

QUALITY OF MINIMALLY PROCESSED 'FUJI' APPLE UNDER REFRIGERATED STORAGE AND TREATMENT WITH ADDITIVES¹

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ABSTRACT – The aim of this study was to evaluate the ability to prolong the useful life of the minimally processed 'Fuji' apple by applying the individual or combined additives (L-cysteine chloride, L-ascorbic acid and calcium chloride) and to determine the appropriate period of storage of the whole fruit to perform the minimum processing. The experimental design was completely randomized in three-factor design with three replications. Factor A was composed of storage periods of whole apples, pre-processing, in cold chambers (20, 78, 138 and 188 days); the factor B was represented by storage periods minimum post-processing, simulating shelf life (3, 6, 9 and 12 days), and factor C was represented by chemical additives (distilled water, as control, 0.5% L-cysteine chloride, 1% L-ascorbic acid, 0.5% L-cysteine chloride along with 1% calcium chloride and 1% L-ascorbic acid together with 1% calcium chloride). The evaluated dependent variables were pulp color (L^* and h^*), soluble solids, titratable acidity, content of phenolic compounds, antioxidant capacity and quantification of polyphenol oxidase. In addition, was analyzed the presence or absence of *Salmonella* sp. and *Escherichia coli*. The prolongation of the storage time of 'Fuji' apples in a refrigerated atmosphere promotes increased susceptibility to browning and softening after processing from 78 days of storage. The use of additives in the process, helps prevent these problems, especially when combined 0.5% L-cysteine chloride with 1% calcium chloride, achieving an excellent conservation in refrigerated shelf up to 6 days. From a microbiological aspect, minimally processed apples are toxicologically safe.

Index terms: antioxidants, *Malus domestica*, storage, minimally processing.

QUALIDADE DE MAÇÃ 'FUJI' MINIMAMENTE PROCESSADA SOB ARMAZENAMENTO REFRIGERADO E TRATAMENTO COM ADITIVOS

RESUMO - O objetivo do trabalho foi avaliar a capacidade de prolongamento da vida útil da maçã 'Fuji' minimamente processada aplicando os aditivos (cloreto de L-cisteína, ácido L-ascórbico e cloreto de cálcio) individuais ou combinados, e determinar o período adequado de armazenamento refrigerado do fruto inteiro para a realização do processamento mínimo. O delineamento experimental utilizado foi inteiramente casualizado, em esquema trifatorial com três repetições. O fator A foi composto por períodos de armazenamento das maçãs inteiras, pré-processamento, em câmara fria (20, 78, 138 e 188 dias); o fator B foi representado por períodos de armazenamento pós-processamento mínimo, simulando vida de prateleira (3, 6, 9 e 12 dias) e, o fator C foi representado por aditivos químicos (água destilada, como controle; L-cisteína a 0,5%, ácido ascórbico a 1%, L-cisteína a 0,5% com cloreto de cálcio a 1%, ácido ascórbico a 1% com cloreto de cálcio a 1%). As variáveis dependentes avaliadas foram coloração da polpa (L^* e h^*), teor de sólidos solúveis, acidez titulável, teor de compostos fenólicos totais, capacidade antioxidante e a quantificação da polifenoloxidase. Além disso, foi analisada a presença ou não de *Salmonella* sp. e *Escherichia coli*. O prolongamento do tempo de armazenamento das maçãs 'Fuji' em atmosfera refrigerada promove aumento da suscetibilidade ao escurecimento e amolecimento após o processamento a partir de 78 dias de armazenamento. O uso de aditivos, no processo, contribui para evitar esses problemas, principalmente quando se combina cloreto de L-cisteína a 0,5% com cloreto de cálcio a 1%, alcançando excelente conservação em prateleira refrigerada até 6 dias. Sob o aspecto microbiológico, as maçãs minimamente processadas são toxicologicamente seguras.

Termos para indexação: antioxidantes; *Malus domestica*; armazenamento; processamento mínimo.

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INTRODUCTION

The apple tree (*Malus domestica* Borkh.), is a typical fructiferous of temperate climates, deciduous leaf of the Rosaceae family, and has great social, environmental and economic importance in the south of Brazil, besides promoting several health benefits (FACHINELLO et al., 2011). Changes in consumption habits have been increasing, boosting the minimally processed fruit sector (GOODBURN; WALLACE, 2013), and thereby increasing competitiveness in the productive sector.

