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## MICROBIOLOGICAL AND PARASITOLOGICAL QUALITY OF FRUIT PULPS AND JUICES USED FOR DETOXIFYING PURPOSES

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### ABSTRACT

The juice consumption with detoxifying properties has increased in the last few years, but inadequately produced food may transport pathogens. This study aimed to investigate the occurrence of parasitological and microbiological contamination in fruit juices and fruit pulp used for detoxifying purposes. Twenty samples were analyzed, 10 of fruit pulp and 10 juices from July to August 2018. Thermotolerant coliform, mold, and yeast were counted. Parasitological analysis was performed by spontaneous sedimentation method and sedimentation by centrifugation techniques. Results showed that 20% and 60% of the fruit pulp and juice samples exhibited thermotolerant coliform counts above 1 log CFU/mL, respectively. The results showed that 60% of the pulp samples presented mold and yeast counts above 3 log CFU/mL. The protozoa found in the samples were *Endolimax nana*, *Entamoeba coli*, and *E. histolytica/E. dispar* cysts. The presence of thermotolerant coliforms in juices is an indicator of the presence of enteropathogens, mainly *Escherichia coli*. The high mold and yeast populations and the presence of protozoa in the fruit pulp and juices indicate hygienic and sanitary failures in the food processing. It may be concluded that there is a need for supervision and training in Good Manufacturing Practices by health agencies, aiming at the food safe production.

KEY WORDS: Coliforms; parasites; food hygiene; food safety.

### INTRODUCTION

In an expanding search for a healthy life, the demand for natural food that provides health benefits has been increasing over the years. Detox diets are popular diet strategies that aim to facilitate the toxin elimination and weight loss (Brasil, 2016).

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Given this scenario, food with detoxifying properties, also known as *detox*, have been gaining adherents. During their preparation, the use of vegetables is highlighted, which provide nutrients, like vitamins, minerals, and fibers, besides having about 70% of water in their composition (Embrapa, 2012), likewise acting as antioxidants and helping the body fight against the free radicals (Brasil, 2016).

The most used vegetables and fruits on detox juices are mint (*Mentha spicata*), watercress (*Nasturtium officinale*), cabbage (*Brassica oleracea*), pineapple (*Ananas comosus*), ginger (*Zingiber officinale*) and orange (*Citrus sinensis*). Mint, watercress, and cabbage are part of the green juice group because they are leafy vegetables that activate metabolic pathways to start the detoxification process (Gaiolla, 2015).

The Food Guide for the Brazilian population highlights the importance of food based on *in natura* (fresh) and minimally processed foods (Brasil, 2014a). Therefore, when it is not adequately sanitized, vegetables and fruit can be vehicles of pathogens associated with Water and Food Transmission Diseases (WFTD). These vegetables contamination occur mainly by water irrigation or cultivation on soil contaminated by fecal material, incorrect handling, and transport (Soares & Cantos, 2006).

Parasitological and microbiological intestinal infections related to the raw vegetables' consumption have grown mainly in developing countries (Luz et al., 2017). The incidence of infections happens in areas with poor basic sanitation and precarious socioeconomic conditions (Cavagnoli et al., 2015).

Studies on the presence of pathogens *in detox* juices are scarce on published results. Therefore, this topic is in need of critical discussion, considering that the juice is ready for consumption and it might have parasitic and/or microbial agents.

Assuming that there are few studies involving the sanitary quality of the fruit pulps and the detox juices, this study aimed to investigate the occurrence of parasitological and microbiological contamination in juices and fruit pulps used for detoxifying purposes.

## MATERIAL AND METHODS

### *Design and study area*

This is a cross-sectional study developed in two cities in the *Recôncavo* of the State of Bahia region (Nazaré city and Santo Antônio de Jesus city), involving the hygienic-sanitary aspects of the fruit pulps and the detox juices marketed in different establishments, from July to August 2018.

### *Sampling collection*

Four commercial establishments in two cities from the *Recôncavo* of Bahia region that sell juices or fruit pulps for detoxifying purposes were selected. The choice of the samples considered the characterization of the fruit pulp or juice which have a detoxifying characteristic by the food handlers. The juices were produced at the time of the purchase with a blender. Five types of fruit pulps and five types of juices were randomly chosen for two weeks, 20 samples were analyzed, 10 per product type.

