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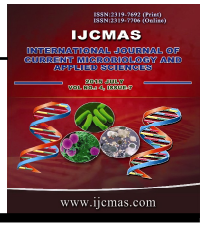

Original Research Article

Potential of chitosan as an Acerola (*Malphighia glabra L.*) juice natural preservative

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Keywords

Biopolymer, tropical fruit juice, shelf life, physico-chemical properties, Sensory Analysis

A B S T R A C T

The objective of the present study was evaluate the potential of the biopolymer chitosan in extending the shelf-life of acerola juice, addressing their physico-chemical, microbiological and sensory aspects. The commercial chitosan of crustacean was dissolved in 1% (20mg/mL) of acetic acid. Acerola juice was tested with chitosan concentrations of 2.5 and 5 mg/ml with storage of 0, 7 and 14 days to evaluate the physic-chemical and microbiological parameters. Sensory parameters were evaluated above 0, 5 and 7 days with 60 untrained sensory panelists with age between 18 and 25 years. The chitosan extended the shelf-life of acerola juice, by the control of acidity, browning and microbial growth during the storage times. In the days 5 and 7 of storage, the chitosan increased the sensory acceptance due to its action on the stability of juice quality. The polymer provided extension of shelf-life of acerola juice, having the potential for use as preservative and stabilizer of natural origin.

Introduction

The interest in natural foods has grown and contributed to the increased consumption of fruit juices. The exotic tropical fruits are ideal for the growing juice market due to its diversity of aromas and flavors, in addition to its nutritional value. In this group, there is the *acerola, Malphighia glabra*, which is a fruit native to the West Indies, but also cultivated in South America, Florida and Texas. The acerola products stand out for its

high ascorbic acid content and sensory quality. The most traded are the frozen pulp and the pasteurized juice (Gomez et al, 1999). Acerola juice, like other fruit juices, has limited shelf life, up to 10 days after processing. The microbial deterioration production of unpleasant odors and degradation of ascorbic acid are among the main causes for loss of quality shelf-life (Raybaudi-Massilia et al, 2009).

In order to prolong the juices life, chemical additives are used; however some countries limit their use because of possible health damage. In this sense, the use of sulfites is restricted in the United States since 1990 because of dangerous side effects for people with asthma. These facts, combined with growing consumer demand for natural foods, encourages the study of the area of food science to gain new perspectives preservatives that meet market demands without neglecting the quality of consumers (Raybaudi-Massilia et al, 2009).

In this context chitosan which is a natural heteropolymer, comprising units β-1, 4 D-glucosamine and N-acetylglucosamine has gain much impact. Its unique biological features include non-toxicity, biodegradability and uphold chitosan to be applied widely in the pharmaceutical, food and environment. Chitosan is obtained from the deacetylation of chitin, a natural oligosaccharide which is the major cell wall component for fungi and exoskeletons of insects and arthropods (No et al, 2007). Is the most abundant organic material after cellulose. The antimicrobial activity of chitosan have been demonstrated against bacteria, yeasts and molds, with a broad spectrum of activity against Gram positive and Gram negative bacteria with low toxicity in mammalian cells (Martín-Diana et al, 2009).

These properties make chitosan suitable for use as an additive in the processing of fruit juices. Can be used in the clarification process, with the reduction of turbidity (Soto-Peralta, Miller and Knorr, 1989, Chen and Li, 1996; Chatterjee et al, 2004; Rungsardthong et al, 2006, Wang, Guan and Yang, 2007), reduction of acidity (Imeri and Knorr 1988), reduction of browning (Sapers, 1992), as antimicrobial agent (Roller and Covill, 1999 ; Rhoades and Roller, 2000; Kisko, Sharp and Roller, 2005; Malinowska-Panczyk et al, 2009), antioxidant agent (Chien et al, 2007) and as a shelf life extender (Martín-Diana et al, 2009).

The study aimed to verify the effectiveness of commercial chitosan in the extension of shelf life of acerola juice, addressing their physic-chemical, microbiological and sensory aspects.

Materials and methods

Materials

Commercial chitosan of crustacean (Sigma ®) with a degree of deacetylation (DD) of 85% was dissolved in 1 % acetic acid (20mg/mL) with pH adjusted to 5.5 (Shigemasa and Minami, 1996).

To prepare acerola juice, fruits were washed, disintegrated and pulped in a blender. Acerola juice was diluted at 60% (w/v), after filtration was pasteurized at 62 °C for 30 min, then added chitosan gel and stored at 4 °C in plastic bottles and refrigerated until the moment of the analysis.