A series of steps characterize the minimum processing as sanitization, peeling, cutting and / or abrasion, which promote the convenience of fruit and vegetable consumption, at the expense of reducing its post-harvest shelf life. For reduction of the enzymatic browning, loss of nutritional quality, and maturation of plant tissues several additives may be applied, such as citric acid, ascorbic acid, calcium chloride and L-cysteine chloride (MANOLOPOULOU; VARZAKAS, 2011, GHIDELLI et al., 2013).

The use of auxiliary agents to prolong the useful life of food is on great interest, since they can maintain the mechanical properties of the food system, control the loss of water, taste, texture and avoid the occurrence of browning reactions (MADANI et al., 2014). The aim of this study was to evaluate the ability to prolong the useful life of the minimally processed 'Fuji' apple by applying the individual or combined additives (L-cysteine chloride, L-ascorbic acid and calcium chloride) and to determine the appropriate period of storage of the whole fruit to perform the minimum processing.

MATERIAL AND METHODS

The experiment was conducted with 'Fuji' apples from the commercial orchard of the company Randon Agrosilvopastoral S.A. (RASIP), in Vacaria, RS, Brazil (50°56'02"S, 28°30'14"W and 900m altitude). The apples went through selection, trying to make the lot uniform as to the degree of maturation and the absence of visible mechanical damages or rot. The fruits were in cold store at $1 \pm 1^\circ\text{C}$ with a relative humidity (RH) of 85-95% and analyzed in the Post-Harvest Laboratory of Embrapa Clima Temperado (Pelotas-RS / Brazil).

The experimental design was completely randomized in three-factor design with three replica-

tions. Factor A was composed of storage periods of whole apples, pre-processing, in cold chambers (20, 78, 138 and 188 days); the factor B was represented by storage periods minimum post-processing, simulating shelf life (3, 6, 9 and 12 days), and factor C was represented by chemical additives (distilled water, as control, 0.5% L-cysteine chloride (LC) (m/v), 1% L-ascorbic acid (AA) (m/v), 0.5% LC along with 1% calcium chloride (CC) (m/v) and 1% AA (m/v) together with 1% CC (m/v)).

At each storage period of the whole fruit (20, 78, 138 and 188 days), the fruits were minimally processed. First, apples underwent sanitization with sodium hypochlorite (200 mg L⁻¹, pH 6.0) for 10 minutes at ambient storage conditions. The apples were cut into four pieces, shaped like buds, removing the central part containing the seeds, all apples had the same caliber. Afterwards, the apples were treated by one minute immersion, with subsequent one minute drainage, in the treatments with: distilled water as control, 0.5% LC; 1% AA; 0.5% LC + 1% CC; 1% AA + 1% CC.

The apples were packed in polystyrene trays measuring 150x150x20mm, which each contained a one apple in the buds shape, packed with nine micron stretchable polyvinyl chloride (PVC) film. The trays were stored for 3, 6, 9 and 12 days in a cold chambers at $4 \pm 1^\circ\text{C}$, under RH from 85 to 95%, simulating the commercialization time of the product. The temperature and RH of the chambers were monitored by computerized system. For each analysis, 12 trays were prepared per treatment, adding 60 trays in each period, totaling 240 trays for the four post-processing analysis times. As treatments were performed on apples stored for four periods, the total design completed 960 trays.

The coloration of the pulp was measured using the Minolta CR-300 colorimeter, with a CIE reading system L*a*b*, and the hue, or chromatic hue, represented by the hue (h°) angle, using the tangent arc formula b^*/a . The result of this equation, expressed in radians, was then converted to degrees, according to Minolta (1994).

The soluble solids content (SS) was quantified with a manual digital refractometer (ATAGO®) and the results were expressed in °Brix. For titratable acidity (TA), 10 mL of apple juice was added in 90 mL of distilled water. The titration of the sample was done with the aid of a digital burette (Brand®), containing sodium hydroxide solution (0.1 N) until

reaching pH 8.1. The titratable acidity was expressed as percentage of malic acid (INSTITUTO ADOLFO LUTZ, 2008).

