Effects of antibrowning solution and chitosan-based edible coating on the quality of fresh-cut apple

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Abstract: Fresh-cut apples were treated with an antibrowning solution and with a chitosan-based edible coating associated to antibrowning agents and the effects on the deterioration at low temperature were studied. The combined effect of ascorbic acid (AA), citric acid (CA) and chitosan was also evaluated. Control, coated and uncoated apples were packed in polyethylene terephthalate trays and the changes in headspace atmosphere, colour, firmness and microbial growth measured along ten days of storage at 5°C. The samples coated with 1% AA + 2% CA + chitosan maintained a good colouration until day 6, similar to those treated with 1% AA + 2% CA without chitosan. Afterwards, the fruits became darker and similar to the control. A slight reduction in the rates of CO_2 production was observed in all samples. Ethylene increased continuously during storage although gas production was extremely low. Texture did not change for all treatments; showing values around 9.0 to 10.0 N. Growth of *Salmonella*, and total and fecal coliforms were not detected. The chitosan coating treatment was the most effective in inhibiting growth of moulds and yeasts, with count of 1.7 CFU g^{-1}. Chitosan coating could be an alternative for preserving quality of fresh-cut 'Gala' apples. Results from this study suggest that.

Keywords: apple; chitosan; ascorbic acid; citric acid; firmness; colour; CO_2; ethylene; microorganisms.

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1 Introduction

Fresh-cut differ from whole fruit and vegetables in terms of their physiology, handling and storage requirements. In general, the disruption of tissue and cell integrity resulting from processing decreases the product shelf life. Besides, these products require more attention due to the magnitude of enzymatic and respiratory factors and microbial growth that may impact consumer safety (Lamikanra, 2002). Many techniques such as low temperature and high relative humidity, use of additives, controlled and modified atmosphere packaging and edible coating, have been applied in order to minimise these factors (Cantwell and Suslow, 2002; Sonti et al., 2003).

Edible coatings, for example, have been suggested as low-cost alternative technology for preserving intact and fresh-cut fruit and vegetables since they can create semi-permeable barriers to gases and water vapour avoid microorganism contamination and help the preservation of produce quality (Olivas and Barbosa-Canovas, 2009). When the coat is formed, a modified atmosphere is developed and along with relative humidity and optimum refrigeration temperature, so fresh-cut products can have their shelf life extended significantly (Rojas-Grau et al., 2008). Polysaccharide-based coatings have been one of the most used materials to formulate preservative coating on fresh-cut products. Chitosan, a deacetylated polysaccharide from chitin, is able to coat and preserve quality of fruit and vegetables by forming films, which decreases respiration rates, inhibits microbial growth and consequently delays ripening. The film forming ability and antimicrobial properties of chitosan is well reported and successful tested in the storability of perishable foods (No et al., 2007; Olivas and Barbosa-Canovas, 2009).

A combination of elements can be used to improve preservation of fresh-cut products. In this sense, edible coatings can also be used as carriers of additives, including chemical agents with inhibitory action on enzymatic browning such as ascorbic acid that has action in reducing o-quinones to colourless diphenols and, citric acid that presents dual effects as lowering pH and as a copper chelate from polyphenol oxidase active site (Garcia and Barrett, 2002). In postharvest practice, ascorbic acid is currently applied in combination with others organic acids to prevent enzymatic browning and maintain fruit firmness (Qi et al., 2011).

Fresh-cut apple in slices form appears in the first place amongst minimally processed fruit or vegetable products found in the market (Toivonen et al., 2010), and their sale are projected to continue growing (Wang et al., 2007).

In this work, the preservative coating of fresh-cut 'Gala' apples was compared between samples dipped in a citric-ascorbic acid solution and samples coated with chitosan associated with citric and ascorbic acids and their main quality attributes investigated.