The fruit pulps were stored into plastic packaging containing 100g and the juices were purchased in 500 mL plastic cups. All samples collected aseptically were packed in plastic bags for first use from snack bars in the two cities, identified, stored, and refrigerated in isothermal boxes with recyclable ice at 4°C. Then, they were transported to the Microbiology and Parasitology laboratories of the Multidisciplinary Complex for Studies and Research in Health (COMEPS) on the Center of Health Sciences (CCS) at the Federal University of the *Recôncavo* of Bahia (UFRB).

Samples were divided into equal portions; one part being used for microbiological analysis and the other part for parasitological analysis. The analysis was performed no later than four hours after sample acquisition.

### *Microbiological analysis*

Thermotolerant coliforms, molds, and yeasts were estimated by the rapid method Petrifilm™ with Petrifilm CC plates (AFNOR 3M 01/2 - 09/89C) for thermotolerant coliforms and Petrifilm YM plates (AOAC 2014.05) for molds and yeasts, which were incubated at 25°C for 5 days and 44°C for 24 hours, respectively, which follows the manufacturers' instructions. Colonies were counted with colony count model CP600 Plus (Phoenix ®), calculating colony formation units (CFU), and results were expressed in log CFU/ mL (Silva et al., 2017).

Priority was given to the research of microorganisms that are indicators of hygienic-sanitary failures of the processing of fruit pulps and juices used for detoxifying purposes, according to Franco & Landgraf (2008).

### *Parasitological analysis in the juices and the fruit pulps*

Initially, samples were filtered using sieves with gauze inside identified chalices according to the sample. From the contents of the chalices, aliquots were taken from the sample for effective research techniques.

Techniques usually used for fecal parasitological examinations were adapted for the enteroparasite research in the juices and fruit pulps, which comprised spontaneous sedimentation method (Hoffmann et al. 1934) and sedimentation by centrifugation. It is emphasized that using these techniques allows the research of helminths eggs and larvae, protozoa cysts and oocysts, insects' and arachnids' pieces, and various dirt.

For the spontaneous sedimentation and centrifugation sedimentation techniques, the juice liquid itself was used as a principle without the other substances' addition such as water. This procedure was done since the juice liquid could be contaminated at the origin point, during its distribution, and in the private reservoirs (Yamaguch et al., 2013), thus the samples were thoroughly analyzed.

In the spontaneous sedimentation technique, samples were filtered by gauze and maintained under spontaneous sedimentation for 24 hours in chalices at room temperature. For the centrifugation sedimentation technique, sediment was collected by sterile droplet counter, taking 9 mL of the liquid obtained from filtration in microtubes of 1.5 mL each. Then, it was centrifuged at 2,500 rpm for 2 minutes. Sterile straws collected the sedimented material, and then slides were prepared in triplicates. Thus, one drop was removed from each sample, which was stained with Lugol, transferred to a slide, and observed under an optical microscope.

## RESULTS

The thermotolerant coliforms, molds and yeasts populations in the analyzed samples ranged from  $<1 \log \text{CFU/mL}$  to  $2.96 \log \text{CFU/mL}$  (mean  $1.5 \log \text{CFU/mL} \pm 0.76$ ) and  $2.5 \log \text{CFU/mL}$  to  $5.99 \log \text{CFU/mL}$  (mean  $4.30 \log \text{CFU/mL} \pm 1.11$ ), respectively (Table 1; Table 2).

The thermotolerant coliforms, molds and yeasts populations in the analyzed fruit pulps samples ranged from  $<1 \log \text{CFU/mL}$  to  $1.7 \log \text{CFU/mL}$  (mean  $1.1 \log \text{CFU/mL} \pm 0.28$ ) and  $2.15 \log \text{CFU/mL}$  to  $5.08 \log \text{CFU/mL}$  (mean  $3.56 \log \text{CFU/mL} \pm 1.01$ ), respectively.

The thermotolerant coliforms, molds and yeasts populations in the analyzed juice samples ranged from  $<1 \log \text{CFU/mL}$  to  $2.96 \log \text{CFU/mL}$  (mean  $1.81 \log \text{CFU/mL} \pm 0.61$ ) and  $4.11 \log \text{CFU/mL}$  to  $5.99 \log \text{CFU/mL}$  (mean  $5.03 \log \text{CFU/mL} \pm 1.01$ ), respectively.

The results showed that 20% and 60% of the fruit pulps and juice samples presented thermotolerant coliform counts above  $1 \log \text{CFU/mL}$ , respectively.