Quantification of the polyphenol oxidase enzymatic activity (PPO) was done using the methods adapted from Siriphanic and Kader (1985) and Flurkey and Jen (1978), expressed as g^{-1} fresh mass (FW) min^{-1} absorbance. The total phenolic compounds (FC) were quantified using the Folin-Ciocalteu reagent according to the protocol described by Swain and Hillis (1959), expressed in mg of chlorogenic acid equivalent (CAE) per 100g^{-1} FW. The antioxidant capacity was determined by spectrophotometry according to a method adapted from Brand-Williams et al. (1995), the results were expressed in μg of trolox equivalent antioxidant capacity (TEAC) g^{-1} FW.

To verify the safety and microbiological quality, the minimally processed apples were evaluated for the presence of *Enterobacteriaceae* family bacteria, such as the genus *Escherichia coli* and *Salmonella* sp. The trays were disinfected externally with 70% alcohol before being opened. For the identification of the genus *Salmonella*, the samples were diluted with 25g of apple and 225mL of lactose broth, instead of sterile water, in a homogenizer (BegMixer®). Therefore, the samples were enriched in an oven at 37°C for 24 hours. In order to identify the presence of *E. coli* the plate counting technique with selective culture medium (Chromocult agar - Merck®) was used. Microbiological data were analyzed as the presence or absence of the microorganism in colony forming units per gram of sample (CFU g^{-1}).

Data obtained were analyzed for normality by the Shapiro-Wilk test, homoscedasticity by the Hartley test and the independence of residues was graphically checked. Later, they were submitted to analysis of variance. In case of significance, the effects of the minimum pre and post-processing periods and the chemical additives were analyzed by confidence intervals. The differences between the treatments were considered significant ($p \leq 0.05$), when there was no overlap between the confidence intervals at 95% probability. The presence of correlations between the variables dependent on the study was analyzed using Pearson's correlation coefficient.

RESULTS AND DISCUSSION

The assumptions of the mathematical model were all met and data transformation was not necessary for all variables. The results in the analyzes of variance for the variables L^* ($F = 5.87, p < 0.0001$), hue ($F = 9.65, p < 0.0001$), soluble solids ($F = 3.06, p = 0.0007$), titratable acidity ($F = 6.37, p < 0.0001$) (Figure 1 A, C, E and G) total phenolic compounds ($F = 5.56, p < 0.0001$) and capacity antioxidant ($F = 4.88, p < 0.0001$) (Figure 2 A and C) showed significance for the double interaction between periods of pre-processing storage and additives. In addition to this pair, there was a significant interaction between post-processing storage periods and additives for the variables L^* ($F = 5.51, p < 0.0001$), hue ($F = 15.81, p < 0.0001$) (Figure 1 B and D), total phenolic compounds ($F = 6.75, p < 0.0001$) and antioxidant capacity ($F = 5.28, p < 0.0001$) (Figure 2 B and D). The enzymatic activity of polyphenol oxidase ($F = 2.28, p = 0.0104$) showed only significance for the interaction between periods of post-processing storage and additives (Figure 1 F).

The elongation of the apples storage period from 20 to 78 days favored the occurrence of browning of the pulp (Figure 1 A). From this period, the differences were not significant between the periods in all the additives. When the additives were applied, the browning was prevented (Figure 1 A and B) and among these, what gave preservation of the light coloring of the fruits was LC+CC, maintaining the average values of L^* between 76.4 and 77.94 (Figure 1 A). When comparing single use of LC or AA, or combined use with CC, the highest values were when LC was present. Corroborating with these results, the control of browning the minimally processed (MP) 'Williams' pear also occurred with the application of L-cysteine chloride, was possible to observe a reduction in L^* values (VILAS BOAS et al., 2015).

By prolonging the storage period of pre-processed apples in cold chambers has increased susceptibility to chromatic changes (h°). The prevention of browning was done with LC and LC+CC until the sixth day of storage (Figure 1 D). In MP strawberries, reductions in the hue angle value over the storage time were reported, and there were no significant differences in the coating treatments of the same (LEITE et al., 2015).

