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Original Paper

Cinnamic acid use in the conservation of minimally processed pineapple

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Abstract

The present work aimed to evaluate the influence of cinnamic acid on the physico-chemical and microbiological characteristics of minimally processed pineapple. After the minimum processing, the fruits were immersed in the following treatments: water (control) and doses of 50, 100, 150 and 200 mg L^{-1} of cinnamic acid. At the end they were packed in expanded polystyrene cartons covered with polyethylene film and stored at 5 °C and 85-90% relative humidity for a period of 10 days. The microbiological analyzes of total counts of mesophiles, psychotrophs, molds and yeasts and total coliforms were performed at 0, 3, 6 and 10 days of storage and physical chemical analyzes of fresh mass loss, pH, soluble solids content (° Brix) and titratable acidity were performed at 0, 2, 4, 6, 8 and 10 days storage. The data were submitted to analysis of variance and the averages were compared by the Tukey test at 5% of probability. The application of cinnamic acid doses showed a positive effect on the maintenance of quality and reduction of microbial contamination of minimally processed pineapples, with 50, 150 and 200 mg L^{-1} doses of cinnamic acid being the most suitable for this experiment.

Key-words: *Ananas comosus* L. Merril., Natural Antimicrobial, Minimally Processed Fruits, Useful Life

Introduction

Pineapple (*Ananas comosus* (L.) Merril) is a fruit consumed worldwide, both *in nature* and industrialized, due to its excellent sensorial and nutritional characteristics (Antoniali and Sanches, 2008).

The concept of "minimally processed product" is used to define fresh, cleaned and pre-prepared commercialized fresh fruits and vegetables. A fresh food, whose cell tissues are alive, is defined as minimally processed, since these characteristics determine the freshness of the foods (Bastos, 2006).

Minimally processed vegetables are those that undergo physical changes, such as removal of bark, cuts, among other processes, but kept in the fresh state and metabolically active (Moretti, 2007).

When cutting is done, physiological changes occur in fruits caused by mechanical injuries, which increase the perishability and reduce the useful life. Therefore, minimally processed fruits need care in the handling and during their preparation and processing (Bastos, 2006). The need for new treatments that promote an increase of the useful life and that guarantees the microbiological and physical-chemical characteristics of this product causes that studies take place on this subject. An alternative to this is the use of natural antimicrobials, which significantly reduce the microbial population of the product, making it safe for consumption and with its guaranteed qualities.

Starting from this problem, and having knowledge of the great demand for the fruit due to its nutritional qualities, the purpose of this work was the elaboration of the minimally processed fruit, submitted to the treatment in different concentrations of cinnamic acid, with the purpose of increasing its

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useful life maintaining the characteristics, both physical-chemical and nutritional.

Material and Methods

The fruits of pineapple cv. Smooth Cayenne *in nature* were purchased and transported to the Plant Laboratory of the Federal Technology University of *Paraná* (*Medianeira Campus*). The fruits were then selected and washed with water and neutral detergent, immersed for 1 minute in 5 °C water with 200 mg L^{-1} of sodium hypochlorite (pH 6.5), in order to remove microorganisms and residues that may have adhered to the surface. The fruits were then peeled, with the removal of the central cylinder and manually chopped, in trapezoidal sections of 3.0 x 2.6 cm. After cutting, the minimally processed fruits were immersed in the different natural antimicrobial solutions for 1 minute at 5 °C: control (water); cinnamic acid (50 mg L^{-1}); cinnamic acid (100 mg L^{-1}); cinnamic acid (150 mg L^{-1}); cinnamic acid (200 mg L^{-1}).

The fruits were packed in expanded polystyrene packages with dimensions of 3 cm high x 10 cm long x 10 cm wide and covered with 15 mm thick polyethylene film, with approximately 80 g each. They were then stored under refrigeration at 5 °C ± 1 °C for a period of 10 days, and the physical-chemical analyzes were performed every two days, and the microbiological tests were carried out at 0, 3, 6 and 10 days of storage to follow up the performance of cinnamic acid in fruits.

