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Perfil microbiológico, parasitológico e análise de sujidades de saladas de frutas minimamente processadas vendidas no comércio ambulante

Microorganisms, parasites, and nonbiological contaminants in minimally processed fruit salads sold by street vendors

Perfil microbiológico, parasitológico y análisis de sujetos de ensalada de frutas minimamente procesados vendidos en el comercio ambulante

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Resumo

Há uma grande variedade de riscos biológicos que podem estar associados aos alimentos e acometer a saúde dos consumidores, como os micro-organismos e parasitos. Com o aumento da demanda pelos alimentos minimamente processados, verifica-se o aumento da oferta de saladas de frutas comercializadas pelo mercado ambulante. Sendo assim, o objetivo do presente trabalho foi traçar o perfil microbiológico, parasitológico e macroscópico (ou das sujidades) de saladas de frutas minimamente processadas comercializadas por ambulantes. Para tanto, cinquenta amostras de saladas comercializadas por diferentes ambulantes e selecionadas aleatoriamente. Foi constatada presença de *Salmonella* spp em 2% das amostras e elevadas quantidade de micro-organismos do grupo dos coliformes, sendo a presença de *Escherichia coli* confirmada em 10% das saladas. Além disso, verificou-se crescimento de *Staphylococcus* coagulase positivo em 14% das amostras, e bolores e leveduras variando de 3,77 a 7,78 log_{10}UFC/g. Não foi observada presença de parasitas e sujidades em nenhuma das amostras analisadas. As amostras de salada avaliada apresentaram contaminação microbiana e pode representar riscos à saúde dos consumidores pela possibilidade de veiculação de micro-organismos patogênicos de importância em saúde pública.

Palavras chave: Alimentos; Riscos biológicos; Saúde pública.

Abstract

Several biological contaminants, such as microorganisms and parasites, can be found in food and affect the health of consumers. The demand for minimally processed foods is increasing, including fruit salads that are sold by street vendors. The objective of this research was to evaluate microorganisms, parasites, and nonbiological contaminants in minimally processed fruit salads sold by street vendors. Fifty fruit salad samples marketed by different street vendors were randomly collected. Presence of *Salmonella* spp. was found in 2% of the samples. High number of microorganisms of the coliform group was found; *Escherichia coli* was found in 10% of the samples, growth of coagulase-positive staphylococcus was found in 14% of the samples, and molds and yeasts in the samples ranged from 3.77 to 7.78 log_{10}CFU g^{-1}. No parasites or nonbiological contaminants were found in any sample analyzed. The salad samples presented microbial contamination and represent risks to the health of the consumers due to the possibility of transmitting pathogenic microorganisms of importance to public health.

Keywords: Food; Biological risks; Public health.

Resumen

Existe una amplia variedad de riesgos biológicos que pueden asociarse con los alimentos y afectar la salud de los consumidores, como los microorganismos y los parásitos. Con el aumento de la demanda de alimentos mínimamente procesados, hay un aumento en la oferta de ensaladas de frutas que vende el mercado callejero. Por lo tanto, el objetivo del presente trabajo fue rastrear el perfil microbiológico, parasitológico y macroscópico (o de suciedad) de ensaladas de frutas mínimamente procesadas vendidas por vendedores ambulantes. Con este fin, cincuenta muestras de ensaladas vendidas por diferentes proveedores y seleccionadas al azar. La presencia de Salmonella spp se encontró en el 2% de las muestras y en grandes cantidades de microorganismos en el grupo de coliformes, con la presencia de Escherichia coli confirmada en el 10% de las ensaladas. Además, hubo crecimiento de Staphylococcus coagulasa positivo en el 14% de las muestras, y mohos y levaduras que oscilaron entre 3.77 y 7.78 log10UFC / g. No se observaron parásitos ni suciedad en ninguna de las muestras analizadas. Las muestras de ensalada evaluadas mostraron contaminación microbiana y pueden presentar riesgos para la salud de los consumidores debido a la posibilidad de propagar microorganismos patógenos de importancia para la salud pública.

Palabras clave: Comida; Riesgos biológicos; Salud pública.

1. Introduction

Food safety is a subject that has great worldwide relevance (Bastos, 2010; Jucene, 2013), mainly due to the transmission of diseases to consumers through food products. The good quality of the food requires good practices during all stages of production, and commercialization, such as the use of clean and sanitized utensils and adequate storage temperature, since food products can be contaminated in different stages (Lopes & Ferreira, 2016).