Physico-chemical parameters

The following analyzes were evaluated: total soluble solids (TSS) determined by reading on a refractometer (aus jena Modelo II) and expressed in °Brix; titratable acidity, with results expressed in acetic acid, determined by titration with 0.1 N NaOH; the pH was

analyzed using a digital potentiometer (pH Meter Tec-2 , Tecnal) and ascorbic acid was measured by titration with 2,6 dichlorophenolindophenol (AOAC, 2002).

The turbidity was measured by direct reading on the spectrophotometer (Bioespectro – spectrophotometer SP-22) at 600 nm, distilled water used as a blank and the results expressed in absorbance units (AU) mL^{-1} of acerola juice.

The browning degree was measure by absorbance at 320nm and expressed in absorbance units (AU) mL^{-1} acerola juice. To evaluate the viscosity was used a Brooksfield viscometer (LVT, number 86910), spindle nº 1. The color parameters L*a*b was analysed in a colorimeter (Minolta) (Martín-Diana et al, 2009).

Microbiological parameters

The enumeration of lactic acid bacteria, mesophilic aerobic bacteria, molds and yeasts, total and fecal coliforms, and detection of *Salmonella* were performed according Vanderzant and Spplittstoesser (1992). The results were expressed as log colony forming units per milliliter (log CFU/mL), except for total and fecal coliforms that were expressed in MPN/mL and *Salmonella* analysis expressed by presence or absence.

Sensory analysis

The project was approved by Ethics Commitee in Research of the UFPE/CCS under protocol number 050/2010. The sensory evaluation of juices was performed during the shelf-life, immediately after preparation, with 5 and 7 days of storage. The selection of this period was based on physico-chemical and microbiological parameters. To this experiment were only included the juices with parameters recommended by RDC nº 12 for Microbiological Standards (Brazil, 2001) and by the Standards of Identity and Quality of acerola juice (Brazil, 2000).

The appearance, viscosity, overall preference, taste, color and odor were evaluated by the test of acceptability using a nine-point hedonic scale (1 = dislike extremely, 2 = dislike moderately, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like / or dislike, 6 = like slightly, 7 = like moderately; 8 = liked very much, 9 = like extremely). At the same time was also assessed the intention to purchase, using a five-point hedonic scale (1 = never buy, 2 = possibly not buy, 3 = maybe buy / might not buy, 4 = possibly buy, 5 = buy). Sixty untrained sensory panelists with age between 18 and 25 years (students and staff of UPFE) were selected based on habits and interest in consuming acerola juice. The tests were conducted in the Laboratory of Sensory Analysis of UPFE, in individual booths using white light, far from noise and odors in pre-established schedules.

Experimental design and statistical analysis

In shelf life tests, the storage time at 4 ºC was during to 14 days after processing. The assays were performed, in triplicate, in time intervals of 0 (immediately after preparation), 7 and 14 days after the preparation of the juice. Four experiments were performed: in the absence of chitosan (T1) and in the absence of chitosan and presence of acetic acid 1% (T2) and concentrations of chitosan 2.5 mg/mL (T3) and 5 mg/mL(T4).

Tests of descriptive statistic (mean and standard deviation) and inferential statistic (Tukey test) were used to determine significant statistically differences ($p<0,05$) between the treatments applied. Statistical

analyses were carried out using the software Sigma Stat.2.03.

Results and Discussion

Physico-chemical parameters

The treatment with chitosan 2.5 and 5 mg/mL (T3 and T4) decreased significantly the Total Soluble Solids ºBrix TSS($p < 0.05$), compared to control without chitosan (T1) and with acetic acid (T2), as shown in Table 1. The reduction of TSS with the addition of chitosan can be explained by the ability of this positively charged polymer coagulate the suspend solids through binding to the negatively charged sugars (Sapers, 1992; Barry-Ryan et al, 2009).

The sample T4 in 14 days of storage (Table 1) showed a significant reduction ($p<0.05$) in acidity compared to controls T1 and T2. Imeri and Knorr, (1988) observed similar results in apple and carrot juice. The pH of T4 increased significantly with the concentration of chitosan compared to T1. The increase of pH and stability of juice during shelf life can be due the ability of chitosan to reduce acidity (Imeri and Knorr, 1988), which confirms the reduction of acidity evidenced in this study. This effect was explained by the ability of chitosan, positively charged at low pH, binding to acids, negatively charged (Einbu and Varum, 2003).

