

Quality of Minimally Processed Products Marketed in Cuiabá, Mato Grosso, Brazil

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Abstract

Consumers have been increasingly seeking healthier foods without sacrificing sensory satisfaction and convenience, which are highly acclaimed attributes in modern times. Minimally processed products can meet these demands. The present study evaluated the microbiological, microscopic and physicochemical quality of minimally processed fruits and vegetables marketed in Cuiabá, Brazil. A total of 36 samples, consisting of sliced melon, fruit salad in pieces, grated carrot, diced melon, sliced papaya and pineapple rounds were subjected to microbiological, microscopic and physicochemical analyses. *Salmonella* spp. was absent in all samples, while 27.8% (10/36) of the samples showed coliform counts at 45 °C over 2.0 and 2.7 log CFU.g⁻¹ in fruit and vegetables, respectively. Light dirt and foreign matter were present in 55.5% of the samples (20/36), including wood fragments and insect/animal excrements (rat hair) in 13.9% (5/36) of the samples. The pH and soluble solids (°Brix) results ranged from 3.84 to 6.66 and from 8.19 to 10.24, respectively. The products were in different stages of maturation and 27.8% (10/36) of the sliced papaya and grated carrots were in unsatisfactory sanitary conditions under the current Brazilian legislation.

Keywords: microbiological, microscopic, physicochemical analyses; fruits; vegetables; minimal processing

1. Introduction

According to the IFPA (2013) (International Association of Minimally Processed Products), minimally processed products are defined as any fruit or vegetable, or even a combination of both, which has been physically altered from its original form, while maintaining freshness. In general, the minimally processed vegetable sector faces major challenges, such as maintaining fresh products without loss of sensory and nutritional quality, in addition to guaranteeing that the products will not result in potential health risks to consumers (Huxsoll & Bolin, 1989).

According to organizations such as Food Standards Agency – FSA(United Kingdom), and Centers for Disease Control and Prevention – CDC (United State America), the frequency of foodborne illness outbreaks associated with the consumption of fresh products, especially minimally processed ones, has increased notably (CDC, 2009; Paula, Vilas Boas, Rodrigues, Carvalho, & Picolli, 2009; FSA, 2007). The Food and Drug Administration (FDA) states that, of 72 cases of outbreaks related to fresh products, 25% (18 cases) are associated to minimally processed products (FDA, 2008).

The presence of injured cells and loss of its components (nutrients and enzymes) during processing operations provide optimum conditions for microorganism growth. The microbial type and species, as well as levels, in minimally processed food products vary according to the fruit or vegetable, cultivation practices, sanitary conditions during handling and processing, and storage temperature, among other factors (Smaniotto, Pirolo, Simionato, & Arruda, 2009).

Microorganism indicators are an important verification tool of food sanitary hygienic conditions. This group consists mainly of coliform and bacteria from the *Salmonella* genus, which is a group of gram-negative, facultative anaerobic bacteria. Coliforms are an indicator of fecal contamination. They are lactose fermenters and, therefore, produce CO₂, used in the identification of these bacteria (Menezes & Moreira, 2012). Pathogens can originate from this microflora and lead to potential food safety problems. Neglecting to maintain appropriate utensils and handler hygiene conditions can contribute to the transmission of pathogenic and spoilage microorganisms (Xisto, Vilas Boas, & Nunes, 2012). Minimally processed vegetables evaluated in southeastern Brazil presented the high coliform bacteria counts in 50% of the samples, revealing neglect regarding hygiene precautions during the processing of these products (Albuquerque, Santos, & Rall, 2013; Pereira & Hoffmann, 2011; Pires, Donadone, Chaud, & Pereira, 2011; Silva & Rall, 2011; Furlaneto, Santini, & Velasco, 2005).

The aim of this study was to evaluate the quality of minimally processed products during different periods which were marketed in two supermarkets at the city of Cuiabá, Mato Grosso, Brazil.

2. Material and Methods

2.1 Sampling

A total of 36 fresh-cut fruit and vegetable samples were collected in refrigerated counters of two large supermarkets (A and B) located at Cuiabá, Brazil. The samples were composed of fruit salad, melon slices and grated carrot, collected in supermarket, A as well as cut melon, papaya slices and pineapple slices, collected at supermarket B, with all samplings conducted weekly from June 2013 until August of the same year. Samples were represented by two packets of minimally processed vegetables during the period, weighing approximately 300 grams. Six samples consisted of fruit salad, melon slices and grated carrot from supermarket A, and six samples consisted of diced melon, sliced papaya and pineapple rounds from supermarket B. All samples weighed approximately 300 grams, collected weekly from June until August 2013. The samples were placed in isothermal bags and sent to the Nutrition School laboratories at the Federal University of Mato Grosso for analysis. The applied parameters were the maximum permissible microbiologic and microscopic limits contained in Brazilian resolutions RDC No. 12 from January 2, 2001 (BRASIL 2001) and RDC No. 175 from July 8, 2003 (BRASIL 2003), which follow the World Health Organization (WHO) guidelines.

