muestras, coliformes fecales y *S. aureus* en el 35.2%, *Salmonella* ssp. en el 16.1% y *Shigella* ssp. en el 2.9%. Los resultados del presente estudio revelan la necesidad de implementar medidas preventivas, con especial enfoque en las prácticas de higiene y manipulación, para prevenir un gran número de enfermedades transmitidas por alimentos.

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### Keywords:

Fruit salads; flavored waters; ready-to-eat food; fecal coliforms; aerobic mesophiles.

### Palabras Claves:

Ensalada de frutas; aguas de frutas; comida lista para comer; coliformes; mesófilos aerobios.

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## INTRODUCTION

Preparing and selling food in the street is an ancient activity, particularly widespread in developing countries. The food preparation, handling and servicing at large scale provides an ample opportunity for the spread of foodborne diseases, either sporadically or on epidemiological scale (Food and Agriculture Organization [FAO]/ World Health Organization [WHO], 1986). Street-vended foods are often the main source of cheap, convenient and significantly nutritious food for both urban and rural populations (Mensah, Yeboah-Manu, Owusu-Darko & Ablordey, 2002). The food and beverages defined as “ready-to-eat” (RTE) are prepared for being sold especially in streets and similar public places (Samapundo, Climat, Xhaferi & Devlieghere, 2015). There is an increasing global interest in RTE items as part of a general concern for

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health and food safety (Canet & N'Diaye, 1996), particularly those which are composed of meat/poultry and salads, having been recognized as potential vehicles of bacterial foodborne pathogens (Campos, Gil, Mourão, Peixe & Antunes, 2015), generally associated with consumption of food that has been exposed to pathogenic microorganisms such as bacteria, viruses and parasites, and to their metabolic toxins, which results in health disorders, either chronic or acute (Ossai, 2012). Some of the microorganisms associated with these diseases are bacteria, such as *Escherichia coli*, *Salmonella* spp., *Clostridium* spp., *Staphylococcus* spp., *Bacillus cereus*, etc. (Food and Agriculture Organization [FAO], 1982, Campos *et al.*, 2015). The aim of this study was to evaluate the microbiological quality of street-vended flavored water and fresh fruit by determining the presence of aerobic mesophilic microorganisms, fecal coliforms and pathogens such as *Escherichia coli* (*E. coli*), *Salmonella* spp., *Shigella* spp. and *Staphylococcus aureus* (*S. aureus*).

## MATERIALS AND METHODS

### Collection of Samples

This research was conducted in the city of Reynosa, state of Tamaulipas, Mexico. 48 samples of flavored water and 20 samples of fruit salad were collected from some ice cream stores and supermarkets, respectively, summing a total of 28 establishments. The samples were preserved in their original containers, transported to the laboratory in a cooler and immediately analyzed.

### Sample Preparation

In the case of liquid samples, 60 ml of each sample were analyzed, and then homogenized by stirring for 1 min. In the case of solid samples, 60 g of each sample were analyzed, and then liquefied with diluent for not more than 2 min. After that, decimal and seeded broth dilutions were performed according to the required analysis.

### Aerobic mesophiles

The presence of these microorganisms was determined according to the NOM-092-SSA1-1994 standard (DOF, 1994), using the technique of culture in depth; this method consists in counting colonies grown on agar after an incubation period of 48 h at 35 °C ± 2 °C. The results were expressed in CFU/ml or g of sample. The official NOM-093-SSA1-1994 standard (DOF, 1994) establishes the allowable limits for these microorganisms.

### Fecal coliforms and *Escherichia coli*

The MPN (most probable number) technique, described by Camacho *et al.* (2009), was used; this method relies on the ability of some coliform bacteria to ferment lactose when incubated at 44 °C for 24 h. To determine fecal microorganisms, a presumptive test in sodium lauryl sulfate broth was conducted, including incubation for 48 h at 35 °C ± 2 °C, as well as a confirmatory test in EC-MUG broth, including also incubation but in water bath for 24 h at 44 °C. To determine the presence of *Escherichia coli*, positive confirmatory tests were irradiated with a UV lamp; to isolate and confirm the strain, Eosin Methylene Blue Agar (Levine) was used, incubating samples for 24 h at 35 °C ± 2 °C, and subsequent biochemical tests. The official NOM-093-SSA1-1994 standard (DOF, 1994) establishes the allowable limits for these microorganisms.