2 Materials and methods

2.1 Materials

Chitosan (Medium molecular weight, 75–85% degree of deacetylation, viscosity of 200–800 cP, Sigma-Aldrich, Inc., St. Louis, MO, USA) was the primary ingredient for the coating formulation. Citric acid (Synth Ltd., Diadema, SP, Brazil) was added to chitosan and keep a low pH for a complete dissolution. Ascorbic acid (Synth Ltd., Diadema, SP, Brazil) was used as antibrowning agent. Sodium dichloroisocyanurate dehydrate (Sumaveg®, JohnsonDiversey Brasil Ltda., Sao Paulo, SP, Brazil) was used to sanitise apples before dipping.

2.2 Preparation of the aqueous and coating solutions

The antibrowning solution (pH 2.08) (1% AA + 2% CA) was prepared by dissolving ascorbic acid (10 g L^{-1}) and citric acid (20 g L^{-1}) in distilled water. The chitosan coating formulation (1% AA + 2% CA + chitosan) was prepared by dissolving chitosan (2 g L^{-1}) in citric acid solution (20 g L^{-1}), while stirring at 70°C. The ascorbic acid (20 g L^{-1}) was

added to the solution at room temperature prior to processing the fruit. The final pH of this formulation was measured as 2.17.

2.3 Fresh-cut fruit coating

Before processing, all utensils and surfaces that would be contact with the fruit during processing were washed and sanitised with 200 mg L^{-1} sodium hypochlorite solution (pH 7). 'Gala' apple fruit (*Malus x domestica* Borkh.) were purchased from a local wholesale distributor at commercial maturity. Apples were selected by uniform size, discarding those with mechanical or any signal of pathological injuries. The fruit were stored at 5 ± 1°C until being processed. The fruits were then washed, sanitised by immersion in a 200 mg L^{-1} sodium dichloroisocyanurate dehydrate solution for 3 min and rinsed prior to slicing. Then, apples were cored and manually cut into wedges (average weight at 25 ± 2 g) with a sharp stainless steel knife. The apple wedges were then rinsed in a 20 mg L^{-1} sodium dichloroisocyanurate dehydrated solution for 3 min. After, rinsing slices were separated in three lots and treated as follow:

1 immersion into 2% citric acid for 2 min

2 immersed into the coating formulation 1% ascorbic acid + 2% citric acid + 2 g L^{-1} chitosan for 2 min

3 as control lot: the wedges conserved as only sanitised.

Excess solution was allowed to drain and the samples, portions of approximately 200 g of fresh-cut apples placed into polyethylene terephthalate trays (160 urn, 750 mL, Galvanotek®, Brazil) and stored in a cold room at 5 ± 1°C. Analyses were carried out every other day for ten days.

2.4 Headspace gas analysis

Headspace gases within the sealed trays were sampled to determine the levels of CO_2 and ethylene during storage. A septum consisted of a polyamine screw and a nut with a 7 mm hole filled with silicone adhesive was placed in the tray lid to collect the gases. The septum was sealed with two flexible silicone rings on the inside and outside of the lid. To measure the atmosphere, a 1-mL headspace sample was withdrawn from the trays through the septum by piercing it with a needle on a syringe. The contents of these trays were used for subsequent fruit analysis. The gas samples were then injected into a C.G 3537-D gas chromatograph (C.G. Scientific Instrument, Sao Paulo, SP, Brazil) equipped with a 200 cm Porapak N column (Sigma-Aldrich, Inc., St. Louis, MO, USA), a thermal conductivity detector for CO_2 and flame ionisation detector for ethylene. The injector, oven, and detector temperatures were held at ambient temperature (23°C). The carrier gas was hydrogen for CO_2 and nitrogen for ethylene, with a flow rate of 30 mL min^{-1}. Five measurements were made and CO_2 and ethylene levels were calculated in comparison to a standard (ethylene: 87,681 uL L^{-1} and CO_2: 3,49 mL L^{-1}).

2.5 Texture measurement

Texture of apple slices was determined using a TA.XTPlus Texture Analyser (Stable Micro Systems Ltd., England, UK) by measuring the maximum penetration force

required for a 4 mm diameter stainless probe to penetrate into an apple wedge to a depth of 5 mm at a rate of 5 mm s^{-1}. Apple wedges were placed perpendicular to the probe to allow penetration in the centre. Five pieces from each tray were used per treatment.