The ascorbic acid (AA) ranged from 302.6 mg/100g to1611.54 mg/100g of vitamin C (Table 2). There was a significantly reduction in the AA from acerola juice along the shelf life on all treatments (T1, T2, T3 and T4). The concentration of chitosan 5 mg/mL (T4) had significant effect ($p<0.05$) in reduction of AA, compared to control (T1), according to Freitas et al (2006) that observed a reduction of 42.12% of AA in the end of storage. This is explaining by the capability of chitosan to bind the acid (Imeri and Knorr, 1988), beyond to the ability to accelerate the process of oxidation of AA (Zoldeners, Kiseleva and Kaiminch, 2005).

Turbidity

The addition of chitosan 5 mg/mL (T4) to juice, significantly reduced ($p<0.05$) the turbidity (Table 2). This may occur due to the presence of positive charges in the molecule of chitosan (amines). Chitosan is able to control turbidity if binding to small sugars, coagulatin them (Grassin and Fauquembergue, 1996). The use of chitosan as a clarifying agent has been demonstrated by Wang, Guan and Yang (2007). This polymer shown antimicrobial activity in juices (Roller and Covill, 1999; Rhoades and Roller, 2000; Kisko, Sharp and Roller, 2005; Malinowska-Panczyk et al, 2009), contributing for turbidity reduction. Renaud et al (2009) suggested that as chitosan is a cationic flocculant; it can be combined with the negatively charged pectin, soluble starch, protein and microparticles through positive and negative charge attraction to form floc precipitation.

Browning potential

The chitosan concentration of 2.5 mg/mL (T3) and 5 mg/mL (T4) significantly reduced the browning potential of acerola juice (Table 2). The control of browning could be associated with the capacity to coagulate solids to which browning-related enzymes are bound. The antioxidant capacity of chitosan, similar to the capacity associated with phenolic compounds (Barry-Ryan et al, 2009). The control of browning may be associated with the capacity of chitosan to coagulate solids, in which the enzymes that cause the browning are linked. Chitosan also presents antioxidant activity,

which may explain the decrease of browning potential, by inhibition of oxidative process (Park, Je, and Kim, 2004; Chien et al, 2007).

Viscosity

The viscosity values of acerola juice ranged from 1.73 to 11.07 mPas (Table 2), Adding chitosan in the form of gel increased viscosity of the juice. On the other hand, the viscosity decreased significantly during storage. This is mainly due to degradation of pectin, resulting in a reduced capacity for water retention and release of free water to the system (Abdullah et al, 2007).

Color

The values found for the parameter a * ranging from 2.48 to 19.46, the b * 25.94 to 40.63 and the L * 48.09 to 72.28 (Table 3). The chromatic characteristics of acerola juice showed positive values of a * and b *, i.e., the colors red and yellow, respectively, are related to the anthocyanins and carotenoids. However, it appears that the intensity of the yellow component b * is greater than the red component a *, revealing that the juice had an orange coloration.

Chitosan had a significant effect on the a* with values lower in T4 compared to T1. In chitosan b * T4 reduced compared to the T1 time 0. Considering the L * a significant increase in T3 in time 14 and in T4 in time 0 compared to control T1 was found. The effect of chitosan on the a* occur primarily by the ability of chitosan to bind anthocyanins.

This was evidenced demonstrated by Horst et al (2009) who observed that the natural dye anthocyanin interacts physically with the polymeric matrix. Knorr (1983) in turn suggests that the chitosan is capable of binding to stains, which is consistent with the results shown in this study. The initial reduction in b * with the addition of chitosan is mainly due to its ability to sequester carotenoids (Martín-Diana et al, 2009). The increase in luminosity L* occurred due to the fining properties of chitosan.

There was also a significant reduction of a* over 14 days. The reduction of this parameter is associated with increased enzymatic and non-enzymatic browning, which is consistent with the increased potential for browning observed in the present study. The reduction of L * is during storage according to Martín-Diana et al (2009) that assigned this effect to the settling unstable particles of juice and the loss carotenoid pigments.

Abd and Niamah (2012) related a significant relationship between chitosan concentration and inhibition of browning. The authors suggested the control of browning could be associated with the capacity to coagulate solids to which browning related enzymes are bound.

The antioxidant capacity of chitosan, similar to the capacity associated with phenolic compounds, could also explain this browning reduction by inhibiting the oxidative process.

