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In Mexico City, fresh-squeezed street-vended orange juice is contaminated with fecal coliforms, *Escherichia coli*, and Shiga toxin-producing *E. coli*: A potential risk for acquiring foodborne diseases

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Abstract

This study evaluated the microbiological quality and prevalence of *Escherichia coli* and diarrheagenic *E. coli* pathotypes (DEP) among fresh-squeezed orange juice from street vendors and their hygienic practices. Sixty orange juice stalls in Mexico City were visited, and 60 samples were purchased. Most (65%) of the street vendors had poor hygienic practices. Twelve (20%) prepared orange juice at the time of purchase, while the other stalls sold already-squeezed orange juice. All samples analyzed were positive for aerobic mesophilic bacteria, with limits ranging from 1 to 6.8 log CFU/mL. A total of 35 (58.3%), 27 (45%), and 5 (8.3%) samples were positive for total coliforms, fecal coliforms, and *E. coli*, respectively, present at concentrations ranging from < 3 to > 1100, < 3 to > 1100, and < 3 to 53 MPN/mL, respectively. Two samples harbored DEP at concentrations of 3.6 and 9.2 MPN/mL. Both samples harbored Shiga toxin-producing *E. coli* strains, positive for the Shiga toxin 2 (*stx*2) locus. Our results suggest that consumption of fresh-squeezed street-vended orange juice may pose a health risk for consumers, revealing the necessity to implement adequate hygiene and safety practices to protect the health of consumers.

Keywords: street-vended orange juice; hygienic practices; microbiological quality; *Escherichia coli*; Shiga toxin-producing *E. coli*.

Practical Application: Street-vended orange juice is of poor microbiological quality and some samples harbor foodborne pathogens, hence the implementation of adequate hygiene and safety practices is very important to reduce health risks for consumers.

1 Introduction

Street vendors in Mexico provide ready-to-eat food for people from different economic backgrounds (Rosales Chavez et al., 2021). In a global food intake survey, 30% of Mexican respondents reported consuming street food. This is stated in the most recent Nielsen global study on food trends outside the home, applied to 30,000 respondents in 61 countries around the world (Nielsen Corporation, 2016). Consumption of fresh-squeezed orange juice is part of the traditional breakfast in Mexico. Therefore, as expected it is one of the most popular beverages sold by street vendors.

Orange juice is the most consumed fruit juice worldwide, particularly appreciated by consumers for its organoleptic properties, and is replete with nutrients and antioxidants (Galaverna & Dall'Asta, 2014). An 8-ounce serving of orange juice provides 122 calories provided almost entirely by carbohydrates such as sucrose, fructose, and glucose. Additionally, orange juice provides a small amount of protein (2 g/8 ounces) and is fat-, saturated fat-, cholesterol-, and sodium-free. Moreover, it is an excellent source of vitamin C, and a good source of folate, potassium, vitamin B6, thiamin, niacin, riboflavin, and vitamin A. Magnesium, iron and calcium are other minerals present in orange juice (Rampersaud & Valim, 2017). Despite the abundance of nutrients and antioxidants, fresh orange juice has been associated with outbreaks of diseases (Vojdani et al., 2008; Krug et al., 2012). One major factor in the occurrence of outbreaks is the lack of pasteurization during orange juice elaboration (Martínez-Gonzales et al., 2003).

Escherichia coli is a normal inhabitant of the gastrointestinal tract; however, some *E. coli* strains have horizontally acquired virulence characteristics that enable them to cause intestinal and extraintestinal illness in humans and other animals (Jesser & Levy, 2020). The diarrheagenic strains of *E. coli* are a very homogeneous group of intestinal pathogens, with different characteristics regarding the antigen structure, virulence mechanisms, host colonization sites, and clinical evolution (Gomes et al., 2016). Based on the presence/absence of virulence factors, the diarrheagenic strains of *E. coli* are categorized into six pathotypes, collectively known as diarrheagenic *E. coli* pathotypes (DEP). DEP include enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC),