2.6 Colour measurement

Colour values of the cut apple surfaces were measured with a colorimeter HunterLab MiniScan XE Plus (Hunter Associates Laboratory, Inc, Reston, VA, USA). Colour was measured using the CIELAB, L* (lightness) C* (chroma) h (hue angle). Illuminant D65 and 10° observer angle were used. The instrument was calibrated using a standard white reflector plate. Samples were taken from three trays per treatment, and five readings (five slices) were made in each tray. The browning index (BI) was calculated as follows: $BI = [100(x - 0.31)] / 0.172$ where: $x = (a + 1.75\ L) / (5.645\ L + a - 3.012\ b)$.

The browning index (BI) is defined as brown colour purity and usually used as indicator of the browning extent in sugar containing food products (Buera et al., 1986; Guerrero et al., 1996).

2.7 Microbiological analysis

The microflora was measured every two days for ten days of storage at 5°C. One slice of apple from each treatment was dipped in equivalent volume of sterile peptone water 1% (e.g., a slice of 10 g was dipped in 1 mL of peptone water) for 1 min. A 1 mL aliquot of the solution was then serially diluted in sterile peptone water 1% in (1:10) to a final dilution of 10^3. Separate 1 mL aliquots of each of the dilutions from 10^0 to 10^3 were withdrawn and poured on 3M™ Petrifilm™ to detect mould and yeast (three plates per dilution), and 3M™ Petrifilm™ to detect E. *coli*/coliform (three plates per dilution), and 3M™ Tecra™ Salmonella Visual Immunoassay for *Salmonella spp.* (three kit wells per sample). The plates for yeast and mould were incubated at 25°C for 72 h; plates for E. *coli* and coliform were incubated at 45°C and 35°C for 24 h, respectively. The microorganisms were counted with an automated colony counter Phoenix CP 600 Plus (Araraquara, SP, Brazil). The results were expressed as CFU g^{-1} of apples. For Salmonella, the samples were primary enriched in Buffered Peptone Water for 24 h at 35°C followed by the secondary enrichment in Rappaport Vassiliads Broth for 20 h at 42°C. For post-enrichment, samples were enriched in M-Broth for 8 h at 36°C. The results were interpreted comparing wells of the samples to the well of positive control.

2.8 Statistical analysis

A completely randomised design was used with three treatments, six days (ten days of storage with analyses every two days: 0, 2, 4, 6, 8 and 10) and five replications, a total of 90 samples. Each tray constituted on experimental unit. The effects of treatments, storage days and interaction for each original isolated variable were tested by general linear model (GLM), using a complete factor analysis in the analysis of variance (ANOVA). Significant differences among the levels of main effects (treatment and storage day) were compared by Tukey's tests at the 5% level of significance. The multivariate structure contained in the original data was explored by multivariate analysis of factors. Analyses were run using the software Statistica 7.0 (STATSOFT, Inc., 2004).

3 Results and discussion

In Table 1 is summarised the main results from samples colorimetric, texture and headspace gases analysis. In these data, two factors account for 63.4% of the variability. Residues were normally distributed with zero mean and constant variance for both factors F1 (lightness, chroma and hue angle) and F2 (CO_2 and ethylene).

Table 1 Factor analysis loadings of colour (lightness, chroma and hue angle), headspace gas composition (CO_2 and ethylene) and texture of fresh-cut gala apples stored for ten days at 5°C

	Factor 1 *Lightness, chroma and hue angle*	*Factor 2* *CO_2 and ethylene*
Lightness	*0.864468*	0.095477
Chroma	*–0.889532*	0.076536
Hue angle	*0.933229*	–0.101667
CO_2	0.102662	*–0.796527*
Ethylene	0.004539	*0.839777*
Texture	0.079827	0.121183
*Expl. var.	2.426421	1.379676
**Prp. totl.	40.44%	22.99%

Notes: *Explained variance; **proportion of variation explained.