Microbiological parameters

The total counts of lactic acid bacteria, molds and yeast were up to 6 log CFU/mL (Figure 1 and 2) and of the aerobic mesophilic were up to 5 log CFU/mL (Figure 3). These results were lower than those found by Rhoads and Roller (2000) who found a maximum count of yeasts, molds, lactic acid bacteria and aerobic mesophilic bacteria of 7 log CFU/mL.

Table.1 The total soluble solids, acidity and pH of acerola juice without the addition of chitosan (T1), supplemented with 1% acetic acid (T2), chitosan added to 2.5 mg/mL (T3) and chitosan added to 5.0 mg/mL (T4)

Assay	Time (days)	Treatment							
		T1		T2		T3		T4	
		Average	SD	Average	SD	Average	SD	Average	SD
Total Soluble Solids (ºBrix)	0	$8.00^{a(A)}$	±0.00	$7.67^{ab(A)}$	±0.58	$7.00^{b(A)}$	±0.00	$6.00^{c(A)}$	±0.00
	7	$7.33^{a(AB)}$	±0.58	$7.00^{a(A)}$	±0.00	$5.67^{b(B)}$	±0.58	$5.00^{b(B)}$	±0.00
	14	$6.33^{a(B)}$	±0.58	$6.00^{a(B)}$	±0.00	$5.33^{ac(B)}$	±0.58	$4.33^{bc(B)}$	±0.58
Titable Acidity (%)	0	$1.14^{a(C)}$	±0.16	$1.16^{a(B)}$	±0.15	$1.11^{a(A)}$	±0.12	$0.87^{a(A)}$	±0.00
	7	$1.57^{a(B)}$	±0.03	$1.52^{a(A)}$	±0.21	$1.35^{a(A)}$	±0.54	$0.88^{a(A)}$	±0.02
	14	$1.96^{a(A)}$	±0.05	$1.97^{a(A)}$	±0.22	$1.40^{ac(A)}$	±0.53	$0.92^{bc(A)}$	±0.10
pH	0	$3.18^{b(A)}$	±0.08	$3.17^{b(A)}$	±0.04	$3.40^{ab(A)}$	±0.15	$3.58^{a(B)}$	±0.18
	7	$2.80^{b(B)}$	±0.56	$2.73^{b(A)}$	±0.41	$3.63^{ab(A)}$	±0.20	$3.85^{a(AB)}$	±0.12
	14	$2.50^{b(C)}$	±0.47	$2.07^{b(B)}$	±0.08	$3.72^{a(A)}$	±0.13	$4.00^{a(A)}$	±0.01

Values in the same line for each medium, followed by different lower case letters and values in the same column, for each medium, followed by different capital letters differ significantly ($p < 0.05$) according to the Tukey test.

Table.2 Ascorbic acid, turbidity, viscosity and browning potential of the acerola juice with no added chitosan (T1), supplemented with 1% acetic acid (T2), chitosan added to 2.5 mg/mL (T3) and chitosan added to 5.0 mg/mL (T4)

Assay	Time (days)	Treatment							
		T1		T2		T3		T4	
		Average	SD	Average	SD	Average	SD	Average	SD
Ascorbic acid mg/100g	0	1585.89$^{a(A)}$	±104.5	1611.54$^{a(A)}$	±167.64	1539.52$^{a(A)}$	±52.62	874.84$^{b(A)}$	±110.82
	7	947.05$^{a(B)}$	±71.62	915.25$^{a(B)}$	±76.35	640.92$^{b(B)}$	±50.75	487.59$^{b(B)}$	±162.93
	14	648.80$^{a(C)}$	±64.92	646.80 $^{a(C)}$	±47.43	488.57$^{a(C)}$	±97.56	302.60$^{b(B)}$	±61.51
Turbidity (UA)	0	0.085$^{a(B)}$	±0.015	0.065$^{a(A)}$	±0.012	0.059$^{ab(B)}$	±0.008	0.037$^{b(C)}$	±0.004
	7	0.152$^{a(A)}$	±0.012	0.099$^{b(A)}$	±0.024	0.073$^{b(B)}$	±0.008	0.071$^{b(B)}$	±0.004
	14	0.163$^{a(A)}$	±0.010	0.128$^{a(A)}$	±0.033	0.150$^{a(A)}$	±0.010	0.120$^{a(A)}$	±0.007
Browning potential (AU)	0	0.47$^{a(A)}$	±0.03	0.40$^{a(C)}$	±0.00	0.24$^{c(C)}$	±0.02	0.12$^{d(B)}$	±0.00
	7	0.61$^{a(A)}$	±0.01	0.60$^{a(B)}$	±0.00	0.32$^{b(B)}$	±0.03	0.23$^{c(A)}$	±0.01
	14	0.71$^{ac(A)}$	±0.22	0.85$^{a(A)}$	±0.00	0.46$^{bc(A)}$	±0.01	0.30$^{b(A)}$	±0.07
Viscosity (mPas)	0	2.68$^{c(A)}$	±0.59	3.04$^{c(A)}$	±0.04	8.60$^{b(A)}$	±0.29	11.07$^{a(A)}$	±0.91
	7	2.73$^{c(A)}$	±0.04	2.71$^{c(B)}$	±0.15	5.58$^{b(B)}$	±0.17	7.20$^{a(B)}$	±0.06
	14	1.89$^{c(A)}$	±0.14	1.73$^{c(C)}$	±0.11	3.22$^{b(C)}$	±0.09	4.12$^{a(C)}$	±0.09