The physico-chemical and microbiological analyzes carried out in this experiment were: loss of fresh mass, pH, soluble solids content, titratable acidity, total counting of mesophilic and psychotrophic bacteria, determination of total coliforms and counts of molds and yeasts. The physical-chemical analyzes were performed in triplicate and the microbiological analyzes were performed in duplicate.

Fresh weight loss was determined by weighing the trays containing the chopped fruits in a semi-analytical balance. The results were expressed as a percentage, considering the difference between the initial weight and the weight obtained at each sampling of time interval.

The pH was measured in the crushed pulp of the fruits using pH-meter (Brasil, 2005).

The soluble solids content was determined in the pulp by refractometer refractometry with measurements of 0 - 32 ° Brix at 25 °C, according to the procedures described by Tressler and Joslyn (1961). The results were expressed in degrees of Brix.

The titratable acidity of the samples was determined by titration according to the methodology proposed by the Adolfo Lutz Institute Analytical Standards (Brasil, 2005). Five grams of the sample were homogenized in 100 ml of water, transferred to a 125 mL Erlenmeyer flask, 2 to 4 drops of the phenolphthalein solution were added and the solution titrated with 0.01 M sodium hydroxide solution. The results were expressed as grams of citric acid per 100 ml of pulp.

For the counting of mesophilic and psychotrophic bacteria, 2 replicates were withdrawn for each treatment per day of analysis, where 25 g of sample were placed in sterile stomacher bags, followed by addition of 225 mL of sterile peptone saline solution 0.1% and homogenized for 1 minute in an electric Stomacher sample homogenizer. Serial dilutions with 0.1% peptone saline were prepared according to the growth of the microbial population (this procedure was also used for the counting of molds, yeasts and total coliforms). The PCA medium (Scharlau) was used to count mesophilic and psychotrophic microorganisms. The incubation conditions were: 30 ± 1 ºC for 72 hours (mesophiles) and 2 to 8 ºC for 7 days (psychotrophic) (ISO, 2013) (APHA, 2001). The counts of mesophilic and psychotrophic bacteria were expressed in log CFU g^{-1}.

For the analysis of mold and yeast counts, DRBC Agar medium (Rosa Bengala Chloranfenicol Base) 0.1 g L^{-1} was used, incubation was performed at 25 ± 1 ºC for 5 days in accordance with ISO (2008). The result of the counts of molds and yeasts was expressed in log UFC g^{-1}.

The determination of total coliforms, Petrifilm™ 3M™ plates were used for the quantification of total coliforms and incubated at 37 ºC for 24 hours (AOAC, 2012), the enumeration being expressed in UFC g^{-1} log.

The data obtained in the physical chemical analyzes were submitted to analysis of variance (ANOVA) and the means compared by the Tukey test at 5% of probability by means of the Infostat program.

Results and discussion

Table 1 presents the results for the loss of fresh mass obtained during the ten days of storage of minimally processed pineapple. In general, minimally-processed pineapples of the control presented greater loss of fresh mass in relation to the minimally processed fruits of the other treatments.

Table 1. Fresh mass loss (%) in minimally processed Smooth cayenne pineapples treated with cinnamic acid, stored at 5 ± 1 °C for 10 days.

Fresh weight loss (%)					
Treatments	**Day 2**	**Day 4**	**Day 6**	**Day 8**	**Day 10**
Control	0.77^{a}	1.51^{a}	2.71^{a}	3.71^{a}	4.98^{a}
50 mg L^{-1}	0.75^{a}	1.30^{ab}	2.30^{bc}	3.10^{a}	3.97^{a}
100 mg L^{-1}	0.62^{ab}	1.22^{b}	2.38^{b}	3.35^{a}	4.37^{a}
150 mg L^{-1}	0.65^{ab}	1.10^{bc}	1.72^{c}	2.32^{b}	2.99^{a}
200 mg L^{-1}	0.53^{b}	0.91^{c}	1.55^{c}	2.15^{b}	2.79^{a}
CV(%)	19.89	17.34	14.16	17.74	18.86

Means followed by the same letter in the column do not differ significantly from each other by the Tukey test at the 5% probability level.