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enteroinvasive *E. coli* (EIEC) and Shiga toxin-producing *E. coli* (STEC, also referred to as enterohemorrhagic *E. coli* (EHEC)) (Gomes et al., 2016). DEP are important etiologic agents of diarrhea worldwide (Jesser & Levy, 2020). Several DEP have been involved in outbreaks of diseases where unpasteurized fruit juices have been identified as the vehicle. The DEP involved in these outbreaks included STEC (mainly associated with serotype O157:H7) and ETEC (Singh et al., 1995; Centers for Disease Control and Prevention, 1996; Parish, 1997; Cody et al., 1999). Street-vended fresh-squeezed orange juice has also been shown to harbor human pathogens in Mexico (Castillo et al., 2006; Cerna-Cortes et al., 2016; Dominguez-Gonzalez et al., 2022; Ocaña De Jesús et al., 2022b). These studies show that the consumption of fresh-squeezed orange juice may pose a risk to the health of consumers. Therefore, the microbiological quality of these products must constantly be evaluated to supply safe orange juice to the public. Due to the paucity of data on the microbial contamination of fresh orange juice in Mexico, we conducted the current study with the following aims: i) to evaluate the overall microbiological quality and prevalence of *E. coli* and ii) to identify the occurrence of DEP in fresh-squeezed orange juice purchased from street vendors in Mexico City, and iii) to record the hygienic practices of street vendors.

2 Materials and methods

2.1 Area of study and sample collection

The selected area of study was Mexico City, a large urban area that, although it has almost 9 million registered inhabitants, reaches a population of nearly 25 million during working hours. A total of 60 samples of fresh-squeezed orange juice, were purchased from street vendors located outside Mexico City metro system stations, between February and October 2019. A total of 33 stations of the 12 metro system lines, located at eight boroughs of Mexico City (Figure 1), were selected at random. The metro stations were selected due to the large number of users: the total number of passengers traveling by metro during 2021 was 837,473,413 (Sistema de Transporte Colectivo, 2021).

The sampling schedule was from 7 to 10 am, which is when there is a greater influx of people who go to work and buy orange juice, sometimes as their only breakfast. Each sample consisted of 1 L of orange juice, which was poured by the vendor into a disposable plastic cup. After purchasing, the juice was immediately poured into a sterile polypropylene bag (Whirl-Pak, Nasco, Madison, WI, USA), which was placed into a rack for transportation to the laboratory. We recorded whether the orange juice was extracted at the time of purchase or if it was already prepared. Samples were analyzed no more than 2 h after purchase.

2.2 Hygienic practices of street vendors

The hygienic practices of street vendors were recorded at the time of purchase. Three criteria were considered: 1) cleanliness of the stall, 2) cleanliness of the food handler, and 3) quality and cleanliness of the raw material (oranges). A score of 1 point was assigned for the correct criteria while a score of 0 points was assigned for the wrong criteria. For the registration of hygienic conditions, evaluation forms were used with a score of 0 to 3. According to the scores, two levels of hygienic conditions were