3.1 Colour changes

The first factor (colour) accounts for 40.44% of data variability. Positive scores indicate lighter slices. Scores closer to zero or negative indicates that apple slices developed a browning colouration (Figure 1). These results agree with the results from browning index for each treatment shown in Figure 2.

Figure 1 Colouration scores in dipped (1% AA + 2% CA), coated (1% AA + 2% CA + chitosan) and uncoated (control) apple slices during storage at 5°C

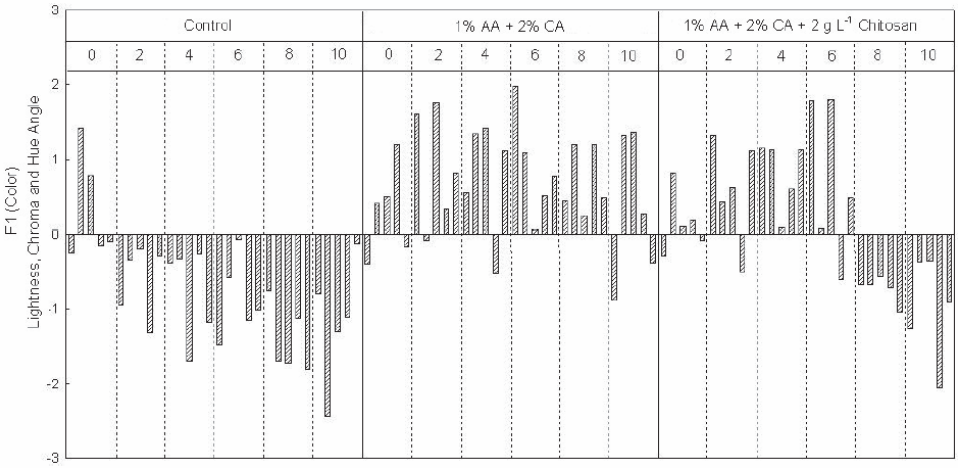
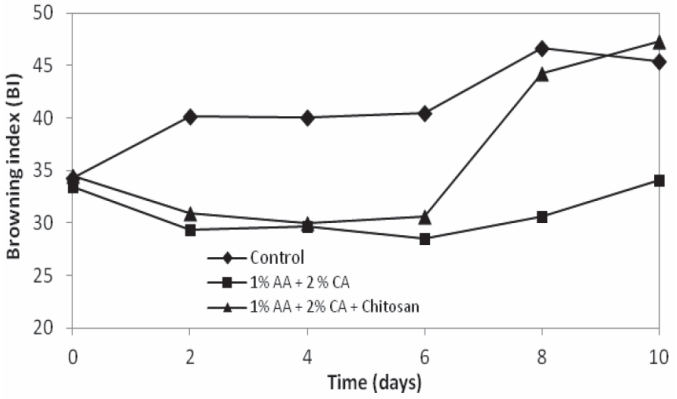


Figure 2 Browning index (BI) from apple slices dipped in 1% AA + 2% CA; 1% AA + 2% CA + 2 g L^{-1} chitosan and control stored for ten days at 5°C



Lightness varies from black to white on a scale of 0 to 10 and represent lighter or darker colours, hue angle is the attribute of colour perception by means of which and an object is judged to be red, yellow, green, blue, etc., and chroma represents degree of departure from grey toward pure chromatic colour (McGuire, 1992). The increase in chroma means an increase in pigments and it is directly related to the browning of apple slices.

In this study, there is a positive correlation with lightness and hue angle and negative correlation for chroma (Table 1); evidencing that if the values of chroma increase during storage, the lightness and hue angle values tend to decrease. In fact, Rocha and Morais (2003) observed the same pattern of changes in colouration during storage of fresh-cut apples for ten days at 4°C.

The samples initially showed weak yellowish hue (~87°) and tended to weak orange hue (~82°) during storage. The control showed the lowest values of lightness and hue angle, and highest values for chroma factors. Apple slices from the control treatment were the darkest suggesting that this treatment was the least efficient for maintaining the quality of apple slices (Figure 1).

