



# Microbiological quality and presence of enteropathogenic bacteria in orange juice sold in popular markets

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## Abstract

Worldwide, there are a large number of retailers in popular markets offering fresh orange juice, which is preferred for its nutritional and health value. Incorrect management during the preparation, the presence of pests, and inadequate environmental conditions can affect the microbiological quality of the juices sold in popular markets and can even make them a reservoir for enteropathogenic bacteria. The aim of this study was to evaluate the microbiological quality and presence of enteropathogenic bacteria in freshly prepared orange juice sold in popular markets, by quantifying the fungi, yeasts, aerobic mesophilic bacteria, and total and fecal coliforms. The fecal colonies were subjected to tests to confirm the presence of enteropathogenic bacteria. The results showed that none of the juice sold at popular markets had fungi, while all of them had yeasts, mesophilic bacteria, and total coliforms, and only 14% did not present fecal coliforms. The identification of the fecal bacteria was positive for *E. coli* and *Salmonella*. The microbiological quality of the orange juices sold in popular markets needs to be improved, since the high microbial load makes them a health risk.

**Keywords:** contamination; yeasts; aerobic mesophiles; coliforms; *E. coli*; *Salmonella*.

**Practical Application:** The selling of natural orange juice in popular markets is not regulated and does not meet microbiological parameters; in addition, the presence of *E. coli* and *Salmonella* affects their microbiological quality and makes them a health risk.

## 1 Introduction

The consumption of minimally processed foods, like vegetables, fruits, and juices made from them has increased due to their high nutrient content. Juices of fresh citrus fruits are a source of Vitamin C, potassium, folic acid, Vitamin A, magnesium, carotenoids, and antioxidants, which favor the prevention of disease and are very important in the face of the current Coronavirus 2019 (COVID-19) pandemic, in which the state of health is related to the severity of illness (Mosqueda-Melgar et al., 2012; Rampersaud & Valim, 2017; Iddir et al., 2020).

Mitchell et al. (2020), reported that 100% natural fruit juice contains over 5% of the recommended dose of Vitamin C, folic acid, magnesium, and potassium. In addition, street foods have acquired relevance in consumption for its freshness, pleasant flavor and low cost (Asiegbu et al., 2016; Cortese et al., 2016; Gupta et al., 2018; Soon, 2019; Ferrari et al., 2021).

The nutrient contribution of consuming minimally processed products like juices is very important, making it necessary to ensure the quality and safety of these products, since their management and preparation makes them highly perishable due to the presence of fungi and yeasts (Das et al., 2010). The presence of coliforms and antimicrobial-resistant pathogens have been identified in fresh foods and unpasteurized fruit juices have been reported as vehicles of foodborne outbreaks of *Escherichia coli*, *Staphylococcus aureus*, *Cryptosporidium*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Candida*, *sp.*, and *Acetobacter*, among others

(Reinders et al., 2001; Hanashiro et al., 2005; Ramos et al., 2010; Guven et al., 2010; Baragón et al., 2013; Aneja et al., 2014a; Callejón et al., 2015; Hossen et al., 2020).

Citrus juices are characterized by their low (acidic) pH; however, human pathogens have managed to break that barrier, in addition to the fact that their characteristic acidity makes them ideal for the growth of certain kinds of fungi and yeasts (Food Drug Administration, 2021).

Good manufacturing practices play a preponderant role during the production of this type of product, since they are directly related to microbiological quality (Cheng et al., 2018).

The microbiological quality of minimally processed products (like 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 (knives, juice extractors, and cutting boards) (Aneja et al., 2014a; Hossen et al., 2020). In addition to not complying with health regulations on the waste generated, the product is prepared in areas near sewer drains that are a focus of contamination due to the presence of pests. Currently, no process of washing and disinfection is 100% effective in eliminating all pathogens that could adhere to the surface of fresh produce (Mandrell et al., 2006; Callejón et al., 2015). Given the issues detailed above and the growing demand for the consumption of natural juices, the aim of this study was to evaluate the microbiological quality and

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the presence of enteropathogenic bacteria in freshly prepared orange juice sold in popular markets.

## 2 Materials and methods

The present study monitored establishments selling unpasteurized orange juice in different markets. The following physicochemical analyses were done on the samples: pH, titratable acid, total soluble solids (°Bx) and maturity index, as well as microbiological determinations of aerobic mesophiles (AM), total coliforms (TC), fecal coliforms (FC), fungi (F) and yeasts (Y). The orange juice samples were placed in sterile sampling bags, sealed and labeled, and transported under refrigeration to the Food Safety Laboratory of the Faculty of Agricultural Sciences, where the aforementioned analyses were carried out (Mexico, 1994c).