In general, there was a gradual increase of the values over time and the maximum loss was reached on the tenth day. Oliveira Júnior et al. (2007) working with minimally processed pineapple ‘pearl’ and stored for 6 days under refrigeration, observed the highest rate of fresh mass loss at day 4.

In this experiment, on the 2nd day, the minimally processed fruits of the control treatments and 50 mg L^{-1} were statistically superior to the minimally processed fruits treated with 200 mg L^{-1} of cinnamic acid. While on the 4th day, minimally processed control pineapples differed statistically from the minimally processed fruits of the 100 mg L^{-1}, 150 mg L^{-1} and 200 mg L^{-1} treatments. For the 6th day, a statistical difference was observed for the minimally processed fruits of the control, which differed from the other treatments. While for the 8th day, minimally processed pineapples of control, 50 mg L^{-1} and 100 mg L^{-1} of cinnamic acid differed statistically from the minimally processed fruits of treatments 150 mg L^{-1} and 200 mg L^{-1}. On the 10th day, there was no significant difference between the samples at the 5% probability level.

The results concerning the pH of minimally processed pineapples as a function of days are shown in Table 2. It was verified that during the days of analysis there was a decrease in pH values of minimally processed fruits, with day 0 showing a pH value statistically superior to the other days.

Table 2. PH values, soluble solids and titratable acidity (g citric acid 100 mL^{-1} pulp) in minimally processed Smooth cayenne pineapples treated with cinnamic acid stored at 5 ± 1 °C for 10 days.

Storage days	**pH**	**Soluble solids (º Brix)**	**Titratable acidity**
0	3.8^{a}	14.20^{c}	0.47^{d}
2	3.73^{b}	14.47^{bc}	0.55^{cd}
4	3.73^{b}	14.85^{ab}	0.55^{cd}
6	3.70^{b}	14.81^{ab}	0.60^{c}
8	3.71^{b}	15.20^{a}	0.64^{b}
10	3.60^{c}	14.61^{abc}	0.66^{a}
CV(%)	1.49	3.87	6.45

Means followed by the same letter in the column do not differ significantly from each other by the Tukey test at the 5% probability level.

In this work, pH values of minimally processed pineapples varied from 3.60 to 3.80, values similar to those found by Sarzi, Durigan and Possi Junior (2002), who worked with minimally processed ‘pearl’ pineapple stored for 12 days, found values ranging from 3.70 to 3.90.

The drop in pH values suggests an advance in the ripening of the fruits, since the organic acids are being produced. This is due to degradation reactions. According to Silva et al. (2005) when working with minimally processed 'pearl' pineapple, reported that this trend could also be influenced by the degree of fruit maturation and microbiological attack.

Table 3 presents the results concerning the pH of the minimally processed fruits as a function of the different doses of cinnamic acid used.

Table 3. PH values, soluble solids and titratable acidity (g citric acid 100 mL^{-1} pulp) in minimally processed Smooth cayenne pineapples, stored at 5 ± 1 °C for 10 days as a function of cinnamic acid treatments.

Treatments	**pH**	**Soluble solids (º Brix)**	**Titratable acidity**
Control	3.73^{a}	14.62^{bc}	0.52^{c}
50 mg L^{-1}	3.75^{a}	15.24^{a}	0.57^{b}
100 mg L^{-1}	3.72^{ab}	13.53^{c}	0.59^{b}
150 mg L^{-1}	3.68^{b}	15.10^{b}	0.58^{b}
200 mg L^{-1}	3.70^{ab}	14.96^{b}	0.66^{a}
CV(%)	1.49	3.87	6.45

Means followed by the same letter in the column do not differ significantly from each other by the Tukey test at the 5% probability level.