The medium values of SS were between 14.72 and 15.65° Brix obtained with MP 'Fuji'

apples. In the case of MP pear stored at different temperatures, the highest values of SS were reported for fruits treated with ascorbic acid (VILAS BOAS, et al., 2015), which are very similar to those observed in this work, depending on the pre-processing period evaluated. In the periods from 20 to 138 days there were no significant variations between the LC and AA treatments when the cold chamber was removed from the MP. On the other hand, LC+CC reduced SS from 15.58 to 15.09°Brix between periods of 20 to 78 days, and AA+CC decreased SS from 15.40 to 14.77°Brix between the periods of 78 to 138 days. The increase observed in SS for the control and LC+CC in the last period may be related to the accumulation of sugars by the loss of moisture. For the other additives, in the last period there was a reduction of SS, this behavior is associated to the consumption of sugars due to the higher respiratory metabolism of the fruit in the presence of the temperature (Figure 1 E).

In relation to acidity, the influence of the storage period factor of the pre-processed apples and of the additives used (Figure 1 G), it was possible to observe that the TA decreased as the storage periods of the fruits were prolonged. This loss of organic acids occurs with the metabolic processes of fruit maturation and senescence, as a result of its use as a substrate in the respiratory process or its conversion into sugars. Similar behavior, decrease of acidity during storage, was found in 'Reubennel' plums subjected to alternative treatments (BENATO et al., 2015).

In this study, the variations of medium TA values for storage periods of pre-processed apples were 0.26 to 0.33% malic acid for 20 days, 0.21 to 0.23% for 78 days, from 0.25 to 0.26% for 138 days and from 0.13 to 0.20% for 188 days (Figure 1 G). These values are in agreement with those found in fresh and MP apples treated with calcium chloride and ascorbic acid, ranging from 0.22 to 0.23% of malic acid, with decreases in the presence of storage (SABA; SOGVAR, 2016).

In general, the activity of the polyphenol oxidase enzyme decreased with prolongation of the refrigerated storage time, remaining at higher levels in the fruits processed with the application of adjuvants. The increase in PPO activity was observed in the control, a condition in which there was also greater browning, represented by the hue angle value (Figure 1 F). All the additives reduced the PPO activity when compared to the control, thus

demonstrating that the additives used may act on this enzyme. The decline in the activity of this enzyme throughout the storage was also reported in 'Louis' melons submitted to different temperatures, this fact justified the absence of browning in the fruit pulp (MORGADO et al., 2015).

The enzymatic activity in these products was also influenced by the antioxidant treatments used, presenting browning control of MP 'Fuji' apple (AUGUSTO et al., 2016). In MP 'Fuji' apples, the most efficient PPO reduction treatment during the entire storage period of the processed product was AA with medium values of 0.14 to 0.16, followed by AA+CC with mean values of 0.17 to 0.19 g⁻¹ FW min⁻¹ absorbance. Contrasting with the control, whose mean values varied from 0.20 to 0.25 g⁻¹ FW min⁻¹ absorbance, they are considered relatively high, providing favorable conditions for browning.

The additives used in this study were efficient to maintain the total phenolic compounds content. The AA had medium values of CF ranging from 331.96 to 363.58 mg CAE 100g⁻¹ FW, and the control ranged from 269.26 to 294.98 mg CAE 100g⁻¹ FW, compared to storage periods of pre-processing apples (Figure 2 A). In MP apples treated with chemical additives, the authors Saba and Sogvar (2016) studied the phytochemical variation and found that the total phenolic compounds content decreased during storage, and the treatments were efficient in maintaining these indices, presenting values similar to those verified in this job. Regarding the storage time of the pre-processed apples, after 138 days, the total phenol content decreased. This occurs as a consequence of the maturation metabolism and senescence, probably caused by the oxidation of these compounds by polyphenol oxidases.

Thus, treatments AA, LC+CC and AA+CC were important reducers in the fight against browning of products that depend directly on the amount of enzymes and substrates, both for the storage periods of apples before and after processing (Figure 2 A and B). In a study with MP apples, it was possible to find a high concentration of phenolic compounds, depending on factors such as cultivar, harvest, storage conditions and processing (KARAMAN et al., 2013). Based on this study, it was observed that the CF contents of the MP 'Fuji' apples had mean values from 263.45 to 371.79 mg CAE 100g⁻¹ FW (Figure 2 B) values higher than those found in the whole fruit, favoring thus reducing of the browning.