### Determination of *Staphylococcus aureus*

A total count of *S. aureus* was conducted by direct plating on agar Baird-Parker, with an incubation time of 48 h at 35 °C ± 2 °C. The presence of typical colonies was confirmed using coagulase and thermonuclease tests, as established by the NOM-115-SSA1-1994 standard (DOF, 1995).

### Detection of *Salmonella* spp.

Following the NOM-114-SSA1-1994 standard (DOF, 1995), a pre-enrichment culture with a lactose broth was used, followed by an enrichment culture with tetrathionate broth, subsequent seeding on selective media, such as Hektoen Enteric Agar, Agar XLD, *Salmonella Shigella* Agar and Bismuth Sulfite Agar, and biochemical tests (glucose fermentation, urease, lysine decarboxylase, indole test, H<sub>2</sub>S production and dulcitol fermentation); all cultures were incubated for 24 h at 35 °C ± 2 °C.

### Determination of *Shigella* spp.

An enrichment culture with GN broth was used, following Barrantes & Achí (2011); samples were then replated on selective media such as Hektoen Enteric Agar, XLD Agar, *Salmonella Shigella* Agar, and further biochemical tests were conducted. All cultures were incubated for 24 h at 35 °C ± 2 °C.

## RESULTS

This study analyzed 28 street food establishments, including some ice cream stores and supermarkets. The

water samples included 6 different flavors: "rice water" (*horchata*) ( $n = 8$ ), pineapple ( $n = 9$ ), lemon ( $n = 8$ ), melon ( $n = 8$ ), "roselle flowers" (*jamaica*) ( $n = 8$ ) and tamarind ( $n = 7$ ). As for fruit salads, 20 samples of fruits such as papaya, pineapple, strawberry, mango, peach, watermelon, jicama, orange, apple, melon and banana were collected. These samples were divided into two groups: salads with orange juice ( $n = 7$ ) and salads without orange juice ( $n = 13$ ).

### Determination of aerobic mesophiles

Table 1 shows the overall results of aerobic mesophiles in fruit water and fruit salad samples. Among the total samples analyzed, mesophilic contamination was found in 82.3% ( $n = 56/68$ ). Among the 48 fruit water samples analyzed, mesophilic contamination was found in 77% ( $n = 37/48$ ), with a range of 20 CFU/ml – 940 000 CFU/ml, which exceeded 6.2 times the maximum value dictated by the standard (150 000 CFU/ml). Pineapple water showed a high degree of contamination, with an average of 225 190 CFU/ml; it was also the water flavor with more out-of-standard samples, a 44.4% ( $n = 4/9$ ), while lemon water showed the lowest degree of contamination, with 501 CFU/ml. On the other hand, 95% of fruit salad samples were contaminated ( $n = 19/20$ ), with counts of 95 CFU/g to 240 000 CFU/g, exceeding 1.6 times the maximum value, according to the standard. The group of fruit salads without orange juice showed 92.3% ( $n = 12/13$ ) of positive samples, with a bacterial count average of 41 009 CFU/g, but only one sample was found to be out of standard (14.2%;  $n = 1/7$ ). The group of fruit salads

with orange juice showed 100% ( $n = 7/7$ ) contamination, with a bacterial count average of 69 040 CFU/g and 16.6 % ( $n = 2/12$ ) of out-of-standard samples.

### Fecal coliforms and *Escherichia coli*

Table 2 shows the general data about fecal coliforms and *Escherichia coli* isolation. Among the total samples, 35.2% ( $n = 24/68$ ) were found positive for bacterial contamination. Among the flavored water samples analyzed, 41.6% ( $n = 20/48$ ) showed contamination by these microorganisms, with a count of < 3 MPN/ml – 654 MPN/ml. All positive samples were out of the official Mexican standard, which states that there must be no fecal contamination in these foods. *Horchata* water had the largest number of contaminated samples, with an 87.5% ( $n = 7/8$ ) and a contamination average of 640 MPN/ml; however, melon water showed the highest average of fecal contamination with 654 MPN/ml, while *jamaica* water showed the lowest average with 5 MPN/ml. Fruit salads showed less contamination, with only 20% ( $n = 4/20$ ) of the samples showing fecal contamination; again, any positive sample was considered as out of standard. The counts of these organisms were of 9 MPN/g – 23 MPN/g. In the group of fruit salads with orange juice, 28.5% ( $n = 2/7$ ) of the samples tested positive for contamination, with an average of 2.46 MPN/g, while in the group of fruit salads without orange juice, only 15.3% ( $n = 2/13$ ) were positive, with an average of 4.57 MPN/g. No presence of *Escherichia coli* was detected in any sample analyzed in this study.