Statistical differences were observed between the treatments, with the fruits being minimally processed from the control and those treated with 50 mg L^{-1} higher than the minimally processed fruits of the 150 mg L^{-1} treatment. Moreira (2009), working with minimally processed melon, observed that the fruits of treatment with cinnamic acid at 148.16 mg L^{-1} presented higher pH values than the fruits of the other treatments during the ten days of storage.

Regarding the soluble solids contents obtained in the fruits minimally processed and stored for 10 days (Table 2), it can be observed that the minimally processed pineapples stored on the 8th were statistically superior to those stored on the 0 and 2nd days. In the course of days of storage there was increase in the content of soluble solids until the 8th day, occurring then decrease.

According to Chitarra and Chitarra (2005), the soluble solids content tends to increase with maturation due to the increase of the simple sugars content. Soluble solids generally increase with fruit ripening due to biosynthesis, degradation of polysaccharides or loss of fruit water.

In this experiment, the soluble solids values ranged from 14.20 to 15.20 ° Brix, results lower than those found by Silva et al. (2003), when working with minimally-processed 'pearl' pineapple (13.5 ° Brix) and Bonnas et al. (2003) when working with minimally processed Smooth Cayenne pineapple (9.15 º Brix). The high values presented in the tables can be explained by the high degree of maturation of the pineapples used.

For the values of soluble solids obtained in the minimally processed fruits in relation to treatments with cinnamic acid (Table 3), it can be observed that minimally processed fruits and treated with 50 mg L^{-1} of cinnamic acid were statistically superior to minimally processed pineapples of the other treatments. Moreira (2009) working with minimally processed melons treated with cinnamic acid did not observe a statistical difference between treatments.

According to the values of titratable acidity obtained in minimally processed pineapples, in relation to the days of storage (Table 2), it can be observed that with the storage days there was an increase in the values, and the 10th day was statistically superior to the other days. Torres et al. (2002) observed the same acidity increase behavior when storing minimally processed pineapples under a modified atmosphere under refrigeration at 5 °C for eight days.

In this work were observed values of titratable acidity ranging from 0.47 to 0.66 g. citric 100 mL^{-1} pulp. Working with minimally processed pineapples, Figueirêdo et al. (2003) found a variation of 0.44 to 0.39 g citric ac. 100 mL^{-1} pulp.

According to Chitarra and Chitarra (2005), fruits rapidly lose acidity with ripening, however, in some cases, small increases in values may occur with the advancement of maturation.

For the values of titratable acidity obtained considering the different doses of cinnamic acid used (Table 3), the minimally processed fruits treated with 200 mg L^{-1} of cinnamic acid were statistically superior to those treated with the other doses.

Table 4 shows the count of mesophilic bacteria found in minimally processed pineapples treated with doses of cinnamic acid. With the storage time, a gradual increase of mesophilic bacteria counts was observed, as was observed by Moreira (2009), working with application of cinnamic acid in melon Minimally processed toad skin.

Table 4. Total counting of mesophilic bacteria (log UFC g^{-1}) obtained in minimally processed and cinnamic acid treated Smooth cayenne pineapples stored at 5 ± 1 °C for 10 days.

Treatments	Storage days			
	Day 0	**Day 3**	**Day 6**	**Day 10**
Control	4.99	5.17	5.86	8.52
50 mg L^{-1}		5.15	5.76	7.97
100 mg L^{-1}		5.89	5.13	8.45
150 mg L^{-1}		4.89	6.01	8.35
200 mg L^{-1}		5.86	6.12	8.47

It was verified that at the end of the ten days of storage, the most efficient treatment for the minimally processed fruits was the application of 50 mg L^{-1} dose, presenting a population of 7.97 log CFU g^{-1}. The minimally processed fruits of the control were the ones that presented greater development of mesophilic microorganisms (8.52 log CFU g^{-1}) at the end of the storage period.