In order for the browning occur, in addition to high values of the PPO enzyme, three additional conditions are necessary: the substrate (phenolic compounds), oxygen (O_2) and the contact to complete the reaction ($PPO + O_2 + \text{substrate}$) (AUGUSTO et al., 2016). In the case of apples, there is high PPO activity, high CF content, exposure to O_2 and damage caused by fruit cutting, facilitating the occurrence of the browning reaction. Because of this, is important to use resources that hinder or delay the occurrence of this enzymatic reaction. In terms of CF and PPO activity, adequate treatment was AA and in some cases AA+CC, maintaining the lowest PPO activity.

The treatments with coadjuvants used in this study affected antioxidant capacity, and treatment with AA promoted higher values from 3005.49 to 2705.14 $\mu\text{g TEAC g}^{-1}$ FW. On the other hand, the control treatment, in which the lowest antioxidant capacity was obtained, with medium values of 2640.60 to 2341.03 $\mu\text{g TEAC g}^{-1}$ FW (Figure 2 C).

In the last storage period of the pre-processed apples, it was observed that AA, LC and LC+CC were similar and maintained the highest values for the antioxidant capacity at the end of the refrigerated storage. For post-processing periods, similar variations occurred in pre-processing, where the treatment with the highest antioxidant capacity was AA (3070-2876.43 $\mu\text{g TEAC g}^{-1}$ FW), followed by LC+CC (2362.86-2757.14 $\mu\text{g TEAC g}^{-1}$ FW) and AA+CC (2355.45-3151.43 $\mu\text{g TEAC g}^{-1}$ FW) that obtained superior antioxidant capacity to the control (Figure 2 D). Studies on fresh cut apples, Saba and Sogvar (2016) found that the combination of additives such as carboxymethyl cellulose and ascorbic acid maintained antioxidant capacity and quality attributes evaluated during storage, reducing browning.

The presence of *E. coli* bacteria (thermally tolerant coliforms) was determined in all samples and did not exceed 1×10^2 CFU g^{-1} , being in accordance with Brazilian legislation RDC n° 12/2001 - ANVISA, which establishes maximum *E. Coli* of 5×10^2 CFU g^{-1} ($2.7 \log$ CFU g^{-1}) for fresh, prepared, sanitized, refrigerated or frozen fruits for direct consumption (ANVISA, 2001). The presence of bacteria of the genus *Salmonella* was not detected in any of the analyzed samples, being thus in agreement with the current legislation.

Regarding the correlations, the significant results were between total phenolic compounds and antioxidant capacity, which evidenced the highest

coefficient of positive correlation. When there was an increase in the total phenol content, it was also verified an increase in the antioxidant capacity of the fruits. In this context, 20-day period the highest coefficient of correlation was obtained in the AA+CC treatment ($r = 0.99$, $p < 0.0001$), followed by AA ($r = 0.97$, $p = 0.0046$). Already, in the period of 78 days the only correlation verified was in AA+CC ($r = 0.99$, $p = 0.0002$). For the 138-day period all treatments had the same behavior, ie, high correlation coefficient (above 0.90) and all significant. While in the 188-day period the highest correlation coefficient was the LC+CC treatment ($r = 0.99$, $p = 0.0178$), followed by AA+CC and AA, this correlation is due to the high antioxidant power that the phenolic compounds presented.