The results showed that 60% of the fruit pulps samples presented mold and yeast counts above  $3 \log \text{CFU/mL}$ . This result does not comply with ANVISA Normative Instruction IN 60/2019 that determines the maximum limit of  $3 \log \text{CFU/mL}$  of molds and yeasts in fruit-based pulps (Brasil, 2019) and 100% of the juice samples had high counts above  $3 \log \text{CFU/mL}$ . In the case of fruit pulps and juices, this legislation, which establishes the lists of microbiological standards for food, includes research on *Salmonella* and *E. coli*, which were not carried out for operational reasons. In the case of fruit pulps, there is still a need to do the counting of molds and yeasts.

For the parasitological evaluation, the data available on Tables 1 and 2 refers to the results for the adapted techniques of spontaneous sedimentation and centrifugation sedimentation. Parasitological evaluation of detox fruit pulps and juices acquired in different establishments of cities in the *Recôncavo* of Bahia region showed the presence of protozoa cysts, filaments of fungi, and mites in adult form. The forms of enteric parasitic found in the samples were *Endolimax nana*, *Entamoeba coli*, and *Entamoeba histolytica/E. dispar* cysts.

*Table 1.* Ingredients, thermotolerant coliform, mold and yeast counts and distribution of parasitic forms in pulps for detoxifying purposes, Bahia, 2018.

Sample	Type	E* <sup>1</sup>	Ingredients	TC* <sup>2</sup>	MY* <sup>2</sup>	Parasitic forms
P1	Pulp	EA	kiwi, chlorophyll, banana, mint, guarana, catuaba, acidulant, and preservative	<1	2.78	<i>Endolimax nana</i> and <i>Giardia</i> cysts
P2	Pulp	EA	apple, carrot, mint, cabbage, and pineapple	<1	3.3	<i>Entamoeba histolytica/E. dispar</i>
P3	Pulp	EB	beetroot, apple, cabbage, and orange	<1	2.15	<i>Endolimax nana</i>
P4	Pulp	EB	pineapple, cabbage, lemon, and ginger	1.7	4.69	Negative
P5	Pulp	EB	passion fruit, ginger, and mango	<1	4.11	Negative
P11	Pulp	EA	kiwi, chlorophyll, banana, mint, guarana, catuaba, acidulant, and preservative	1.49	4.3	Negative
P12	Pulp	EA	apple, carrot, mint, cabbage, and pineapple	<1	2.73	Negative
P13	Pulp	EB	beetroot, apple, cabbage, and orange	<1	2.48	Negative
P14	Pulp	EB	pineapple, cabbage, lemon, and ginger	<1	5.08	Negative
P15	Pulp	EB	passion fruit, ginger, and mango	<1	4	<i>Entamoeba coli</i>

Source: From the authors.

\*<sup>1</sup>E Establishment; \*<sup>2</sup>Thermotolerant coliforms TC; \*<sup>3</sup> MY Molds and yeasts.

Table 2. Ingredients, thermotolerant coliform, mold and yeast counts and distribution of parasitic forms in juices for detoxifying purposes, Bahia, 2018.

Sample	Type	E* <sup>1</sup>	Ingredients	TC* <sup>2</sup>	MY* <sup>2</sup>	Parasitic forms
S6	Juice	EC	mint, kale, spinach, carrot, honey, quinoa, apple, and mango	2.23	4.98	<i>Entamoeba histolytica/dispar</i>
S7	Juice	EC	grape, seed, strawberry, and lemon	<1	4.34	Fungi
S8	Juice	EC	grape, lemon, ginger, and cinnamon	<1	4.11	Negative
S9	Juice	ED	pineapple, ginger, and lemon	2.96	5.26	Adult form of mite
S10	Juice	ED	pineapple, carrot, and ginger	2.63	5.17	<i>Endolimax nana</i> and Fungi
S16	Juice	EC	mint, kale, spinach, carrot, honey, quinoa, apple, and mango	2.08	4.95	<i>Entamoeba coli</i>
S17	Juice	EC	grape, seeds, strawberry, and lemon	<1	4.53	Negative
S18	Juice	EC	grape, lemon, ginger, and cinnamon	<1	5.08	Negative
S19	Juice	ED	pineapple, ginger, and lemon	2.66	5.91	<i>Endolimax nana</i>
S20	Juice	ED	pineapple, carrot, and ginger	2.78	5.99	Negative

Source: From the authors.

\*<sup>1</sup>E Establishment; \*<sup>2</sup>Thermotolerant coliforms TC; \*<sup>3</sup>MY Molds and yeasts.