Values in the same line for each medium, followed by different lowercase letters and values in the same column, for each medium, followed by different capital letters differ significantly ($p < 0.05$) according to the Tukey test.

Table.3 Color a*, b* and L* of acerola juice without chitosan (T1), with acetic acid 1% (T2), with 2.5 mg/mL of chitosan (T3) and with 5.0 mg/mL of chitosan (T4)

Assay	Time (days)	Treatment							
		T1		T2		T3		T4	
		Average	SD	Average	SD	Average	SD	Average	SD
Ascorbic acid mg/100g	0	$1585.89^{a(A)}$	±104.5	$1611.54^{a(A)}$	±167.64	$1539.52^{a(A)}$	±52.62	$874.84^{b(A)}$	±110.82
	7	$947.05^{a(B)}$	±71.62	$915.25^{a(B)}$	±76.35	$640.92^{b(B)}$	±50.75	$487.59^{b(B)}$	±162.93
	14	$648.80^{a(C)}$	±64.92	$646.80^{a(C)}$	±47.43	$488.57^{a(C)}$	±97.56	$302.60^{b(B)}$	±61.51
Turbidity (UA)	0	$0.085^{a(B)}$	±0.015	$0.065^{a(A)}$	±0.012	$0.059^{ab(B)}$	±0.008	$0.037^{b(C)}$	±0.004
	7	$0.152^{a(A)}$	±0.012	$0.099^{b(A)}$	±0.024	$0.073^{b(B)}$	±0.008	$0.071^{b(B)}$	±0.004
	14	$0.163^{a(A)}$	±0.010	$0.128^{a(A)}$	±0.033	$0.150^{a(A)}$	±0.010	$0.120^{a(A)}$	±0.007
Browning potential (AU)	0	$0.47^{a(A)}$	±0.03	$0.40^{a(C)}$	±0.00	$0.24^{c(C)}$	±0.02	$0.12^{d(B)}$	±0.00
	7	$0.61^{a(A)}$	±0.01	$0.60^{a(B)}$	±0.00	$0.32^{b(B)}$	±0.03	$0.23^{c(A)}$	±0.01
	14	$0.71^{ac(A)}$	±0.22	$0.85^{a(A)}$	±0.00	$0.46^{bc(A)}$	±0.01	$0.30^{b(A)}$	±0.07
Viscosity (mPas)	0	$2.68^{c(A)}$	±0.59	$3.04^{c(A)}$	±0.04	$8.60^{b(A)}$	±0.29	$11.07^{a(A)}$	±0.91
	7	$2.73^{c(A)}$	±0.04	$2.71^{c(B)}$	±0.15	$5.58^{b(B)}$	±0.17	$7.20^{a(B)}$	±0.06
	14	$1.89^{c(A)}$	±0.14	$1.73^{c(C)}$	±0.11	$3.22^{b(C)}$	±0.09	$4.12^{a(C)}$	±0.09

Values in the same line for each medium followed by different lower case letters and values in the same column for each medium followed by different capital letters differ significantly ($p < 0.05$) according to Tukey test.