The gradual increase in mesophilic bacteria counts was also observed by Moreira (2009), when working with minimally processed melons treated with cinnamic acid. However, melons treated with cinnamic acid alone, in two different concentrations, presented lower counts of mesophilic microorganisms.

For psychotrophic microorganisms (Table 5), there was a gradual increase in counting over the days of storage. The fruits minimally processed and treated with 150 mg L^{-1} presented the lowest population (4.17 log CFU g^{-1}) at the end of the storage period. According to Moreira (2009), when working with minimally processed melons treated with cinnamic acid, the psychotrophic count was 3.04 ± 0.13 log CFU g^{-1}, at 3.28 ± 0.1 log CFU g^{-1}.

Table 5. Total count of psychotrophic bacteria (log UFC g^{-1}) in minimally processed Smooth cayenne pineapples treated with cinnamic acid stored at 5 ± 1 °C for 10 days.

Treatments	Storage days			
	Day 0	Day 3	Day 6	Day 10
Control	<1	3.84	4.30	4.50
50 mg L^{-1}		4.20	4.58	5.03
100 mg L^{-1}		4.05	4.52	4.82
150 mg L^{-1}		3.91	4.06	4.17
200 mg L^{-1}		3.93	4.26	4.36

High populations of psychotrophic bacteria reduce the shelf-life of minimally-processed vegetables, which may occur due to raw material with high contamination, lack of cleaning and disinfection of surfaces, poor hygiene during processing and inadequate storage conditions (Bruno et al., 2005).

Table 6 shows the values obtained in the determination of total coliforms. It was verified that in the 10th day of storage, the minimally processed pineapples of the control group presented 1 log CFU g^{-1} and those treated with 50 mg L^{-1} presented 2.44 log UFC g^{-1}.

Table 6. Determination of total coliforms (log UFC g^{-1}) in minimally processed and cinnamic acid-treated Smooth cayenne pineapples stored at 5 ± 1 °C for 10 days.

Treatments	Storage days			
	Day 0	Day 3	Day 6	Day 10
Control	<1	<1	<1	<1
50 mg L^{-1}		<1	<1	2.44
100 mg L^{-1}		<1	<1	<1
150 mg L^{-1}		<1	<1	<1
200 mg L^{-1}		<1	<1	<1

Although there are no standards for total coliforms, it is generally recommended that foods containing microbial counts in the range of 5 to 6 log CFU g^{-1} are unfit for human consumption (ANVISA, 2002), thus minimally pineapple is within the limits allowed for consumption.

According to Gava and Silva (2008), the pineapple is a very acidic fruit and, therefore, there is great restriction to the growth of microorganisms, being more common the presence of acetic bacteria, filamentous fungi and yeasts. The fungi presented a constant proliferation in the minimally processed fruits of all the treatments, being observed on the last day of experiment, a higher count in the minimally processed fruits treated with 50 mg L^{-1} of cinnamic acid (Table 7). Moreira (2009), working with minimally processed melon and treated with two different cinnamic acid formulations, after 10 days storage, observed a count of 2.1 ± 0.1 log CFU g^{-1} at 2.5 ± 0.2 log UFC g^{-1}.

Table 7. Mold and yeast counts (log UFC g^{-1}) in minimally processed and cinnamic acid treated Smooth cayenne pineapples stored at 5 ± 1 ° C for 10 days.

Treatments	Storage days			
	Day 0	Day 3	Day 6	Day 10
Control	4.25	5.22	5.11	8.60
50 mg L^{-1}		5.23	5.61	8.73
100 mg L^{-1}		5.33	5.65	8.53
150 mg L^{-1}		5.06	5.66	8.42
200 mg L^{-1}		5.26	5.34	8.40

Conclusion

The application of cinnamic acid doses showed a positive effect on the maintenance of quality and reduction of microbial contamination of minimally processed pineapples, with 50, 150 and 200 mg L^{-1} doses of cinnamic acid being the most suitable for this experiment.

Conflict of interest: All authors declare no conflict of interest.

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