The Resolution N<sup>o</sup>. 14/2014 of ANVISA (03/28/2014) provides the presence and maximum limits of extraneous matter (any macroscopic and/or microscopic material not constituent of the product) in food and beverages. It includes helminths and protozoa as aggravating factors for human health, so their tolerance in juices and fruit pulps is zero (Brasil, 2014b).

The P4, S8, P14, and S18 samples (Tables 1 and 2) did not present parasitic forms but are unsuitable for consumption, as they presented high molds and yeasts counts. It is emphasized that the ED establishment showed parasitological and microbiological contamination, with the highest counts for the indicator microorganisms evaluated.

## DISCUSSION

According to Gomes et al. (2021), who completed a systematic review on the microbiological profile of fruit pulps from northeastern Brazil, the main microbiological parameters were thermotolerant coliforms (80%) and mold and yeast counts (70%), given the importance of these indicator microorganisms for verifying the hygienic-sanitary quality of food.

Then, the high thermotolerant coliform, mold and yeast counts, and the presence of enteric parasites in fruit pulps and juices are indicators of poor hygienic and sanitary conditions in their processing.

This result suggests possible incorrect hygiene of vegetables used in their preparation, and/or poor hygiene of equipment and utensils, and/or lack of personal hygiene of handlers, especially hands, which should be sanitized every 30 minutes with the antiseptic product (Brasil, 2004; Gomes et al., 2005).

The presence of thermotolerant coliforms in the analyzed samples indicate hygienic-sanitary failures and the probable presence of enteropathogens, mainly *Escherichia coli*, representing a risk to human health (Franco & Landgraf, 2018).

The importance of the correct hygiene of vegetables is emphasized, especially because juices and fruit pulps usually have raw materials such as raw vegetables. Furthermore, the hands hygiene of food handlers is an essential practice for ensuring the quality of food. Hygiene is responsible for removing dirt, secretions, dead cells, and, especially, for eliminating or reducing the microbial load present in the hands, preventing microbiological and parasitological infections (ANVISA, 2007). The hygiene of equipment and utensils also contributes to food safety, preventing cross-contamination (APN, 2018).

The most important factor to control the safety of refrigerated or frozen food is temperature (James & James, 2014). In this study, it was possible to notice that the frequency of parasitic forms and the indicator microorganisms analyzed were less observed in the fruit pulp samples than in juice samples. This result may be due to the conservation of the fruit pulps as they are frozen, this prevents microbial multiplication, due to more intense manipulation for the production of the juice.

Another relevant fact is associated with the higher prevalence of microbial and parasitic agents in samples that had vegetables into their composition, which may be due to failures in the cleaning process of these vegetables, since they are usually consumed raw, as well as the hands of handlers and equipment and utensils. In developing countries, the main causes of infections by pathogens are related to the lack of basic sanitation and the consumption of raw food without sanitization (Barros et al., 2019, Dantas et al, 2020).

In this way, it is essential to guarantee food safety and to prevent foodborne disease that food handlers comply with hygiene rules during the phases of production, processing, transport and storage of food (Gallo et al., 2020).

Divergent results were found by Pinheiro et al. (2006) in a study conducted in raw cashew, pineapple, and passion fruit juices sold in the city of Fortaleza (State of Ceará) market. The authors found low counts of thermotolerant coliforms in juice samples and  $<1 \log$  CFU/mL for molds and yeasts in pineapple and cashew juices, and only one sample of passion fruit juice presented a count of  $2.40 \log$  CFU/mL, being very low the average count of molds and yeasts in the present study ( $5.03 \log$  CFU/mL).

Jesus et al. (2020) analyzed the thermotolerant coliform counts in fruit pulps (passion fruit, tamarind, umbu, and cajá) in municipal schools in the city of Santo Antônio de Jesus (State of Bahia). They observed that only one sample of juice (passion fruit) did not comply with the current legislation (Brasil, 2019), it presented a value above  $2 \log$  (most probable number - MPN /mL) for thermotolerant coliforms.

It is suggested that the divergence of the results of this present study with the research by Pinheiro et al. (2006) and Jesus et al. (2020) refers to the diversity of ingredients used for the preparation of the detox juices since at least three ingredients were found in the analyzed samples, which increases the possibility of food contamination. Thus, the importance of hygienic and sanitary care in the entire production process of juices and the fruit pulps is emphasized, as established by ANVISA through resolution RDC No. 216/2004 (Brasil, 2004).