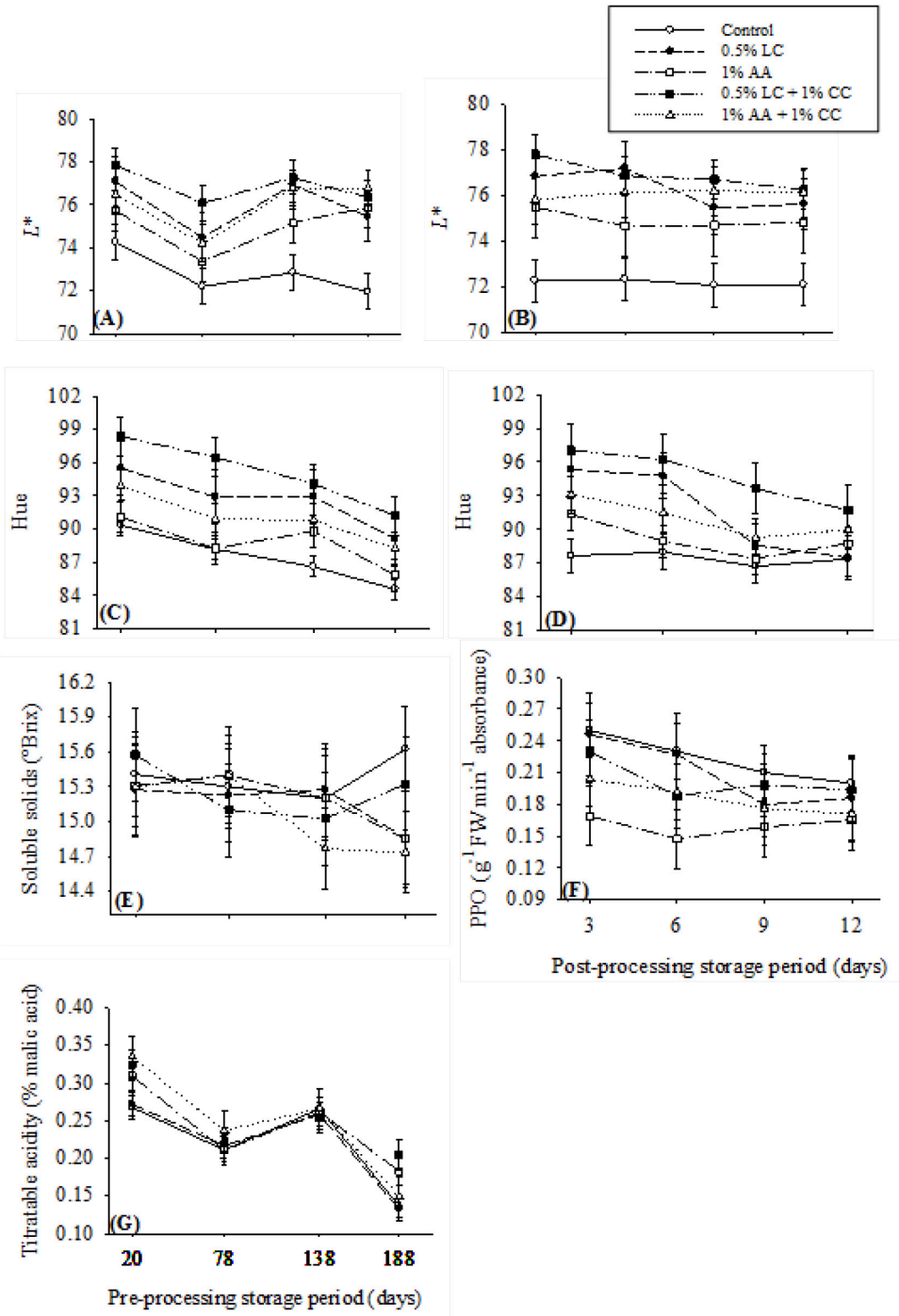


FIGURE 1 - L^* (A and B), Hue (C and D), soluble solids ($^{\circ}\text{Brix}$ - E), polyphenol oxidase (PPO - $\text{g}^{-1} \text{FW min}^{-1}$ absorbance - F) and titratable acidity (% malic acid - G) of 'Fuji' apples submitted storage periods pre-processing (20, 78, 138 and 188 days) and post-processing (3, 6, 9 and 12 days), after minimally processed and treated with: distilled water (Control); 0.5% L-cysteine chloride (0.5% LC); 1% ascorbic acid (1% AA); 0.5% L-cysteine chloride + 1% calcium chloride (CC) (0.5% LC + 1% CC) and 1% ascorbic acid + 1% calcium chloride (1% AA + 1% CC). Embrapa Clima Temperado, Pelotas, 2012/13. (The vertical bars represent the 95% confidence intervals).