### 2.1 Physicochemical analyses

The pH of each sample was measured with a potentiometer (Thermoscientific stard 215). The titratable acid was represented as % of anhydrous citric acid (Association of Official Analytical Chemists, 1984; International Standard Organization, 1998), the total soluble solids (TSS) was measured with a digital refractometer (Atago 3810 PAL-1) and are reported as °Bx. The maturity index was established as the TSS/acidity ratio. All analyses were done in triplicate for each sample.

### 2.2 Microbiological analyses

The samples were transferred to sterile containers and processed according to the Official Mexican Law NOM-110-SSA1-1994 (Mexico, 1994d). Dilutions were prepared by aseptically taking 10 mL of the juice sample and mixing with 90 mL of sterile Saline Peptone Water BPA (0.1% eptone + 0.85% NaCl), 1 mL of that mixture was transferred to tubes with 9 mL BPA (primary dilution). From the primary dilution, serial dilutions were prepared to  $10^2$ . Then, 1 mL of the dilutions was seeded onto plates in triplicate on plate count agar (BD BIOXON®), violet red bile agar (VRBA) (BD BIOXON®), potato dextrose agar (BIOXON) and yeast extract agar (BIOXON), for the quantification of AM, TC, FC, F and Y, respectively. Then, the plates were incubated at the temperatures and times corresponding to each microbial group, the colonies formed and the media analyzed were enumerated and presented as Log CFU/mL (Mexico, 1994a, e; Mexico, 1978; Food Drug Administration, 2017). The colonies of fecal origin were subjected to a Gram stain test and seeded on selective media for the detection of *E. coli* and *Salmonella* spp. The agars used were MacConkey (BIOXON), chromogenic O157:H7 (DIBICO), eosin methylene blue (BIOXON), brilliant green (BIOXON), *Salmonella Shigella* (BIOXON) and Xylose Lysine Desoxycholate (DIBICO). The plates were incubated at 35 °C for 24 h.

Seeding on selective media was complemented with IMViC biochemical reactivity tests (indole production, motility, methylene red, Vogues-Proskauer and Simmons citrate tests), complemented with urease production, hydrogen sulfide, and ornithine decarboxylation tests. The results obtained were compared to the criteria established by Koneman for the identification of *E. coli* and *Salmonella* (Winn & Koneman, 2008).

### 2.3 Experimental design and statistical analysis

The microbe counts (Log CFU/mL) are presented as means  $\pm$  standard deviation and an independent sample t-test was used to compare the quantity of microorganisms present in the samples with the maximum permissible limits established by international and Mexican official norms, which stipulate  $\leq 150000$  CFU/mL (5,2 Log CFU/mL), and for TC and FC the sample should not exceed 100 CFU/mL (2 Log CFU/mL) and the same norms establish that no pathogenic microorganisms should be present (Mexico, 1994b; Food and Agriculture Organization, 2005). A one-way ANOVA was also carried out to compare the degree of contamination among the popular markets analyzed, and upon significant results, a Tukey post hoc test was done ( $p < 0.05$ ). All of the statistical tests were done with the statistical program Stat Graphics (Statgraphics Technologies, 2009).

## 3 Results and discussion

The average concentration of hydrogen ions was  $3,4 \pm 0,23$ , with a minimum of 3,2 and a maximum of  $3,7 \pm 0,10$  with no effect on the microorganisms present. This parameter is reported as one of the characteristics necessary for colonization by some microbial groups such as fungi and yeasts, which are more acid-tolerant, while some bacteria such as *Lycyclobacillus* sp., *Erwinia*, *Enterobacter* sp., *Pseudomonas* sp., and *Bacillus* sp., can also contaminate the product due to their tolerance of acid and the concentration of sugars typical of the product (Aneja et al., 2014b; Kaczmarek et al., 2019). TSS values ranged from 10.8 to 13 °Brix and had an average value of 12.04 °Brix, which are in accordance with Mexican legislation NMX-F-118-1984 (Mexico, 1984), which stipulates values from 10.5 to 13.5, as well as the Codex General Norm for Juices (*Norma General del Codex para Zumos*), which establishes that values should be no lower than 10 °Brix (Food and Agriculture Organization, 2005). TSS are important, since they mostly consist of sugars and organic acids, which are related to the flavor and ripeness of the fruits used to produce the juice (Al-Mouei & Choumane, 2014; Rodríguez et al., 2020).

The analysis of titratable acid showed values of  $0.93 \pm 0.08$  which is within the range of 0.65% to 1.85% established by the norm NMX-F-118-1984 (Mexico, 1984). Organic acids are very important, since they influence sensory properties. Schvab et al. (2013), as well as Arthey & Ashurts (1996) point out that orange juice has better flavor and aroma once acidity reaches values near 1% and pH of 3.5.