**Table 1.**  
Results for aerobic mesophiles by type of water and group of fruits.

	Positive samples	Samples outside the Norm	Average CFU/g or ml	Range
Fruit Waters ( $n = 48$ )	37 (77%)	10 (27%)	95 769	20 - 940 000
<i>Horchata</i> ( $n = 8$ )	8 (100%)	3 (37.5%)	102 802	640 - 340 000
Pineapple ( $n = 9$ )	8 (100%)	4 (44.4%)	225 190	5 100 - 710 000
Lemon ( $n = 8$ )	3 (37.5%)	0	501	110 - 3 400
Melon ( $n = 8$ )	8 (100%)	1 (12.5%)	138 795	2100 - 940 000
<i>Jamaica</i> ( $n = 8$ )	6 (75%)	2 (25%)	101 896	20 - 430 000
Tamarind ( $n = 7$ )	3 (42.8%)	0	5428	1000 - 220 000
Fruit Salads ( $n = 20$ )	19 (95%)	3 (15.7%)	433 209	5 - 240 000
Fruit Salads with orange juice ( $n = 7$ )	7 (100%)	1 (14.2%)	690 409	5 - 240 000
Fruit Salads without Orange juice ( $n = 13$ )	12 (92.3%)	2 (16.6%)	41 009	130 - 220 000

Source: Author's own elaboration.

**Table 2.**  
Results for fecal coliforms by type of water and group of fruits.

	Positive samples	Samples outside the Norm	Average CFU/g or ml	Range	<i>E. coli</i>
Fruit Waters (n = 48)	20 (41.6%)	20 (100%)	294	< 3 - 2400	0
<i>Horchata</i> (n = 8)	7 (87.5%)	7 (100%)	640	< 3 - 2400	0
Pineapple (n = 9)	6 (66.6%)	6 (100%)	323	< 3 - 2400	0
Lemon (n = 8)	0	0	0	0	0
Melon (n = 8)	5 (62.5%)	5 (100%)	654	< 3 - 2400	0
<i>Jamaica</i> (n = 8)	1 (12.5%)	1 (100%)	5	< 3 - 43	0
Tamarind (n = 7)	1 (14.2%)	1 (100%)	100	< 3 - 1000	0
Fruit Salads (n = 20)	4 (20%)	4 (100%)	3.5	< 3 - 23	0
Fruit Salads with orange juice (n = 7)	2 (28.5%)	2 (100%)	2.5	< 3 - 24	0
Fruit Salads without Orange juice (n = 13)	2 (15.3%)	2 (100%)	4.6	< 3 - 25	0

Source: Author's own elaboration.

### Detection of *Staphylococcus aureus*

Table 3 shows the overall results for *S. aureus*; 35.2% (n = 24/68) of the samples showed contamination by this microorganism. 37.5% (n = 18/48) of the flavored water samples showed contamination, with counts of 100 CFU/ml – 200 000 CFU/ml. *Horchata* water showed higher contamination than the rest of the samples, with 62.5% (n = 5/8) and a contamination index of 26 625 CFU/ml. Even so, tamarind water showed the highest contamination level, with an average of 41 428 CFU/ml. Lemon water showed the lowest contamination level, with 1375 CFU/ml. Fruit salads showed less contamination by this microorganism, with 30% (n = 6/20) of positive samples and a contamination range of 100 CFU/g to 10 000 CFU/g. Fruit salads with orange juice showed 42.8% (n = 3/7) of positive samples, with an average of 1471 CFU/g, while fruit salads without orange juice had 23% (n = 7/13) of contaminated samples, with an average of 69 CFU/g.

### Detection of *Salmonella* and *Shigella* spp.

Table 4 shows the overall results. The presence of *Salmonella* spp. was detected in 16.2% (n = 11/68) of all samples analyzed. Among fruit water samples, 16.6% (n = 8/48) were positive for contamination with this microorganism; the most contaminated were *horchata*, melon and *jamaica*, with 25% (n = 2/8). In the case of fruit salads, this microorganism was present in 15% of all samples (n = 3/20); the group of fruit salads with orange juice was the most contaminated with 28.6%

(n = 2/7) of them infected. In the case of *Shigella* spp., only 2.9% (n = 2/68) of all samples tested were contaminated; only flavored water samples showed total contamination by this organism (4.1%; n = 2/48), *horchata* and melon water having one positive sample each (12.5%; n = 1/8).