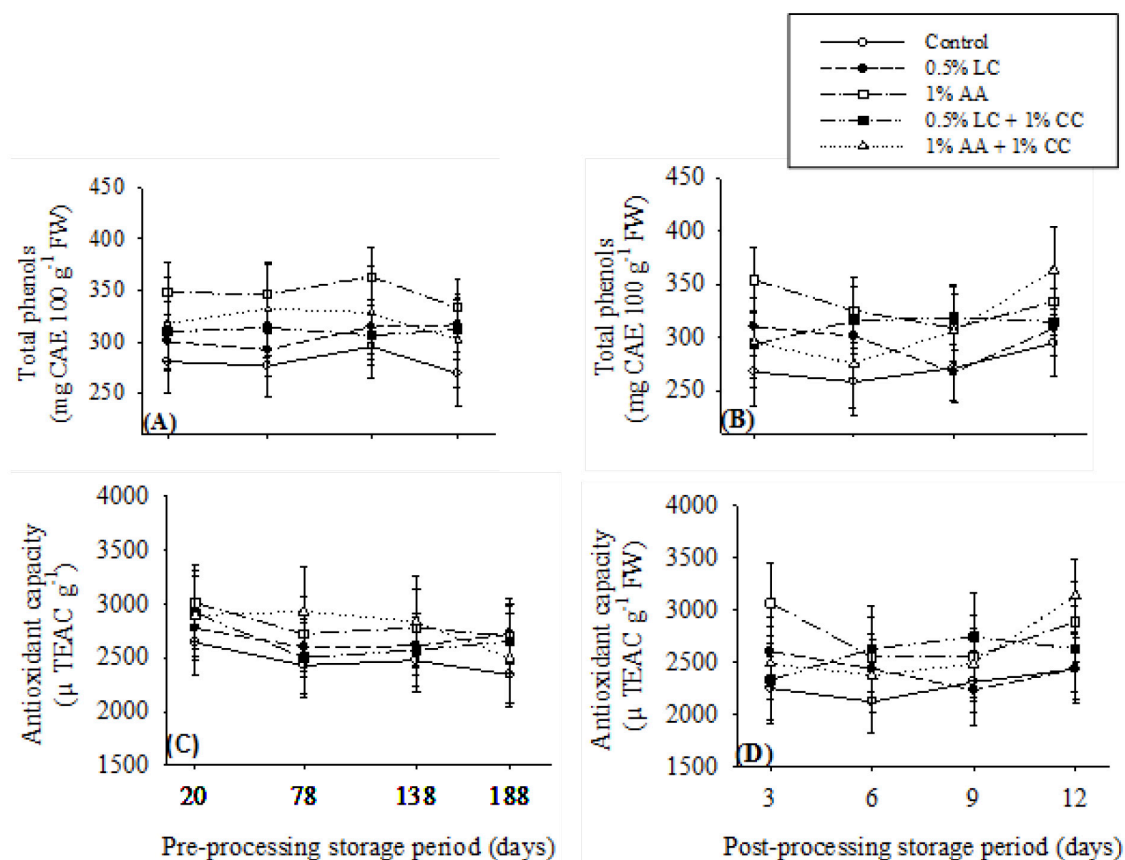


FIGURE 2 - Total phenols (mg CAE 100 g⁻¹ FW - A and B) and antioxidant capacity (μ TEAC g⁻¹ FW - C and D) of 'Fuji' apples submitted storage periods pre-processing (20, 78, 138 and 188 days) and post-processing (3, 6, 9 and 12 days), after minimally processed and treated with: distilled water (Control); 0.5% L-cysteine chloride (0.5% LC); 1% ascorbic acid (1% AA); 0.5% L-cysteine chloride + 1% calcium chloride (CC) (0.5% LC + 1% CC) and 1% ascorbic acid + 1% calcium chloride (1% AA + 1% CC). Embrapa Clima Temperado, Pelotas, 2012/13. (The vertical bars represent the 95% confidence intervals).

CONCLUSIONS

The prolongation of the storage time of 'Fuji' apples in a refrigerated atmosphere promotes increased susceptibility to browning and softening after processing from 78 days of storage. The use of additives in the process, helps prevent these problems, especially when combined 0.5% L-cysteine chloride with 1% calcium chloride, achieving an excellent conservation in refrigerated shelf up to 6 days. From a microbiological aspect, minimally processed apples are toxicologically safe.

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