The results of the parasitological analysis, which showed the presence of enteric parasites in the samples (*Endolimax nana*, *Entamoeba coli* and *Entamoeba histolytica*/*E. dispar* cysts) are worrying, because parasitic infections represent a public health problem in Brazil, by affecting primarily the poorest population, with their little access to education and basic sanitation (Santos et al., 2019).

Carminate et al. (2011) detected parasitic forms in 21.3% of the samples analyzed in their study conducted on vegetables (lettuce and cabbage) sold at the farmers market in the municipality of Pedro Canário (Espírito Santo), which were lower than those obtained in this study. Similarly, Moura et al.



(2015) evaluated the lettuce and the cabbage commercialized at the markets in Anápolis (Goiás) and they observed contamination by parasitic agents in 16% of the analyzed samples.

Lower results were also obtained by Bozzetti et al. (2013) in their study, which has evaluated pineapple, apple, grape, and orange fruits in supermarkets, “*sacolões*” and in the farmers market (traditional markets) in the city of Ilhéus (State of Bahia). According to the authors, 14.6% of the analyzed samples were contaminated by parasitic structures; the fruits are part of the composition of juices and detox fruit pulps on the present study. The percentages of this study and the two previously mentioned, even below 50% of contamination in the samples of vegetables analyzed, reflect the need for sanitary hygienic care when using the fruits for raw consumption.

Higher results were obtained by Santos et al. (2009), who analyzed samples of lettuce and watercress sold in farmers market and supermarkets in the city of Salvador (Bahia). They observed that 95% of the samples contained some type of parasite, among them: *Entamoeba* sp. and *Endolimax* sp. (protozoa also found in this present study) and hookworms’ eggs.

It is observed that the accidental ingestion of parasitic infectious stages with contaminated raw vegetables or fruits can lead to serious health problems on the individual or even result in outbreaks of parasitic diseases (Li et al., 2020).

This study suggests that the establishments do not apply good hygienic practices in processing juices and detox fruit pulps, putting the health of the population at risk since these products are for instant consumption (Brazil, 2014b).

In a research done by Maldonade et al. (2019) with agribusinesses about ready-to-eat vegetables in the Federal District, in Brazil a positive correlation was observed between good hygienic practices with lower contamination rate corresponding to better product reliability.

For example, Almeida et al. (2018) found that 15% of food handlers from schools in Santo Antônio de Jesus city (Bahia) showed little knowledge regarding the quality and safe food handling risks of transmitting pathogens via food. This situation happened with 80% of the interviewees who had already participated in the Training Course on Good Practices in Food Handling. Incorrect practices can lead to food contamination and favor the growth and development of pathogens and cause WFTD. This study, in another publication (Jesus et al., 2020), showed that 20% of these handlers presented contamination of the hands with results above 3 MPN of thermotolerant coliforms/hand; having 100% contaminated with mesophilic aerobics, with results ranging from  $7.0 \times 10^2$  to  $1.4 \times 10^9$  CFU/hand and three bearing parasitic forms (*Endolimax nana* and *Entamoeba coli*) in subungual material in two schools; evidencing possible personal hygiene problems of those food handlers.

For this, ANVISA establishes measures for the correct hygiene of hands in food services to ensure food with quality and safety (ANVISA, 2007). These are current care stemming from the pandemic of the new coronavirus, caused by coronavirus of severe acute respiratory syndrome 2 (SARS-CoV-2) (OPAS, 2021).

For vegetables, resolution - RDC N°. 352 of December 23, 2002, establishes that vegetables should be washed with drinking water added with a chlorinated solution so that the last water of the washing process has a free residual chlorine content ranging from 0.5 to 2.0 ppm (Brasil, 2002).

The establishments must be aware of hygienic failures in food processing, to ensure a safe product for the population.

It is suggested to carry out analysis involving the research of *Salmonella* and *Escherichia coli* in samples of pulp and juices for detoxifying purposes to compare with the maximum limits established by IN 60/2019 of ANVISA, in addition to molds and yeasts only for fruit pulps.

The higher count of microorganisms' as an indicative of precarious hygienic-sanitary quality in juices and fruit pulps used for detoxifying purposes, reveals the need for a closer look and even an intervention of the sanitary organs, through inspection, in industrialization and commercialization establishments of juices and detox fruit pulps.

A continuous training for handlers about the adequate hygiene procedures, in addition to the implementation of the Standard Operating Procedures and the Manual of Good Practices in establishments is necessary, in order to ensure the product quality and consumer health.

## CONFLICT OF INTEREST

The authors declare there is no conflict of interest to disclose.

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