The lack of knowledge of street vendors about the risks associated with the consumption of contaminated foods is concerning (Cintra et al., 2017); they can market products that have inadequate hygienic-sanitary conditions, compromising the food safety and, consequently, the consumers' health (Moura et al., 2017). One of the most marketed foods by street vendors are fruit salads, classified as minimally processed food.

Minimally processed plant products present greater perishability than intact ones, since their production involves peeling and cutting operations that disrupt their biological structures (Barbosa et al., 2017), and removal of inedible parts such as husks, stems, and seeds to make them ready for consumption (Spoto & Miguel, 2006).

The demand for minimally processed foods is increasing in recent years, mainly because they are produced with simple processing operations, maintaining similar qualitative and nutritional characteristics to the raw material used, and are convenient to consumers (Alves et al., 2010). Sanitization of the raw material and other hygienic care during the production of these foods are necessary to guarantee the quality of these food products, since food safety is associated to handling conditions (Pinheiro et al., 2011).

According to the Resolution-RDC 12/2001 of the Brazilian Health Regulatory Agency (ANVISA), peeled and fractionated fresh fruits marketed for direct consumption cannot present more than $5x10^2$ per gram of coliforms at 45 °C (thermotolerant) (Brasil, 2001). These microorganisms are indicators of poor hygienic-sanitary practices in the production of these foods; their presence in food may indicate problems at handling, since the pathogenic microorganisms, such as *Staphylococcus aureus* for example, is frequently related to inadequate handling (Franco & Landgraf, 2008). Moreover, *Salmonella* should be absent in food products (Brasil, 2001) because it is a significant cause of morbidity and mortality, presenting a great importance to public health (Fosythe, 2013).

Other problems associated with the consumption of not cooked foods or foods produced with unsatisfactory hygienic conditions are contaminations by parasites, (CACCIÒ et al., 2018) such as *Ascaris lumbricoides*, *Enterobius vermicularis*, *Strongyloides* spp., *Toxocara* spp., and *Entamoeba coli* (Fallah & Makhtumi & Pirali-Kheirabadi, 2016), and nonbiological contaminants, such as sand, plastic, and soil (Soares et al., 2017).

Thus, considering the precarious conditions of food products marketed by street vendors, the increasing demand for minimally processed foods, and the intense handling during the production of these products, the objective of this work was to evaluate microorganisms, parasites, and nonbiological contaminants in minimally processed fruit salads sold by street vendors.

2. Material and Methods

The objective of researches is to bring new information to society, as described by Pereira et al. (2018). The present study has an experimental character, which proposes to investigate a theme, including variables and forms of control. The databases used for the research were ILACS, PubMed, Scielo, and Google Scholar; scientific papers were acquired by selecting the articles according to the keywords: salad, contamination, parasites, and food. The work consists of qualitative and quantitative evaluations, which allow for more reliable results.

Samples of minimally processed fruit salads sold by randomly chosen street vendors were collected in the commercial center of Mossoró, State of Rio Grande do Norte, from March 2017 to March 2018. Ten samples of fruit salads were purchased in 5 collections (50 samples). These fruit salads were handmade, cooled, and had no labels.

The temperature of the samples was measured using an infrared digital thermometer and the fruit salads and their containers were packed in isothermal boxes with ice and taken to the Food Biotechnology Laboratory of the Center of Agricultural Sciences of the Federal Rural University of the Semi-Arid Region. The utensils used in the collection were previously sterilized in a laminar flow cabinet.

The samples were subjected to microbiological analysis (presence of *Salmonella* spp., coagulase-positive staphylococcus count, most probable number of total and thermotolerant coliforms, presence of *Escherichia coli*, and yeast and mold counts), parasitological analysis, and presence of nonbiological contaminants.

Microbiological analyzes followed methodologies described by the APHA (1998). It was carried out with sterilized materials, using aseptic procedures in a laminar flow cabinet. Twenty-five grams of each sample was diluted into 225 mL of peptone saline solution to obtain a 10^{-1} dilution, and serial dilutions up to 10^{-8} were obtained from these samples.