Factor 1 scores have similar characteristics at the beginning of the evaluation for all treatments. However, during storage was noticed that the control gradually became darker. Darkening of the colour increased by 32% at the of the storage period as compared to initial values (Figure 2).

The apple slices dipped in 1% AA + 2% CA maintained their colouration score during storage with lighter slices (Figures 1 and 2). Browning in apples is caused by the enzymes polyphenol oxidase and peroxidase. When slices are in contact with oxygen, production of dark pigments occurs affecting flavour and aroma (Rojas-Grau et al., 2008). Treatment with 1% AA + 2% CA was the most effective in preserving the colour (Figure 1).

The samples coated with 1% AA + 2% CA + chitosan maintained good colouration until the sixth day of storage, similar to the 1% AA + 2% CA treatment. Afterward, they became darker and similar to the control. Chien et al. (2007) likewise reported that colour was preserved for seven days at 6°C when fresh-cut mango was coated with different concentration of chitosan dissolved in acetic acid. Similarly, Olivas et al. (2007) did not find significant differences among alginate-based coating, alginate-acetylated

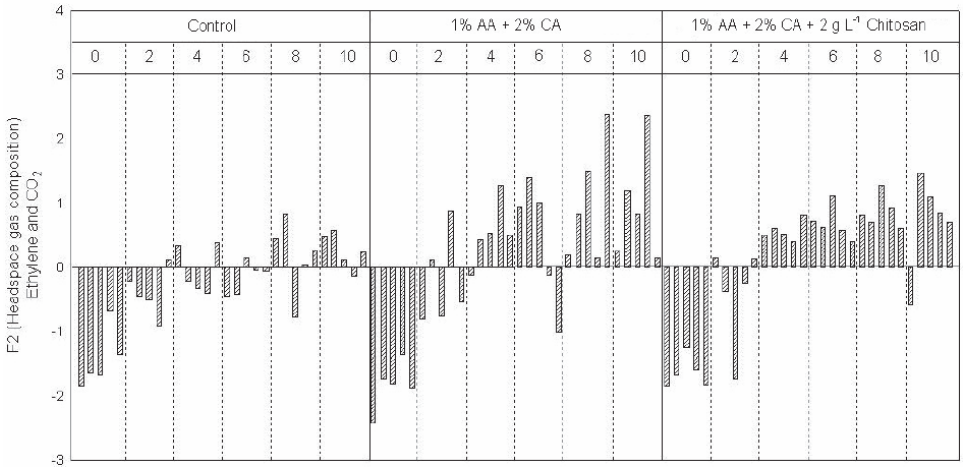
monoglyceridelinoleic acid-based coating, alginatebutter-linoleic acid-based coating, and the control in apple slices during the first six days of storage; however, after day 8 the control was 20% darker.

Nevertheless, the colour preservation measured in samples coated with chitosan associated to AA and AC in the first week of storage is quite superior to similar analyses performed on sliced apples coated only with chitosan with no antibrowning, as reported by Assis et al. (2012).

3.2 Changes in headspace gas composition

The second factor (CO_2 and ethylene) accounts for 22.9% of data variability. It shows a positive correlation for ethylene and negative for CO_2. It evidences that there was an increasing tendency in the ethylene values while CO_2 values showed a slight decrease (Figure 3).

Figure 3 CO_2 and ethylene scores in dipped (1% AA + 2% CA), coated (1% AA + 2% CA + chitosan) and uncoated (control) apple slices during storage at 5°C