### Other isolated pathogens

Table 5 shows the general data. In addition to the microorganisms that were specifically sought in this study, the isolation of some other pathogens, such as *Klebsiella pneumoniae* (*K. pneumoniae*), *Klebsiella oxytoca* (*K. oxytoca*), *Enterobacter aerogenes* (*E. aerogenes*) and *Serratia marcescens* (*S. marcescens*) was successfully achieved. These microorganisms were isolated in 36.8% (n = 25/68) of the samples. The most frequently isolated was *K. pneumoniae*, in 33.8% (n = 23/68) of the samples. Among the flavored water samples, 50% (n = 24/48) showed contamination; *horchata* was the most frequently contaminated with 87.5% (n = 7/8) of the samples. The presence of *K. pneumoniae* was detected in 41.6% (n = 20/48) of the flavored water samples; it was more prevalent in *horchata* water, as 100% of the samples (n = 7/7) were positive for its presence. These microorganisms were found in only 15% (n = 3/20) of the fruit salad samples; the group of fruit salads without orange juice showed greater contamination with 15.3% (n = 2/13). It is worth noting that *K. pneumoniae* was the only bacteria that prevailed in this type of sample.

**Table 3.**  
 Results for *Staphylococcus aureus* by type of water and group of fruits

	Positive samples	Average CFU/g or ml	Range
Fruit Waters (n = 48)	18 (37.5%)	21 963	100 - 200 000
Horchata (n = 8)	5 (62.5%)	26 625	1000 - 100 000
Pineapple (n = 9)	4 (44.4%)	2230	100 - 20 000
Lemon (n = 8)	2 (25%)	1375	1000 - 10 000
Melon (n = 8)	2 (25%)	20 125	1000 - 160 000
Jamaica (n = 8)	2 (25%)	40 000	120 000 - 200 000
Tamarind (n = 7)	3 (42.8%)	41 428	90 000 - 100 000
Fruit Salads (n = 20)	6 (30%)	560	100 - 10 000
Fruit Salads with orange juice (n = 7)	3 (42.8%)	69.2	100 - 400
Fruit Salads without Orange juice (n = 13)	3 (23%)	1471	100 - 10 000

Source: Author's own elaboration.

**Table 4.**  
 Results for *Salmonella* spp. and *Shigella* spp. by type of water and group of fruits

	<i>Salmonella</i> spp. Positive samples	<i>Shigella</i> spp. Positive samples
Fruit Waters (n = 48)	8 (16.6%)	2 (10%)
Horchata (n = 8)	2 (25%)	1 (12.5%)
Pineapple (n = 9)	1 (11.1%)	0
Lemon (n = 8)	0	0
Melon (n = 8)	2 (25%)	1 (12.5%)
Jamaica (n = 8)	2 (25%)	0
Tamarind (n = 7)	1 (14.2%)	0
Fruit Salads (n = 20)	3 (15%)	0
Fruit Salads with orange juice (n = 7)	2 (28.6%)	0
Fruit Salads without Orange juice (n = 13)	1 (7.7%)	0

Source: Author's own elaboration.

**Table 5.**  
 Results of *K. pneumoniae*, *K. oxytoca*, *E. aerogenes* and *S. marcescens* by type of water and group of fruits.

	Positive samples	<i>K. pneumoniae</i>	<i>K. oxytoca</i>	<i>E. aerogenes</i>	<i>S. marcescens</i>
Fruit Waters (n = 48)	22 (45.8%)	20 (42%)	3 (6%)	4 (8%)	0
Horchata (n = 8)	7 (87.5%)	7 (15%)	2 (4%)	0	0
Pineapple (n = 9)	7 (77.7%)	5 (10%)	0	2 (4%)	0
Lemon (n = 8)	0	0	0	0	0
Melon (n = 8)	6 (75%)	6 (13%)	0	1 (2%)	0
Jamaica (n = 8)	2 (25%)	1 (2%)	0	1 (2%)	0
Tamarind (n = 7)	2 (25%)	1 (2%)		0	0
Fruit Salads (n = 20)	3 (15%)	0	0	0	0
Fruit Salads with orange juice (n = 7)	1 (14.2%)	1	0	0	0
Fruit Salads without Orange juice (n = 13)	2 (15.3%)	2	0	0	0

Source: Author's own elaboration.