Table.4 Acceptance testing acerola juice without the addition of chitosan (T1), with chitosan 2.5 mg/mL (T3) and added to the chitosan 5 mg/mL (T4)

Assay	Time (days)	Tratament					
		T1		T3		T4	
		Average	SD	Average	SD	Average	SD
Appearence	0	8.18$^{a(A)}$	±1.06	8.12$^{a(A)}$	±1.18	7.80$^{a(A)}$	±1.44
	5	7.22$^{a(B)}$	±1.22	7.38$^{a(B)}$	±1.03	7.48$^{a(AB)}$	±1.25
	7	5.66$^{b(C)}$	±1.55	6.24$^{b(C)}$	±1.44	6.92$^{a(B)}$	±1.29
Color	0	8.06$^{a(A)}$	±1.17	8.28$^{a(A)}$	±1.23	8.12$^{a(A)}$	±1.37
	5	7.50$^{a(A)}$	±1.17	7.60$^{a(B)}$	±1.28	7.44$^{a(A)}$	±1.16
	7	5.66$^{a(B)}$	±1.76	6.20$^{a(C)}$	±1.65	6.44$^{a(B)}$	±1.85
Aroma	0	8.32$^{a(A)}$	±1.00	7.86$^{ab(A)}$	±1.29	7.44$^{b(A)}$	±2.02
	5	5.86$^{b(B)}$	±1.63	6.90$^{a(B)}$	±1.49	6.74$^{a(A)}$	±1.24
	7	4.56$^{b(C)}$	±1.63	5.88$^{a(C)}$	±1.78	5.56$^{a(B)}$	±1.63
Flavor	0	7.88$^{a(A)}$	±1.51	7.54$^{a(A)}$	±1.42	6.48$^{b(A)}$	±2.23
	5	5.06$^{b(B)}$	±1.65	6.24$^{a(B)}$	±1.67	6.68$^{a(A)}$	±1.60
	7	4.54$^{b(B)}$	±2.27	6.42$^{a(B)}$	±1.43	6.44$^{a(A)}$	±1.30
Viscosity	0	7.40$^{a(A)}$	±1.43	7.34$^{a(A)}$	±1.27	7.04$^{a(A)}$	±1.85
	5	6.04$^{b(B)}$	±1.31	6.90$^{a(A)}$	±1.13	6.60$^{ab(A)}$	±1.51
	7	5.04$^{b(C)}$	±1.77	5.96$^{ab(B)}$	±1.59	6.26$^{a(A)}$	±1.64
General acceptability	0	7.96$^{a(A)}$	±1.09	7.60$^{ab(A)}$	±1.34	6.90$^{b(A)}$	±1.97
	5	5.64$^{b(B)}$	±1.60	7.00$^{a(AB)}$	±1.60	6.38$^{ab(A)}$	±1.59
	7	5.02$^{b(B)}$	±1.55	6.56$^{a(B)}$	±1.16	6.96$^{a(A)}$	±1.26

Values in the same line for each medium followed by different lower case letters and values in the same column for each medium followed by different capital letters differ significantly ($p < 0.05$) according to the test Tukey.

Table.5 Test of intent to purchase the acerola juice without chitosan (T1), chitosan added to 2.5 mg/mL (T3) and chitosan added to 5 mg/mL (T4)

	Time (days)	Treatment					
		T1		T3		T4	
		Average	SD	Average	SD	Average	SD
Intent to purchase	0	4.24$^{a(A)}$	±0.98	4.44$^{a(A)}$	±0.97	3.96$^{a(A)}$	±1.25
	5	2.44$^{c(B)}$	±0.97	3.44$^{b(B)}$	±0.95	3.92$^{a(A)}$	±1.05
	7	2.00$^{c(B)}$	±0.93	3.10$^{b(B)}$	±1.06	3.66$^{a(A)}$	±1.12

Values in the same line for each medium followed by different lower case letters and values in the same column for each medium followed by different capital letters differ significantly ($p < 0.05$) according to the Tukey test.

Figure.1 Total count of lactic acid bacteria in acerola juice with different treatments: T1 without chitosan, T2 with acetic acid, T3 chitosan in concentration of 2.5 mg/mL and T4 chitosan in concentration of 5mg/mL