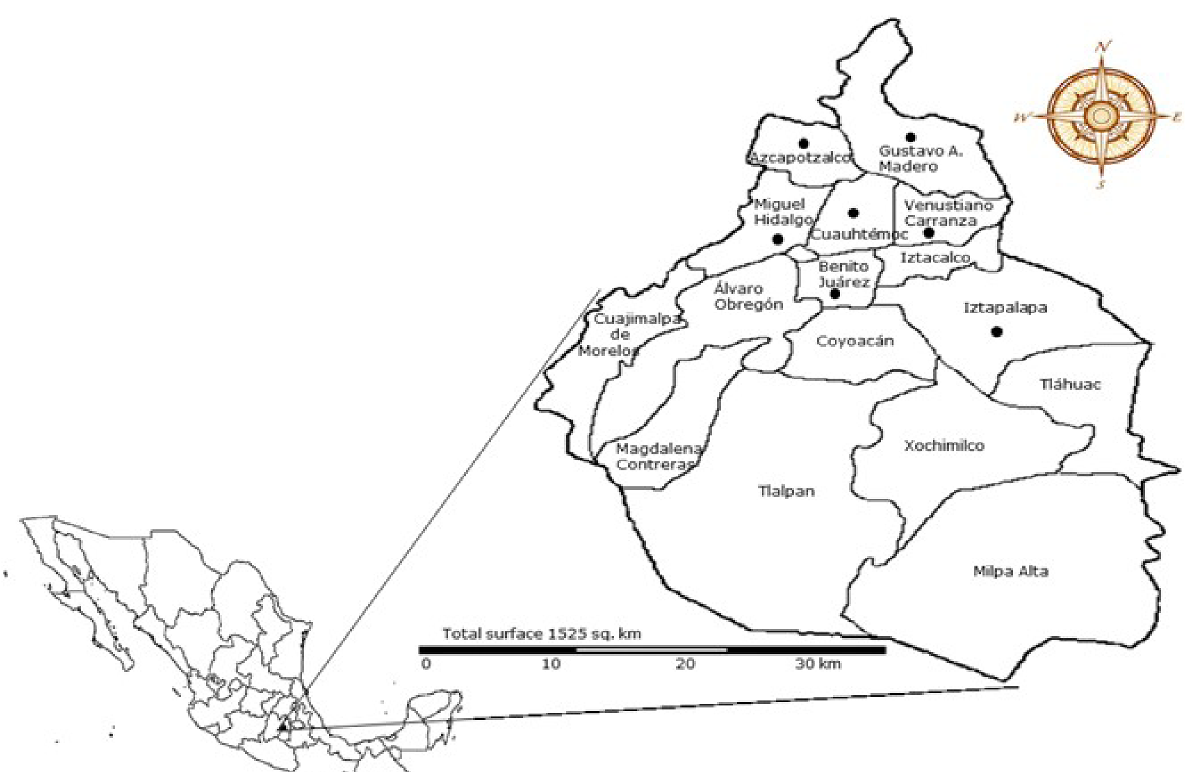

Figure 1. Boroughs in Mexico City where fresh-squeezed orange juice samples were collected (●).

established: good hygiene conditions for a score of 2 to 3 points and poor hygiene conditions for 0 to 1 point.

2.3 Microbiological analysis

A 10 mL portion of orange juice was separated from each sample and used for pH measurement in a pH meter (model pH 209, HANNA Instruments, Sarmeola di Rubano-PD, Italy). Subsamples (50 mL) from each fresh orange juice sample were placed in a sterile plastic bag (Whirl-Pak, Nasco, Madison, WI, USA) and lactose broth (Difco™ BD, Sparks, MD, USA) was added to obtain a final dilution of 1:10 (10^{-1}). Samples were homogenized for 1 min in a stomacher (tissue disrupter) and serially diluted (10^{-1} to 10^{-4}). These dilutions were used for quantification (CFU/mL) and estimation (MPN/mL) of various microorganisms. Each sample was tested for the presence of aerobic mesophilic bacteria (AMB), total coliforms (TC), fecal coliforms (FC), and *E. coli* following the methods approved by the Bacteriological Analytical Manual of the Food & Drug Administration (2019). The methods used for the isolation, confirmation, and identification of the microorganisms found in orange juice samples are shown in Table 1. All confirmed *E. coli* strains were streaked onto tryptic soy agar slants, incubated at 37 °C for 24 h, and maintained at 3–5 °C until they were used for polymerase chain reaction (PCR).

2.4 Multiplex PCRs for DEP locus identification

E. coli strains were further characterized for the presence of genes that define six DEP by two PCRs. The first multiplex PCR assay as described by Lopez-Saucedo et al. (2003) identifies the following loci: intimin (*eae*A) and bundle-forming pilus (*bfp*A) for EPEC, Shiga toxin 1 and 2 (*stx*1, *stx*2) and intimin (*eae*A) for STEC, heat-stable and heat-labile enterotoxins (*st*, *lt*) for ETEC, and invasion-associated loci (*ial*) for EIEC. The second multiplex PCR identifies three plasmid-borne virulence genes of EAEC: the master regulon (*agg*R), dispersin (*aap*), and anti-aggregation transporter (*aat*A) (Cerna et al., 2003), as well as the Afa adhesion usher (*afa*C) for DAEC (Patzi-Vargas et al., 2013). All STEC strains were also characterized for expression of the O157 lipopolysaccharide antigen and enterohemolysin gene (*ehx*A, previously *hly*A), using a latex particle agglutination kit (Oxoid Limited, Basingstoke, UK), and PCR, respectively (Paton & Paton, 1998).