2.2 Microbiological Analyses

Twenty-five grams (25 g) of each sample were diluted in 225 mL of sterile peptone saline solution 0.1% (w/v) in plastic 720 mL *Stomacher* bags (Hexis Scientific S/A, Brazil) and processed in *Stomacher* sample homogenizers (Marconi, Brazil) for 60 seconds. All analyses were performed according to the International Commission on Microbiological Specification for Foods (ICMSF, 1983) and American Public Health Association (APHA, 2001).

Coliforms were determined by analysis of multiple sets of three laurel sulfate tryptose broth tubes (Himedia®, Mumbai, India), inoculated with 1 mL aliquots of the diluted samples at 10, 100 and 1000 times, and incubated at 35 °C for 24-48 hours during the presumptive phase. The confirmation analysis of coliforms was conducted from positive fermentation tubes and gas production at 35 °C and 45 °C in brilliant green bile broth 2% and *Escherichia coli* broth (Himedia®, Mumbai, India), incubated for 24-48 hours. The results were expressed in log MPN.g⁻¹ (APHA, 2001; ICMSF 1983).

The *Salmonella* spp. investigation consisted of three phases: pre-enrichment, selective enrichment and differential isolation. The pre-enrichment was conducted in buffered peptone water (Himedia®, Mumbai, India), which included 25 grams of each sample, incubated at 35 °C for 24 hours. Aliquots of 0.1 to 1 mL of the homogenate were transferred to 10 mL Rappaport-Vassiliadis broth (RV) and tetrathionate broth (TT) (Himedia®, Mumbai, India) and incubated at 42 °C and 35 °C, respectively, for 24 hours. Subsequently, typical *Salmonella* colonies were seeded in rambach (Merck, Germany) and brilliant green agar (Himedia®, Mumbai, India) by groove, incubated at 42 °C and 35 °C for 24 hours and then subjected to biochemical tests for identification (APHA, 2001; ICMSF 1983).

2.3 Physicochemical Analyses

After grinding and homogenizing the tissues in a 1:5 ratio (20 g of diluted pulp in 100 mL of distilled water), titratable acidity (TA), pH and soluble solids (SS) assays were performed. The determination of titratable acidity (% correspondent to citric acid) was performed by titration with 0.1 N NaOH solution using phenolphthalein as an indicator (AOAC, 2000). The pH was determined using a T-1000 Model pH meter (Tecna, Brazil) (AOAC, 2000). The SS were determined by refractometry, using a digital ATAGO PR-100 refractometer (Atago, Ribeirão Preto, Brazil) with automatic temperature compensation at 25 °C (AOAC, 2000).

2.4 Microscopical Analyses

Microscopical analyses were performed by the light debris floating method using a Wildman trap. Sample aliquots of 20 grams were introduced into the Wildman trap and homogenized with distilled water and soybean oil, and the filth contained in the oil phase was captured on a filter paper, dried through a vacuum pump (Tecnal, model TE058, São Paulo, Brazil) and observed by use of a stereoscopic microscope (Olympus, model SZST, Japan). The detected structures were confirmed by observation on an optical microscope (Olympus, model CX40RF100, Japan) (Beux, 1992).

2.5 Statistical Analyses and Experimental Design

The experiment was conducted in a randomized block design (RBD) with three blocks that comprised the manufacturing periods of the minimally processed products (1st, 2nd and 3rd manufacturing dates), performed weekly. The statistical analyses of the physicochemical assays were performed using the Statistical Programme Sisvar 4.3 (Ferreira, 2000). After the analysis of variance, the significance level applying an F test was observed. The treatment means, when significant, were compared by the Scott-Knott test at 5% probability. The results of the microbiological and microscopic analyses were obtained from indicative samples consisting of two portions of fruit and/or vegetables and are expressed as frequency and/or log of Colony Forming Unit (CFU), following Brazilian legislations (BRAZIL, 2003; BRAZIL, 2001) and recommendations from the World Health Organization. The results of the microbiological and microscopic analyses were performed with a 2 fruit or vegetable portion pool, being considered indicative tests. These procedures were performed for all collecting periods.