## DISCUSSION

Food security has been defined as the ability of a society to address the food needs of its people (Barcelo, 1989). However, this definition should not be limited only to the field of food availability, because even if it is enough food available, the biological utility of food can be affected if its safety is not taken into account (Daley, David & Robertson, 1982). Today, it is well known that food-handling personnel play an important role in ensuring food safety, since mismanagement and the breach of hygiene regulations by those engaged in the selling of food can help pathogens get into contact with it, survive in it and multiply to a scale at which they are able to cause diseases in consumers. Thus, the results of this study reflect improper practices of food preparation and the lack of knowledge among employees of food selling establishments about proper food handling, processing and hygiene, which causes food to suffer contamination by the microorganisms mentioned above.

This study showed that flavored waters are more contaminated than fruit salads, which is attributable to the fact that most of the flavored water samples were collected in open-air exposed establishments; in addition, the preparation of flavored water requires a lot of handling. In contrast, fruit salad samples were collected from supermarkets and there was less handling of the fruits compared to fruits for making flavored water. There is a remarkable difference between these results and those reported by Félix, Campas & Meza (2005), who evaluated the presence of aerobic mesophiles, fecal coliforms and *Salmonella* spp. They found a range of 200 – 450 000 and 11 600 – 850 000 of aerobic mesophiles in salads and flavored water respectively. On the other hand, *Salmonella* was present in only 3% of salads. This can be explained because in their study the flavored waters were all collected from fixed establishments, while fruit salads were collected from street establishments; consequently, fruit salads showed higher contamination by mesophiles, fecal coliforms and *Salmonella* spp. than flavored waters, the opposite of what was found hereby.

In the present research work, *S. aureus* was isolated in 37.5% of flavored water samples, largely above the percentage reported by Tambekar, Jaiswal, Dhankar, Gulhane & Dudhane (2009), who isolated this microorganism in only 6% of the fruit juice samples. In this study, the presence of this bacteria was much higher in fruit water samples than in fruit salads, yet the amount of enterotoxigenic *S. aureus* was not enough to be toxic. The reason for the low counts

of *S. aureus* is that this microorganism is not competitive, and in most cases, it was isolated together with other bacteria. The presence of this bacteria is attributable to the fact that it is part of the normal flora of the human body, where it is found in the nasopharyngeal tract and on the skin. When collecting the samples, it was observed that, during the preparation of flavored water, employees tend to use their bare hands when picking the ice, and they also speak during the preparation; it was also realized that, when serving water, it makes contact with their skin, and droplets of it fall into the main container of flavored water.

No *E. coli* was isolated in this study, despite being considered the most important fecal microorganism. However, the isolation of *K. pneumoniae* is not surprising, since this microorganism is considered part of fecal coliforms and its presence is consistent with other studies in which it was isolated in juice samples (Reddy, Chandrakanth, Indu, Venkata & Usha 2009; Tambekar *et al.*, 2009).

*Salmonella* spp. was isolated in 16.6% of the total samples, a very similar result to that reported by Tambekar *et al.* (2009) and Ukwo, Ndaeyo & Udoh (2011), both of whom reported the presence of this microorganism in 17% of the juice samples analyzed. However, only 15% of fruit salad samples showed contamination by this microorganism, a low percentage compared with those reported by Bayona (2009), who presented that 25% of the fruit samples were contaminated by this organism.

In this study, *Shigella* spp. was isolated in 4.1% of fruit water samples, unlike the study by Lewis, Thompson, Rao, Kalavati & Rajanna (2006), which reported contamination by these bacteria in 16.6% of his juice samples. Regarding the absence of this microorganism in fruit salads, this coincides with the studies made by Utzinger, Arias, Monge & Antillón (1992).

In conclusion, street-vended foods have clearly a higher degree of contamination than those sold in closed establishments. Aerobic mesophilic microorganisms were detected in 82.3% of all samples, while contamination by fecal coliforms and *Staphylococcus aureus* was detected in 35.2% of them, *Salmonella* spp. was present in 16.1% of them, *Klebsiella* in 33.8% of the samples and *Shigella* spp. in only 2.9% of them. No presence of *Escherichia coli* was detected. In addition to the excessive handling and the lack of water for washing, the environmental pollution also contributes to contaminate food and drinks. For all these reasons, it is necessary to establish preventive

measures, such as the training of the sales personnel, focusing on hygiene practices in handling and processing of food. These could prevent many foodborne diseases, since access to good quality food is a basic requirement for the preservation of human health.

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