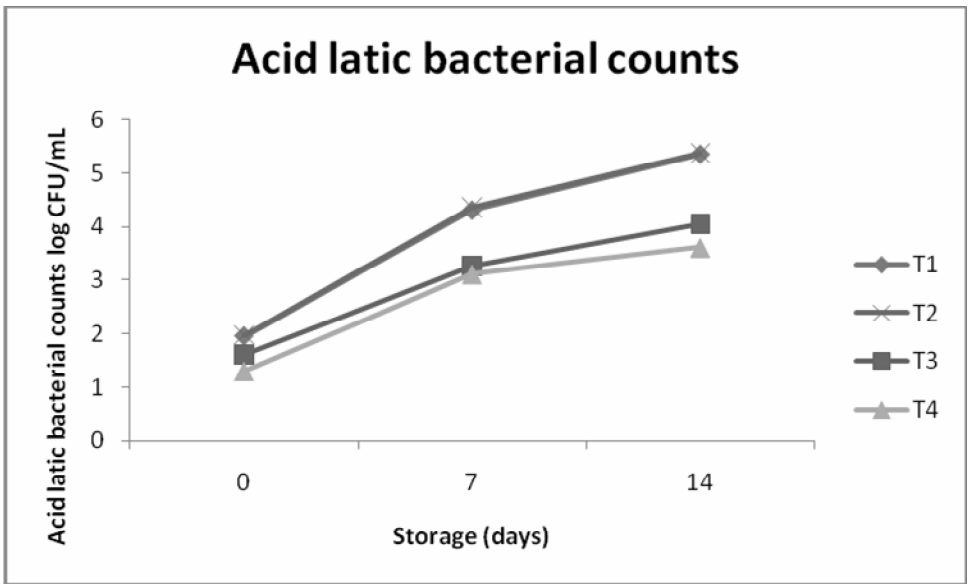


Figure.2 The total count of yeasts and molds in acerola juice with different treatments: T1 without chitosan, T2 with acetic acid, T3 chitosan in concentration of 2.5 mg/mL and T4 chitosan in concentration of 5mg/mL

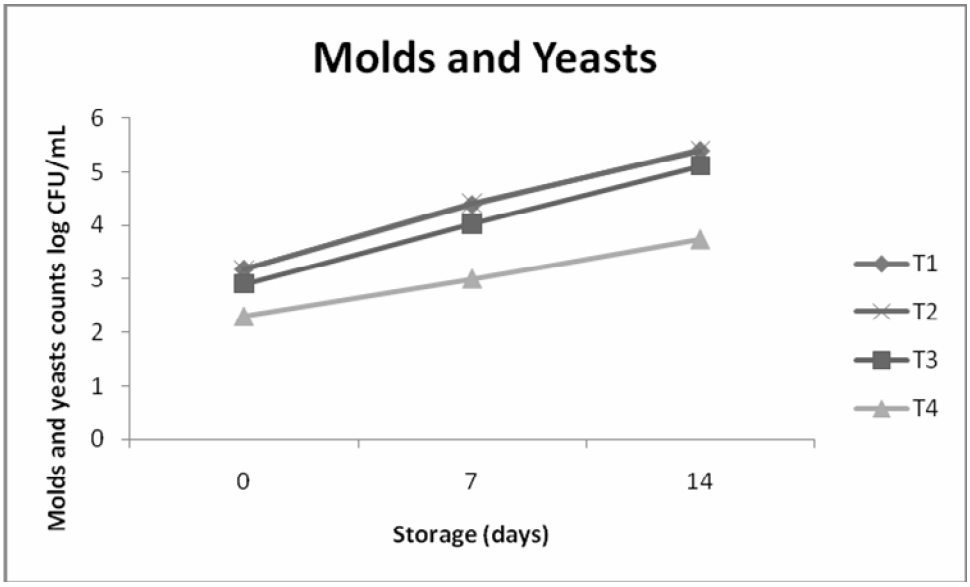
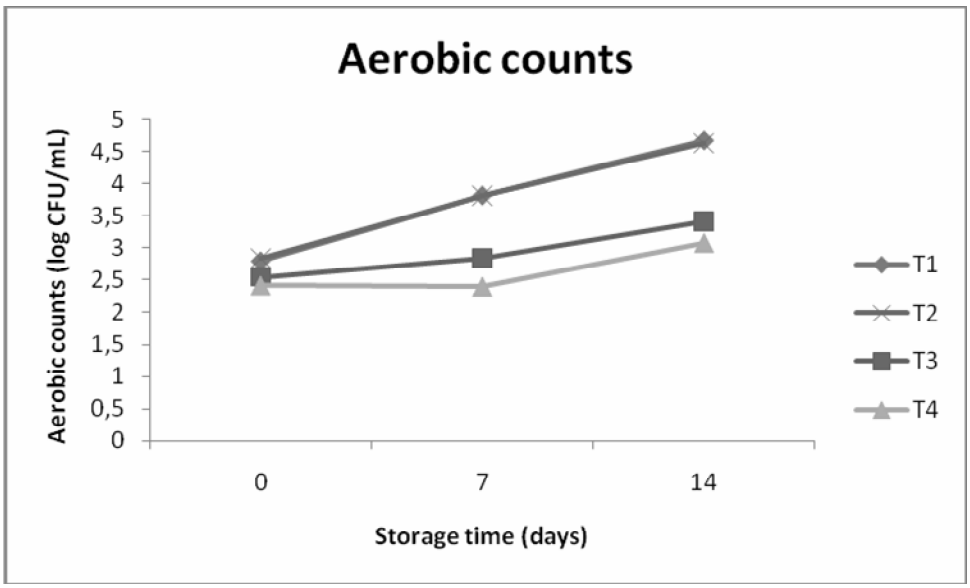