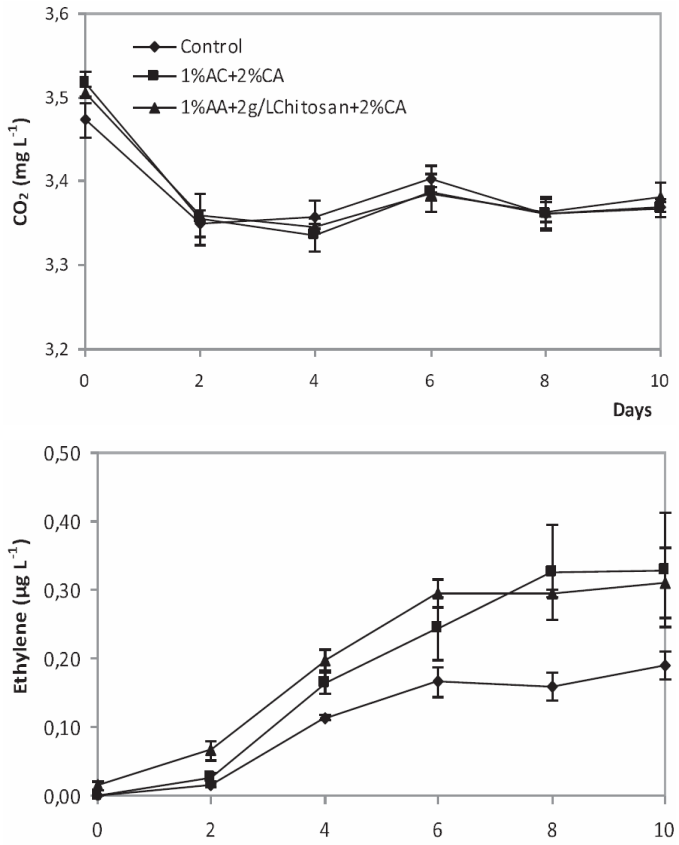


Positive scores indicate that samples had higher concentration of ethylene and negative scores indicate low concentration of this gas. Since CO_2 values remained practically constant, ethylene may be assumed as responsible for the changes observed in the scores for all treatments during storage period (Figure 3).

Despite the small variation in the CO_2 values throughout the storage, an increase in the concentration was detected on the first day of analysis for all treatments (Figure 4), which may be related to the physical stress caused by minimal processing (Rosen and Kader, 1989).

In this study, the three treatments showed the same pattern during the storage time concerning the synthesis of ethylene, characterised by a daily increase of gas content (Figure 4). According to Abeles et al. (1992), the occurrence of injuries in plant tissues increases ethylene production reaching its maximum level within 6 to 12 hours.

Figure 4 CO_2 and ethylene headspace gas concentration in trays containing dipped (1% AA + 2% CA), coated (1% AA + 2% CA + chitosan) and uncoated (control) apple slices during storage at 5°C



Note: Data shown are the means (±standard deviation).

The control samples showed the lowest mean Factor 2, the lower concentration of gases was detected throughout the storage period. For samples dipped in 1% AA + 2% CA and in 1% AA + 2% CA + chitosan, the scores became positive showing a tendency of increase in ethylene concentration from the fourth day. However, the detected concentration of both CO_2 and ethylene were low for all treatments (Figure 4). This agrees with results reported by Rojas-Grau et al. (2007) in which a low level in ethylene production was measured in samples coated with alginate-apple puree-based films. Vargas et al. (2006) found similar behaviour in the respiratory rate of strawberry coated with chitosan combined with oleic acid in first three days of storage at 4°C.

It is well known that ethylene production is stimulated when plant tissues are injured and it can accumulate in packages of fresh-cut product leading to undesirable effects (Watada and Qi, 1999). Thereby, a well elaborated edible coating can assure the formation of a modified atmosphere inside the fruit reducing levels of internal oxygen minimising the ethylene production (Olivas and Barbosa-Canovas, 2009). Storage temperature has a major effect on metabolic activity (Garcia and Barrett, 2002), and should be associated to additional preservative procedures.

3.3 Texture changes

Edible coatings can preserve texture of fruit and vegetables by reducing water loss and preventing dehydration (Lin and Zhao, 2007) and serving as carriers of texture enhancers, such as calcium chloride (Olivas and Barbosa-Canovas, 2009).

Concerning texture analyses, the load values of the variable were very low in both F1 and F2 factors and not considered as relevant in this study. An individual analysis of variance showed no significant differences between means of texture for treatments and storage days ($p > 0.05$) and showed homogeneity in variances. Neither of the treatments affected texture, with all means ranging from 9.0 to 10.0 N.