A 10^{-1} dilution was incubated at 36±1 °C for pre-enrichment for 24 hours in a bacteriological oven to verify the presence of *Salmonella* spp. One milliliter of this content was subsampled in Rappaport Vassiliadis, Selenite Cystine, and Tetrathionate broths for selective enrichment for 24 hours at 41±1 °C in a micro-processed water bath. The streaking was carried out using a microbiological loop coupled with a Kole's cable, and Petri dishes previously prepared with Salmonella-Shigella agar, and Rambach agar. After streaking, the plates were stored upside down at 36 °C in a bacteriological oven for 24 hours, then, the presence of typical colonies was verified. The confirmation of the presence of *Salmonella* spp. was carried out using biochemical tests (decarboxylation of lysine; lactose, and sucrose fermentation capacity; hydrogen sulphide production in Lysine Iron Agar, and Triple Sugar Iron agar; and urease test).

Coagulase-positive staphylococcus counts were performed using surface culture in Petri dishes with Baird Parker Agar with telluride. The Petri dishes were inoculated with 1 mL of each dilution, in duplicates. The sample was then spread with a Drigalsky spatula and the Petri dishes were incubated upside down in a bacteriological oven at 36±1 °C for 48 hours. Gram staining, catalase, and coagulase were performed to confirm the presence of *Staphylococcus aureus*.

Mold and yeast counts were performed using surface culture in potato dextrose agar, and the inoculation of the sample in the medium was similar to that described for the staphylococcus counts. Samples were incubated in a BOH oven at 28±1 ºC for 7 days.

The plates were read for staphylococcus, molds, and yeasts using colony counts and the results were expressed in $\log_{10}$CFU g^{-1}.

Total and thermotolerant coliforms were evaluated using the multiple tubes technique to measure the most probable number (MPN) (APHA, 2001), which is based on the fermentation of lactose with separation of gas inside Duran tubes. Total coliforms were evaluated using the culture in sodium lauryl sulphate broth at 36±1 ºC in water bath for 48 hours as presumptive test. Positive tubes were subsampled using a platinum loop in brilliant green bile lactose broth under the same incubation conditions to confirm the most probable number of total coliforms. The tubes with formation of gas were subsampled in *Escherichia coli* broth and cultured in water bath at 45±1 °C for 48 hours.

The presence of *Escherichia coli* was confirmed using samples of the positive tubes for thermotolerant coliforms streaked on Petri dishes with eosin methylene blue agar to show the typical colonies. These colonies were subjected to confirmatory biochemical tests, according to the methodology described by Santos and Coelho (2016).

Parasitological heavy eggs were evaluated using the Hoffmann method adapted for food samples, which consists of spontaneous sedimentation (Hoffman; Pons; Janer, 1934). One hundred grams of the fruit salad sample were placed into plastic bags, homogenized with 250 mL of distilled water for 5 minutes and transferred to sedimentation containers for 24 hours. An aliquot of the obtained precipitate was taken from the bottom of the containers and placed on a slide for evaluation using an optical microscope.

Parasitological light eggs were analyzed using the Faust flotation methodology (Faust, 1938) adapted for food samples. One hundred grams of the fruit salad sample were added to an aqueous zinc sulfate solution with density of 1.2 g cm^{-3} (Faust et al., 1938). Three slides per sample were used for the analyses. The slides were covered with cover glasses for 10 min to form a film in the slide surface with the analyzed material and examined under an optical microscopy.

Light and heavy nonbiological contaminants were evaluated using the methodology described by the Association of Official Analytical Chemists (AOAC, 2005), with adaptations. A sample 50 g of the fruit salad with 200 mL of distilled water and 10 mL of mineral oil were shaken vigorously for 30 seconds. The solution was placed in a separation funnel to form 3 phases (oil, water, and heavy nonbiological contaminants), which were then separated into different containers. This procedure was repeated for heavy nonbiological contaminants until the liquid became translucent. This liquid was then passed through a filter paper and the water was removed using a vacuum pump. The material contained in the oil container was placed on a filter paper and the oil was removed using vacuum pump. Subsequently, the material was analyzed in an optical microscope with a 40x objective (Soares et al., 2017).

3. Results and Discussion

The results of the microbiology analyses of the fruit salads marketed by street vendors were described in Table 1.

Table 1 - Microbiological analyses of minimally processed fruit salads marketed by street vendors in Mossoró, State of Rio Grande do Norte, Brazil, from March 2017 to March 2018.