3. Results and Discussion

No specific legislation for minimally processed exists in Brazil. Thus, the microbiological standards set out in Resolution RDC No. 12 from 2001 of the Brazilian National Health Surveillance Agency (BRASIL, 2001) are the norm for fruit and/or vegetables "fresh, prepared (peeled, selected or cracked), sanitized, chilled or frozen for direct consumption". The RDC 12/2001 establishes the absence of *Salmonella* spp. in 25 g of fruit or vegetable and a maximum thermotolerant fecal coliform count at 45 °C or *Escherichia coli* of 5×10^2 CFU.g⁻¹ (log 2.7 CFU.g⁻¹) for fruit and 1×10^2 CFU.g⁻¹ (log 2.0 CFU.g⁻¹) for vegetables.

The 36 fruit and vegetable samples evaluated in this study were in accordance to the recommended limits dictated by Brazilian legislation (BRASIL, 2001), such as the absence of *Salmonella* in 25 g of product. However, 27.8% of the samples, represented by five pools of two sample portions (Table 1), totalizing 10 of the 36 samples, were in unsatisfactory sanitary conditions due to the presence of coliforms at 45 °C, with counts above log 2.7 CFU.g⁻¹, detected in sliced papaya (supermarket B) from the 1st and 3rd collection period, and above log 2.0 CFU.g⁻¹ detected in grated carrots (supermarket A) during all three collection periods, with coliforms also detected at 35 °C.

The absence of *Salmonella* spp. associated with a percentage of 71.2% of negative samples for coliforms at 35 °C and 45 °C may indicate that good manufacturing practices were implemented, such as the use of detergents to carefully wash the fruits and vegetables exteriors, as well as environment and utensil sanitizing on the processing site (Shale, Mukamugema, Lues, Venter, & De Smidt, 2012). The absence of *Salmonella* in fresh-cut fruits and vegetables was also observed in Campinas-SP, by Santos et al. (2010), Bauru-SP (Smanioto, Pirolo, Simionato, & Arruda, 2009), in a salad mix at Camburiú and Florianópolis-SC (Marmentini, Felipe, Lemos, Pedrozo, & Bender, 2011) and also in fruit salad evaluated in Fortaleza-CE (Pinheiro, Abreu, Maia, Sousa, Figueiredo, Rocha, & Costa, 2011).

The presence of *Salmonella* spp. in minimally processed vegetables was detected in 25% of fruit samples (Pinheiro, Figueiredo, Figueiredo, Maia, & Sousa, 2005) and in 66.6% of vegetables and tubers (Bruno, Queiroz, Andrade, Vasconcelos, & Borges, 2005) sold in supermarkets in Fortaleza-CE, and in 25% of fruit from Catanduva-SP (Virgolin, Geromel, Manfrin, & Fazio, 2013). The presence of bacteria from this genus reflects poor sanitary conditions and health risks, such as the possibility of infections, that the products may present, due to cross-contamination and inefficient sanitizing techniques (Menezes & Moreira, 2012).

Among the tested samples, only 27.8% (10/36) presented coliform counts of 2.7 and 2.0 log CFU.g⁻¹ at 35 °C and 45 °C in fruits and vegetables, respectively (Table 1). The percentage of samples with high coliform results in the present study was lower than in other studies conducted in Brazil as reported. The occurrence of high coliform counts at 35 °C and 45 °C classifies these products as presenting unsatisfactory sanitary conditions (Franco & Landgraf, 2005), since the occurrence of this bacteria group can indicate the possible presence of pathogenic microorganisms due to possible direct or indirect contact with feces (Oliveira, Costa, & Maia, 2006).

Table 1. Coliforms at 35 °C and 45 °C (log MPN.g⁻¹) in minimally processed vegetables sampled in different manufacturing periods

Supermarket	Product	log MPN.g ⁻¹ of coliforms per period			
		Coliforms	1 st	2 nd	3 rd
A	Fruit salad	35 °C	2.66	1.97	1.54
		45 °C	2.66	1.97	1.54
A	Diced melon	35 °C	1.36	0.48	1.88
		45 °C	0.56	0.48	1.18
A	Grated carrots	35 °C	3.04	3.04	3.04
		45 °C	3.04	3.04	3.04
B	Pineapple rounds	35 °C	0.96	0.48	0.48
		45 °C	0.48	0.48	0.48
B	Sliced papaya	35 °C	3.04	1.63	3.04
		45 °C	3.04	1.63	3.04
B	Sliced melon	35 °C	1.97	0.48	2.18
		45 °C	1.18	0.48	1.43

Table 2. Mean titratable acidity (TA), pH and soluble solids (SS) values in minimally processed vegetables sampled in three different manufacturing periods