Figure.3 The total count of aerobic mesophilic in acerola juice with different treatments: T1 without chitosan, T2 with acetic acid, T3 chitosan in concentration of 2.5 mg/mL and T4 chitosan in concentration of 5mg/mL



It was found that the total count of mesophilc bacteria, yeasts and lactic acid bacteria, reduced by the addition of chitosan representing a positive effect on the extension of shelf life. Chitosan reduced the bacterial count of aerobic mesophilic and lactic acid in a 1 log (T3 and T4) and the count of yeasts and molds in approximately 2 log CFU/mL (T4) compared to T1.

The concentration of 5 mg/mL was more effective in reducing the growth of yeasts and molds than 2.5 mg/mL. This was probably because the yeasts are part of the predominant microbiota of juices present in large quantities and the minimum inhibitory concentration (MIC) ranging from 0.02 mg/mL to 1 mg /mL and for some bacteria and filamentous fungi of 0.01 mg/mL to 5 mg/mL (Rabea et al, 2003).

Over the 14 days of storage there was an increased in count of mesophilic aerobic bacteria, molds, yeasts and lactic acid bacteria, which was also observed in Rhoades and Roller (2000), Martín-Diana et al (2009). The values of total coliforms and fecal coliform bacteria were less than 3 MPN/ mL. It was not detect the presence of *Salmonella* sp. in samples evaluated during the storage.

Sensory analysis

The concentration of chitosan significantly affected ($p<0.05$) the sensory acceptance of acerola juice (Table 4). The sensory scores ranged from 4.54 "dislike slightly" to 8.32 "liked".

The overall assessment at time 0 showed a significant reduction in sensory acceptance with the addition of chitosan to "like moderately" (T1) to "like slightly" (T4). At the time (5), an increase of "neither liked / or disliked" (T1) to "like moderately" (T3) were demonstrated. At the time 7, in turn, the acceptance of "neither liked / or disliked" (T1) increased to "like slightly" (T3 and T4). There was however, no relation between this difference and the increasing concentration of 2.5 (T3) to 5 mg/mL (T4).

Therefore, after the processing initialize

(time 0), the acerola juice was given the highest scores sensory (Table 4) which are however reduced by the addition of chitosan (T3 and T4). These results are in agreement with Martín-Diana et al (2009) who found a reduction in the overall assessment of the juice with the addition of chitosan. This is due to the bitter taste of the juice due to the addition of chitosan gel (Han et al 2005).

Throughout the store there was a reduction of notes sensory acceptance. In T4, however, attributes for appearance, flavor, viscosity and overall assessment there was no significant reduction ($p<0.05$). These results corroborate Freitas et al (2006) who observed a reduction in the acceptance of color and flavor in addition to reducing the overall assessment of the note at the end of shelf life of acerola juice. Chitosan has a positive effect on stability of the juice, mainly divided reducing the acidity of the juice (Imeri and Knorr 1988).

The juices added chitosan were acceptable ("neither liked / or disliked" to "liked") even at the end of storage time in spite of the notes are lower compared to the beginning, being in agreement with Martín-Diana et al (2009).

The intention to purchase the acerola juice ranged from 2 "Possibly not buy" to 4.44 "possibly buy" (Table 5). There was no significant difference ($p <0.05$) in intention to buy the juice at time 0, but the T4 had a grade lower than that of T1. In time 5 and 7 the addition of chitosan (T3 and T4) significantly increased purchase intent ($p <0.05$) compared to control (T1). There was a significant reduction in sensory scores of T1 and T3 during storage, in T4 it was not observed. This may underline the possibility that if the juices with chitosan were available in the market consumers would likely to buy.

Chitosan proved to be a viable alternative to chemical additives in extending the shelf life of acerola juice. Thus, studies with the change in the characteristics of chitosan are needed to reduce the impact on the nutritional value of juice.

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