The maturity index, which is determined by the relationship between total soluble solids and titratable acid, had values of  $12.01 \pm 0.15$ , which is within the range stipulated by Mexican norms of 12.0 to 20.0 and the international limit of 15 (Mexico, 1984; Food and Agriculture Organization, 2005). This parameter is used to determine the state of ripeness of the fruit from which the juice was extracted.

The microbial counts are shown in Table 1, which shows levels of microbial contamination of orange juice sold at different popular markets.

Average microbe counts expressed in Logarithms of Colony Forming Units (CFU/mL).; AM: Aerobic Mesophiles; TC: Total

Coliforms; FC: Fecal coliforms, Y: Yeasts; -: absent. Means followed by the same letter show a lack of significant difference in Tukey tests ( $p < 0.05$ ).

The juice sampled from the different markets had an absence of molds, but there was a high index of Y, with the formation of five statistically different groups ( $p < 0.05$ ). Generally, pre-harvest fungal colonization usually determines postharvest deterioration, the origin of the fruit, and the exposed surfaces of the fruit are contaminated by water, air, animals, insects, contact with processing equipment (Food and Agriculture Organization, 2004). All of the juice samples from the different markets had presence of Y, with 86% having above 2 Log CFU/mL. According to Aneja et al. (2014b), yeasts were the main cause of deterioration of freshly prepared citrus juices, and Alam et al. (2019) report similar results and suggest that high indices of Y, in addition to deteriorating the product, can have adverse health effects in consumers.

All of the juice retailers evaluated had AM counts with an average range of  $3.98 \pm 0.08$  Log CFU/mL, with four statistically significant groups ( $p < 0.05$ ); 14% of the samples exceeded the maximum permitted range of 5.2 Log CFU/mL (Mexico, 1994b; Food and Agriculture Organization, 2005). High AM counts, as occurred in this study, are important since this indicator group is related to overall microbial contamination, lack of hygiene and food safety during the production process (Castillo et al., 2006; Tasnim et al., 2010). Similar counts were reported by Iqbal et al. (2015), who showed higher microbial loads higher than permissible limits in unpasteurized fruit juices, and Mohd Nawawee et al. (2019) in milk- and fruit-based beverages sold on the street and Ferrari et al. (2021), reported unsatisfactory hygiene conditions in the sale of food on the street and 1% showed higher microbial loads higher than permissible limits. According to Afreen et al. (2019), high microbial loads in street foods, including juices, can be attributed to deficient hygiene practices and juices are pointed out as potential reservoirs for foodborne illnesses.

All of the markets evaluated presented TC counts and statistical differences from the permitted parameters; 71% were above the norm limit of 2 Log CFU/mL. High concentrations of TC are indicative of microbiological quality, a lack of sanitary hygiene conditions, and are considered a risk, since they contribute to the deterioration of the product and can affect consumer health (Food and Agriculture Organization, 2005). Counts above the permissible limits coincide with Iqbal et al. (2015) and Mohd Nawawee et al. (2019), who reported counts of coliforms of

$5.45 \pm 1.06$  Log CFU/mL and  $4.75 \pm 0.79$  Log CFU/mL in fruit juices, respectively.

The total counts indicate that 86% of the samples had FC, with a highest average reading of  $4.64 \pm 0.04$  Log CFU/mL, forming three statistically distinct groups ( $p < 0.05$ ) and surpassing the permitted limit of FC  $> 2$  Log CFU/mL. According to Reda et al. (2017), the presence of thermotolerant FC can be attributed to fecal contamination of the water used to wash utensils, fruits, or transferred directly from the vendors, as well as the environment in which the juice is prepared, and leaving food at room temperature, as is the case of these juices, these can multiply to reach high concentrations.

Observation of the bacteria under a microscope allows the bacteria to be grouped as Gram negative, and in the selective media we obtained greenish colonies with a metallic sheen (*E. coli*), transparent colonies on red and black background (*Salmonella* spp.), as well as pink-colored, colonies with lactase production (*E. coli*). The additional biochemical tests (Table 2), confirmed the presence of *Escherichia coli* and *Salmonella* spp (Winn & Koneman, 2008), indicative of recent fecal contamination and unsanitary processing (Food Drug Administration, 2021).