2.5 Statistical analysis

Descriptive statistics were used to summarize data in the form of frequencies and percentages and are presented in Table 2. One-way ANOVA was also carried out to compare the degree of contamination (AMB, TC, FC, and *E. coli*) among the orange juice samples and was corroborated by Tukey's method. The median values of log CFU/mL of AMB and MPN of TC and FC, and good and poor hygienic conditions were compared by the Kruskal–Wallis test. In the same way, the median values of log CFU/mL of AMB and MPN of TC and FC, and the time of orange juice extraction (extracted at the time of purchase or already prepared) were compared by the Kruskal–Wallis test. A p value < 0.05 was considered significant. All statistical analyses were run with the statistical program SPSS for Windows version 21.

3 Results and discussion

In Mexico, where street food vending is prevalent, there is a lack of information on the incidence of foodborne diseases related to street-vended food, mostly due to the lack of an active national surveillance system. Similarly, there is little data available concerning the microbiological quality of fruit

Table 1. Methods of detection, identification, confirmation, and reporting of results of microorganisms in fresh-squeezed orange juice samples.

Microorganisms	Presumptive test or primary enrichment	Confirmatory test or secondary enrichment	Selective medium for isolation	Identification test	Results reported as
Aerobic mesophilic bacteria	NR[a]	Plate count agar (Bioxon™, BD, State of Mexico, Mexico) for 48 h at 35 °C	NR	NR	CFU/mL[b]
Total coliforms	Lactose broth (Difco™, BD, Sparks, MD, USA) for 48 h at 35 °C	Brilliant green lactose bile broth (Difco™, BD, Sparks, MD, USA) for 48 h at 35 °C	NR	Gas production	MPN/mL[c]
Fecal coliforms	Lactose broth (Difco™, BD, Sparks, MD, USA) for 48 h at 35 °C	*Escherichia coli* broth (Difco™, BD, Sparks, MD, USA) for 48 h at 44.5 °C	NR	Gas production	MPN/mL
Escherichia coli	Lactose broth (Difco™, BD, Sparks, MD, USA) for 48 h at 35 °C	*Escherichia coli* broth with MUG (4-methylumbelliferyl-β-D-glucuronide) (Difco™, BD, Sparks, MD, USA) for 48 h at 44.5 °C	Eosin methylene blue agar (Bioxon™, BD, State of Mexico, Mexico) for 24 h at 35 °C	Indole production, methyl red test, Voges-Proskauer test, and citrate utilization (Bioxon™, BD, State of Mexico, Mexico) for 24 h at 35 °C	MPN/mL

[a]NR, not required; [b]CFU/mL, colony-forming units per mL; [c]MPN/mL, most probable number per mL.

Table 2. Aerobic mesophilic bacteria (AMB), total coliforms (TC), fecal coliforms (FC), *Escherichia coli*, and diarrheagenic *E. coli* pathotypes (DEP) concentrations and number of positive samples in fresh-squeezed orange juice samples.[a]

Microorganisms	Minimum	Median	Maximum	Number of positive samples (%)
AMB	1	3.2	6.8	60 (100)
TC	< 3	3.6	> 1100	35 (58.3)
FC	< 3	< 3	> 1100	27 (45)
E. coli	< 3	< 3	53	5 (8.3)
DEP	< 3	< 3	9.2	2 (3.3)

[a]n = 60. Minimum, median, and maximum values are in $\log_{10}$ CFU per mL for AMB and most probable number (MPN) per mL for TC, FC. *E. coli*, and DEP.