Street Vendor	*Staphylococcus* (Log_{10}UFC g^{-1})	*S. aureus*	Molds and yeasts (Log_{10}UFC g^{-1})	Coliforms at 35 °C (MPN g^{-1})	Coliforms at 45 °C (MPN g^{-1})	*E. coli*	*Salmonella* spp.
1	4.49	P	5.47	1100	15	P	A
2	5.21	P	5.69	>1100	20	P	A
3	6.16	A	6.13	>1100	9,2	A	A
4	4.77	P	5.41	>1100	20	P	A
5	3.68	A	5.57	93	<3.0	A	A
6	5.38	A	5.24	290	<3.0	A	A
7	5.33	A	5.27	>1100	7.4	P	A
8	4.41	A	5.70	>1100	<3.0	A	A
9	5.14	A	4.47	93	<3.0	A	A
10	4.08	A	4.98	240	3.6	A	P
11	A	A	6.15	>1100	>1100	A	A

12	5.51	P	7.36	>1100	1100	A	A
13	6.31	A	5.46	>1100	27	A	A
14	5.56	A	6.53	>1100	36	P	A
15	4.50	A	6.12	>1100	>1100	A	A
16	5.65	A	6.42	>1100	240	A	A
17	6.62	A	6.89	>1100	7.2	A	A
18	6.07	A	6.70	210	<3.0	A	A
19	5.90	A	6.08	>1100	<3.0	A	A
20	5.07	A	6.52	>1100	<3.0	A	A
21	3.53	A	5.47	>1100	35	A	A
22	3.20	A	6.11	>1100	11	A	A
23	4.23	A	5.95	460	<3.0	A	A
24	A	A	6.11	43	3.6	A	A
25	A	A	5.35	23	<3.0	A	A
26	2.47	A	5.74	<3.0	<3.0	A	A
27	3.30	A	5.79	460	20	A	A
28	4.09	A	5.98	>1100	15	A	A
29	A	A	5.72	460	<3.0	A	A
30	4.20	A	5.97	>1100	<3.0	A	A
31	4.04	A	4.54	23	3.6	A	A
32	3.47	A	4.38	93	7.4	A	A
33	3.69	A	4.53	9.2	<3.0	A	A
34	3.60	A	4.47	460	3.6	A	A
35	A	A	5.49	210	<3.0	A	A
36	3.69	A	5.46	<3.0	<3.0	A	A
37	3.30	A	3.77	23	<3.0	A	A
38	3.30	P	3.77	460	<3.0	A	A
39	3.84	P	5.56	460	<3.0	A	A
40	3.77	P	5.70	460	<3.0	A	A
41	4.20	A	5.84	460	<3.0	A	A
42	4.11	A	5.63	>1100	<3.0	A	A
43	4.13	A	7.65	1100	20	A	A
44	4.96	A	7.55	1100	1100	A	A
45	4.24	A	7.29	>1100	3.0	A	A
46	4.33	A	7.50	>1100	1100	A	A
47	4.52	A	7.78	>1100	28	A	A
48	3.97	A	6.65	1100	75	A	A
49	3.99	A	7.34	1100	36	A	A
50	4.28	A	7.40	>1100	27	A	A

P = Presence; A = Absence. Source: Authors, 2019.

Total coliforms were found in 96% (48) of the fruit salad samples evaluated, and 62% (31) of them had also growth of thermotolerant coliforms. The MPN of total and thermotolerant coliforms varied from <3.0 to >1100 MPN g^{-1}. Presence of *Escherichia coli* was confirmed in 10% (5) of the analyzed samples (Table 1).

Coliforms are microorganisms of the genera *Escherichia*, *Enterobacter*, *Kleibsiella*, and *Citrobacter*. Analyses of these microorganisms in food products indicate the hygienic-sanitary conditions in their processing and selling (Forsythe, 2013).

Peeled and fractionated fruits for fresh consumption such as fruit salads cannot have coliform contents higher than $5x10^2$ g^{-1} at 45 °C (BRASIL, 2001). Ten percent of the samples had coliform counts higher than this limit established by the ANVISA. This may be due to inadequate conditions during handling and preparation of the fruit salads (Santos and Carvalho, 2017), use of contaminated utensils or devices (Lima et al., 2017), and inadequate exposure of the product for selling, which is often practiced by street vendors (Santos et al., 2015) who are not well informed about these risks (Nascimento et al., 2017).