Product/Supermarket	Period	TA (% citric acid)	pH	SS (°Brix)
Fruit salad/A	1 st	0.35 ^a	4.65 ^b	8.14 ^a
	2 nd	0.35 ^a	4.46 ^a	8.15 ^a
	3 rd	0.39 ^b	4.69 ^b	10.00 ^b
Diced melon/A	1 st	0.16 ^b	6.44 ^a	8.24 ^b
	2 nd	0.13 ^a	6.66 ^b	8.25 ^b
	3 rd	0.15 ^b	6.40 ^a	6.00 ^a
Grated carrots/A	1 st	0.17 ^a	6.44 ^b	4.47 ^b
	2 nd	0.19 ^a	6.36 ^a	4.49 ^b
	3 rd	0.28 ^b	6.41 ^b	4.19 ^a
Pineapple rounds/B	1 st	0.54 ^a	4.01 ^b	10.23 ^b
	2 nd	0.52 ^a	3.84 ^a	10.24 ^b
	3 rd	0.59 ^b	3.98 ^b	8.09 ^a
Sliced papaya/B	1 st	0.12 ^b	5.27 ^b	8.34 ^a
	2 nd	0.10 ^a	5.18 ^a	8.28 ^a
	3 rd	0.12 ^b	5.28 ^b	9.87 ^b
Sliced melon/B	1 st	0.12 ^b	6.41 ^b	8.31 ^b
	2 nd	0.10 ^a	6.22 ^a	8.27 ^b
	3 rd	0.12 ^b	6.39 ^b	8.19 ^a

* Means followed by the same letter represent statistical similarities between the manufacturing periods at 5% probability according to the Scott-Knott test.

The different processing stages of minimally processed products may be responsible for spoilage and contamination by pathogenic microorganisms (Coelho, Scalcon, Guaitanele, & Haida, 2008). According to

Pinheiro et al. (2005), microorganism growth in minimally processed vegetables is influenced by multiple factors, depending on the type of plant (pH, water activity and nutrients), origin, processing steps (washing, sanitizing, peeling, cutting, packaging storage temperature), sanitary conditions of the handler, equipment and utensils, as well as the environment. In the present study, pH, titratable acidity and soluble solids were evaluated (Table 2). Among these parameters, the pH showed the greatest influence on microbial growth, with values ranging from 3.84 in pineapple rounds to 6.66 in diced melon.

The papaya and carrot samples with high coliform counts at 35 °C and 45 °C presented a mean pH of 5.28 and 6.40 (Table 2), respectively. Fruits have low acidity content due to their naturally occurring organic acids (ICMSF, 1980), while greens and vegetables usually present a pH ranging from 5 to 7 (ICMSF, 1985).

According to Franco and Landgraf (2005), the pH of carrot ranges from 4.9 to 6.0. The pH tending to neutrality detected in carrots and papaya are within the minimum and optimum pH values, in which some coliforms, as *Escherichia coli* (4.3 to 8.0) and *Enterobacteriaceae*, such as *Erwinia carotovora* (4.9 to 7.1), *Proteus vulgaris* (4.4 to 7.0), *Serratia marcescens* (4.6 to 7.0) and *Salmonella* spp. (4.5 to 7.5), *S. typhi* (4.0 to 7.2) and *S. choleraesuis* (5.0 to 7.6) can grow (Franco & Landgraf, 2005). Among these, *E. coli* and the genus *Salmonella* can represent consumer health risks.

The results displayed in Table 3 show that 55.5% (20/36) of the vegetable samples contained light dirt and foreign materials. However, 13.9% (5/36) showed filth such as insect or animal droppings (rat hair) and wood fragments (Table 3 and Figure 1), respectively, in sliced papaya and grated carrot. These samples were characterized as unfit for human consumption, since, according to the RDC No. 175 of July 8, 2003, insects, in any stage of development, living or dead, whole or in part; insect or animal excrement; rigid, sharp and/or cutting objects are substances harmful to human health (BRASIL, 2003).

Table 3. Results of the microscopic analyses on minimally processed products sold in two supermarkets at Cuiabá, Brazil

Foreign matter	Incidence	Product
Fiber fragments	2	Pineapple
	3	Grated carrot
	3	Sliced papaya
	2	Melon
	3	Sliced melon
Insect/animal excrements	1	Sliced papaya
Wood fragments	1	Grated carrot



Figure 1. Rat hair found in papaya slices

In the present study, samples considered unsuitable due to the presence of light dirt also showed unsatisfactory sanitary conditions, due to the presence of coliforms at 45 °C. The situation described herein confirms the

statement by Pineli and Araújo (2006) that the contamination of minimally processed vegetables by human pathogens may occur directly or indirectly by different factors during production, acquisition and processing, including by animal or insect excrements.

4. Conclusions

The minimally processed vegetables sold in supermarkets in the city of Cuiabá, sampled from June to August 2013, were in different maturation stages, with 71.2% (26/36) of the samples presenting good quality and being fit for consumption, while 27.8 % (10/36), composed of a pool of sliced papaya and grated carrots, were in unsatisfactory sanitary conditions and unfit for human consumption according to the current Brazilian legislation microbiological and microscopic standards.

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