juices. Therefore, the present study focused on assessing the microbiological quality and presence of DEP in fresh-squeezed orange juice. Regarding street-vended orange juice stalls, most include a small table, and oranges are bought in large sacks where they are kept on the street. Orange juice is simply prepared by mechanical means using an orange juice press to squeeze juice at the street stand, usually from unwashed oranges since street vendors do not have access to running water. At these stands, water is kept in a plastic bucket, which is used for rinsing hands and kitchen utensils and is reused several times. Such conditions and practices are likely to lead to cross-contamination of orange juice. The final product is an unfermented, untreated juice, ready for consumption. Fresh-squeezed orange juice is then kept in jugs and sold in a plastic bag with a straw attached to the bag with a plastic band or in plastic glasses with a lid that has a hole for the straw. Only 12 (20%) of the street vendors prepared orange juice at the time of purchase whereas the remaining 48 (80%) had the orange juice already prepared. Regarding the hygienic practices of street vendors, 39 (65%) had poor hygienic practices and the remaining 21 (35%) had good hygienic practices. These results are similar to those reported by Dominguez-Gonzalez et al. (2022) who reported that street vendors of fresh orange juice from Morelia, Mexico, had poor hygienic practices.

Orange juice pH values ranged from 3.0 to 4.3, where 60% of the samples showed a pH ≤ 4. This result is consistent with previous studies carried out in Mexico and Turkey (Castillo et al., 2006; Bagci & Temiz, 2011; Cerna-Cortes et al., 2016; Dominguez-Gonzalez et al., 2022; Ocaña De Jesús et al., 2022a, b).

The microbiological quality of unpasteurized citrus juices is mainly determined by the correct washing and disinfection of fruits, cleanliness, and washing and disinfection of the surfaces and utensils employed (Ocaña De Jesús et al., 2022b). The level of AMB present is intended to indicate the level of overall bacterial contamination of food or beverage samples tested. In this study, all samples analyzed were positive for AMB, with concentrations ranging from 1 to 6.8 log CFU/mL (Table 2). Unfortunately, in Mexico there is no national guideline that establishes the maximum permissible limits for indicator microorganisms in fresh fruit juice. Studies conducted in different parts of Mexico have also shown similar AMB levels in fresh orange juice samples purchased from street vendors (Castillo et al., 2006; Cerna-Cortes et al., 2016; Dominguez-Gonzalez et al., 2022; Ocaña De Jesús et al., 2022a, b). According to Afreen et al. (2019), high microbial loads in street foods, including juices, can be attributed to unhygienic vending practices, and juices are pointed out as potential reservoirs for foodborne pathogens. Therefore, proper raw material handling and good hygiene practices need to be promoted and implemented. High AMB levels found in the samples analyzed in this work may be due to most of the orange juices (80%) being left at ambient temperature which may have led to the proliferation of AMB resulting in increased bacterial counts. Bryan et al. (1992) reported that the major factor that contributes to outbreaks of foodborne diseases in developed countries is keeping prepared foods at ambient temperature for several hours. Hence, keeping foods while being displayed by street vendors is a critical control point that should be addressed.

Coliform bacteria are commonly used as an indicator of the sanitary quality of foods or to check potential contamination of pathogenic microorganisms. In our study, a total of 35 (58.3%) orange juice samples were positive for TC, with limits ranging from < 3 to > 1100 MPN/mL (Table 2). Our results are consistent with those reported by Cerna-Cortes et al. (2016) who reported that 54% of fresh orange juice samples collected from street vendors harbored TC in similar concentrations. Our results differ from those published by Ocaña De Jesús et al. (2022b) and Dominguez-Gonzalez et al. (2022), who found that 100% and 90% of the fresh orange juice samples evaluated harbored TC, respectively.