Other studies have also found thermotolerant coliforms in fruit salads. Santos et al. (2015) found counts above the maximum allowed by the legislation in 33% of the samples in Juazeiro do Norte, CE, Brazil; and Santos and Carvalho (2017) found thermotolerant coliform counts higher than 1100 MPN g^{-1} at 45 ° C in Ilhéus, BA, Brazil.

Fourteen percent (07/50) of the fruit salad samples had coagulase-positive staphylococcus counts of 3.30 to 5.51 Log_{10}CFU g^{-1} (Table 1).

The occurrence of coagulase-positive *Staphylococcus aureus* in fruit salads may be a risk to the health of consumers due to the possible pathogenicity associated with this bacterium. This bacterium is usually associate with inappropriate handling conditions, since staphylococcus can inhabit the respiratory tract of humans, and food products can be contaminated during processing and selling. This is even more common in food products marketed by street vendors, whose knowledge about good handling practices is usually low (Nascimento et al., 2017).

Molds and yeasts were also found in the fruit salads, with counts of 3.77 to 7.78 log_{10}CFU g^{-1} (Table 1). Although Brazilian legislation does not establish a standard for this group in fruit salads, high incidences of fungi in food products may decrease their quality. They can compromise the safety of these products because many fungi produce mycotoxins that are harmful to human health and alter the microorganism composition, since they are correlated with deterioration of acidic fruits (Santos & Carvalho, 2017). When these fruits are minimally processed for fruit salads, they present high perishability mainly due to the intense handling at peeling and cutting operations (Fagiane et al., 2017).

Salmonella spp. were found in 2% of the fruit salads; one sample (1/50) was not in accordance with the Brazilian legislation, which determines the absence of this pathogen in 25 g samples of the product (Brasil, 2001). Other authors have found similar results in minimally processed foods in Brazil. Fagiane et al., (2017) evaluated minimally processed products from Presidente Prudente, SP, and found *Salmonella* spp. in all samples; Lins et al. (2014) found this pathogen in fruit salads from the Cariri microregion in the state of Ceará; and Smanioto et al. (2009) found *Salmonella* spp. in minimally processed fruits and vegetables from supermarkets in Bauru, SP.

Parasites were absent in 100% (50/50) of the samples. This may be due to the refrigeration or even freezing of the product for the conservation of the fruit salads. However, this refrigeration may have ruptured egg cell walls of the parasites (Neves et al., 2016), leading to false negative results.

Developed countries have experienced remarkable advances in handling and industrialization practices for minimally processed foods (Cacciò et al., 2018), however, food processing in developing countries such as Brazil still have poor general sanitation conditions, which can compromise the quality of food products (Holanda & Vasconcello, 2015). Thus, evaluations of parasites in food products is important as an indicator of their quality and for the public health. They are an important strategy to evaluate hygiene conditions and the potential transmission of these agents by food products (Neres et al., 2011).

Nonbiological contaminants were not found in any sample. According to CNNPA Resolution No. 12 of 1978, samples of processed fruits should not contain earthy substances or foreign bodies. Although no study has found macroscopic nonbiological contaminants in fruit salads, other fresh consumed foods have presented contamination (Soares et al., 2017; Robertson et al., 2018 *In press*), confirming the importance of including macroscopic analyzes in food evaluations.

In this context, carried out researches focused on establishing a strict control of fresh products is necessary, since they can be physical and biological hazards to consumers, and the occurrence of foodborne diseases is a serious public health problem.

4. Conclusion

The samples of minimally processed fruit salads sold by street vendors in the commercial center of Mossoró, State of Rio Grande do Norte, Brazil, presented contamination by *Salmonella* spp., *Staphylococcus aureus*, and total and thermotolerant coliforms, including *Escherichia coli*.

The incidence of molds and yeasts were relatively high in the samples. These results can be associated with the poor hygienic-sanitary conditions during handling and selling of this food product. Parasites and nonbiological contaminants were not found in the samples.

Thus, the adoption of better sanitary conditions throughout the food processing chain is necessary for minimally processed salads, focusing on adequate food safety for consumers. Further research should be carried out to monitor the food processing conditions for these products.

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