*Salmonella* and *E. coli* were present in 85% of the samples. The norms stipulate that these bacteria should be absent (Mexico, 1994b). The positive identification of these bacteria coincides with Aneja et al. (2014b), who reported *E. coli* in 20% of citrus and carrot juices, in addition to suggesting that the presence of these microorganisms is potentially dangerous to public health. Vantarakis et al. (2011) point out that fruit juices facilitate the survival of foodborne pathogens like *E. coli*, *Salmonella*, *Shigella*, and others due to their carbohydrate content and that they can cause serious illness in humans at low doses (10-100) cells (Kaczmarek et al., 2019). The presence of these type of bacteria coincides with studies showing that unpasteurized orange juice has the potential to transmit enteropathogenic organisms such as *E. coli* O157:H7, as well as the survival of this microorganism for up to 8 days after being inoculated into juice (Rojas & Castillo, 2003). Similarly, Sharma et al. (2001) report that an outbreak in the United States of *Salmonella enteritidis* serotype *anatum* was attributable to the improper washing of fruits used for juice. This highlights the importance of the microbiological quality of the raw materials, hygiene of utensils, adequate sanitary conditions, absence of pests, and good manufacturing practices of the vendors, to reduce the risk of contracting foodborne illnesses (Alimi, 2016). In addition,

**Table 1.** Microorganism counts for microbiological quality in orange juice.

Market	Y	AM	TC	FC
	Log <sub>10</sub> CFU/mL	Log <sub>10</sub> CFU/mL	Log <sub>10</sub> CFU/mL	Log <sub>10</sub> CFU/mL
	X ± SD (Standard deviation)	X ± SD	X ± SD	X ± SD
M1	2.40 ± 0.03 a	3.6 ± 0.19 abc	2.33 ± 1.55 abc	2.70 ± 0.18 abc
M2	2.88 ± 0.08 b	4.01 ± 0.29 bc	3.08 ± 1.07 bcd	2.21 ± 1.19 ab
M3	2.30 ± 0.10 a	2.89 ± 0.60 a	0.90 ± 0.14 a	-
M4	1.80 ± 0.12 c	3.40 ± 0.18 ab	1.88 ± 0.84 ab	1.50 ± 0.94 ab
M5	4.73 ± 0.03 d	5.47 ± 0.56 d	3.79 ± 0.19 cd	4.64 ± 0.04 c
M6	2.02 ± 0.13 e	4.22 ± 0.05 c	4.04 ± 0.11 d	4.33 ± 0.04 c
M7	2.23 ± 0.90 a	4.14 ± 0.03 c	3.70 ± 0.18 cd	3.31 ± 0.06 bc

**Table 2.** Biochemical tests used for the identification of enterobacteria using IMViC reactivity.

	I	M	O	Vp	Rm	Cs	U	TSI	H <sub>2</sub> SO <sub>3</sub>	Salmonella	E. coli
M1	-	+	+	-	+	+	+	A/Ac	+	+	
	+	+	+	-	+	-	-	A/A	-		+
M2	-	+	+	-	+	+	+	A/Ac	+	+	
	+	+	+	-	+	-	-	A/A	-		+
M3	-	+	+	-	+	+	+	A/Ac	+	+	
	+	+	+	-	+	-	-	A/A	-		+
M4	-	+	+	-	+	+	+	A/Ac	+	+	
	+	+	+	-	+	-	-	A/A	-		+
M5	-	+	+	-	+	+	+	A/Ac	+	+	
	+	+	+	-	+	-	-	A/A	-		+
M6	-	+	+	-	+	+	+	A/Ac	+	+	
	+	+	+	-	+	-	-	A/A	-		+
M7	-	+	+	-	+	+	+	A/Ac	+	+	
	+	+	+	-	+	-	-	A/A	-		+

I: Indol; M: Motility; O: Ornithine; Rm: Methyl red; Cs: Simmons citrate; U: Urea; H<sub>2</sub>SO<sub>3</sub>: Production of hydrogen sulfide; TSI: Triple sugar iron.

an important risk factor is that juices are generally prepared in bulk before sale by the vendors, an overwhelming factor in the microbiological quality of the product (Umoh & Odoba, 1999; Alimi, 2016). That street-vending food is a source of risk to consumer health and should be treated as an important health problem. Food handlers and vendors sometimes have poor knowledge of safe handling, which is reflected in their inadequate facilities, unhygienic practices during preparation and sale (Hossen et al., 2020; Ferrari et al., 2021).

Given this situation, it is necessary to implement good manufacturing practices and increased vigilance of the sale of natural juices in popular markets, where consumption has been on the rise, as well as more studies offering alternatives to control pathogens like those detected in this study, such as the use of lactic acid, ascorbic acid, or propionic acid, the use of physical technologies, or complementing their consumption with natural products that have bactericidal effects (Uljas & Ingham, 1999) to guarantee the microbiological quality of natural juice.

#### 4 Conclusions

The orange juices for sale at popular markets present adequate physicochemical properties that meet national and international norms; however, the presence of yeasts, aerobic mesophiles, and coliforms decrease the quality of the product, since these microorganisms contribute to the deterioration of the juice and can even expose consumers to a latent risk of developing a disease transmitted by the juice due to the presence of enteropathogenic bacteria such as *E. coli* and *Salmonella* spp.

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