A total of 27 (45%) orange juice samples analyzed by us were positive for FC, at concentrations ranging from < 3 to > 1100 MPN/mL (Table 2). Our results are similar to those reported by Cerna-Cortes et al. (2016) and Dominguez-Gonzalez et al. (2022) who found that 25% and 35% of the fresh orange juice samples analyzed were positive for FC, respectively, at similar concentrations. Our prevalence of FC is lower than that reported by Ocaña De Jesús et al. (2022b), who found that 86% of orange juice samples evaluated in Toluca, Mexico, contained FC. We found no degree of correlation of median concentrations of indicator bacteria (AMB, TC, and FC) among the orange juice samples ($p > 0.05$). Moreover, no significant difference ($p > 0.05$) was found between indicator bacteria median concentrations and the time of orange juice extraction, as determined by Kruskal–Wallis test. In the same way, we found no correlation ($p > 0.05$) between indicator bacteria median concentrations and good or poor hygienic conditions.

E. coli was present in 5 (8.3%) orange juice samples analyzed, at concentrations ranging from < 3 to 53 MPN/mL (Table 2). This prevalence was substantially lower than that reported by Castillo et al. (2006) and Ocaña De Jesús et al. (2022b) who reported that 75% and 86% of orange juice samples analyzed were positive for *E. coli*, respectively. The prevalence and concentrations of *E. coli* found by us coincide with previous studies from Turkey and Mexico (Bagci & Temiz, 2011; Cerna-Cortes et al., 2016; Dominguez-Gonzalez et al., 2022). The presence *E. coli* in a food indicates fecal contamination and is of concern because it can indicate the possible presence of human enteric pathogens, such as *Salmonella*, *Shigella*, *E. coli* O157:H7, etc. (Parish, 1997).

In this study, 18 *E. coli* strains were isolated from 5 orange juice samples. All were genotyped for the presence of 11 characteristic DEP loci; two samples were contaminated with DEP at concentrations of 3.6 and 9.2 MPN/mL. Both orange juice samples harbored STEC strains positive for the Shiga toxin 2 (*stx*2) locus and negative for the O157 antigen and the enterohemolysin gene. Both samples were orange juice samples already prepared, remaining exposed to the street environment, hence, the STEC strains could have come from the air or dust, since it has been reported that *E. coli* was isolated from indoor and outdoor environments in Mexico City (Rosas et al., 1997), hence, we suggest that orange juice should be squeezed at the time of purchase. STEC strains are not part of the endogenous microflora of oranges and thus their presence derives from fecal contamination. Contamination from raw materials and equipment, additional processing conditions, improper handling, and the prevalence of unhygienic conditions contributes substantially to the entry of bacterial pathogens in juices prepared from these fruits or vegetables (Khan et al., 2015). STEC strains are foodborne pathogens that are an important public health concern due to their low infective doses (between 5 and 50 viable cells) and their association with severe forms of infection such as hemorrhagic colitis and, in very severe cases, systemic complications including hemolytic uremic syndrome (HUS). HUS is the leading cause of renal failure in children under 3 years of age (Bagel & Sergentet, 2022). Therefore, measures to eliminate STEC strains from orange juices should be implemented. Although it has been reported that ozone causes a 4.2 to 6.0 log CFU/mL reduction of *E. coli* O157:H7 and *Salmonella* in apple cider and orange juice (Williams et al., 2004), and that treatment with UV radiation is effective for reducing or eliminating *E. coli* O157:H7 and nonpathogenic *E. coli* in orange juice and other juices (Oteiza et al., 2005), these methods are difficult for street vendors to implement. We suggest treating oranges with hot water at 70 °C for 2 min or 80 °C for 1 min, which produces a 5-log CFU reduction in *E. coli* without altering the original sensory quality of fresh juice (Pao & Davis, 1999).

4 Conclusions

This study reveals that, fresh orange juices purchased from street vendors in Mexico City were of poor microbiological quality, possibly due to poor hygiene practices. Two samples harbored STEC strains associated with hemorrhagic colitis and HUS; therefore, their consumption can pose a potential risk of foodborne illness. Much of this risk can be mitigated through properly washing and disinfection of oranges, prevention of contamination by humans during juice preparation, and refrigerated storage of fresh juice. The implementation of adequate hygiene and safety practices is very important to protect the health of consumers by improving the quality of fruit juice.

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