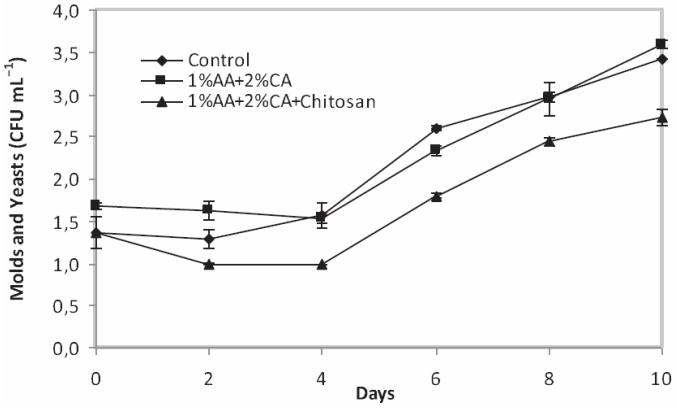
Supapvanich et al. (2011) likewise found that fresh-cut apple texture remained constant when stored at different cold temperature. However, chitosan coating has been reported as efficient in avoiding tissue softening in cut (Qi et al., 2011) and intact apples (Jorge et al., 2011), what can be a factor of polymer concentration in the formed coated. Similarly, Gonzalez-Aguilar et al. (2009) reported that texture of fresh-cut papaya was also maintained for a period when slices were coated with chitosan. Ducamp-Collin et al. (2009) also found that fresh-cut mango texture was preserved for nine days at 4°C when treated chitosan solution (0.75% chitosan + 3% citric acid).

The low concentration of ethylene as found in all treatments in this study may also interfere in delaying the tissue softening. Several studies have showed the effect of ethylene in accelerating softening, as Agar et al. (1999), who reported that the removal of ethylene from the storage atmosphere increased retention of firmness in fresh-cut kiwi.

3.4 Microbiological analysis

Figure 5 shows the effect of chemical dip and chitosan coating on the growth of moulds and yeasts on fresh-cut apple stored for ten days at 5°C. The initial count of moulds and yeast of fresh-cut apple was low for all samples. Samples coated with 1% AA + 2% CA + chitosan and control showed initial count of 1.4 CFU mL^{-1} and samples dipped in 1% AA + 2% CA showed 1.7 CFU mL^{-1}.

Figure 5 Effects of treatments on moulds and yeasts growth (log CFU mL^{-1}) of apple slices



Note: Data shown are the means (± standard deviation).

The chitosan coating treatment was the most effective in inhibiting growth of these microorganisms from the second day of storage until the end of experiment. In fact, the antimicrobial activity of chitosan against a wide range of foodborne filamentous fungi, yeast, and bacteria has made it a potential natural food preservative (Goy et al., 2009; No et al., 2007; Sudharshan et al., 1992). Rojas-Grau et al. (2007) also found low counts of moulds and yeasts, below 2 log CFU mL^{-1}, in apple slices coated with alginate-apple puree coatings containing essential oils. Gonzalez-Aguilar et al. (2009) reported the effect of chitosan in preventing deterioration and found that this coating inhibited the growth of moulds and yeasts on fresh-cut papaya stored at 5°C.

The growth of *Salmonella*, and total and fecal coliforms was also evaluated in this research; however, none of these microorganisms was detected.

4 Conclusions

In conclusion, we can state that samples coated with 1% AA + 2% CA + chitosan and 1% AA + 2% CA treatment do maintain good colouration until the sixth day of storage. Additionally all treatments preserve the texture and low concentration of C 0 2 and ethylene during storage at low temperature. The formulation with chitosan has the additional effect in being effective in inhibiting the growth of moulds and yeast on cut apple surfaces increasing the life-time for consumption. Coatings based on chitosan could be a good choice for fresh-cut coatings by associating antibrowning agent assuring superior activity than simple acid-based dipping.

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