ERYTHORBIC ACID AND SODIUM ERYTHORBATE EFFECTIVELY PREVENT PULP BROWNING OF MINIMALLY PROCESSED 'ROYAL GALA' APPLES

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ABSTRACT

This study aimed to evaluate the effect of erythorbic acid (EA), sodium erythorbate (SE) and kojic acid (KA) to control the pulp browning of minimally processed (MP) 'Royal Gala' apples. Physicochemical and sensorial properties of MP apples were evaluated during a shelf life testing. SE and EA resulted in the highest levels of phenolic compounds, antioxidant activity and PPO and POD inhibition. Sensorial analysis results revealed that SE and EA treatments preserved the flavor, odor, color, succulence, firmness and overall quality of MP apples for up to 9 days. In conclusion, both SE and EA are suitable antibrowning agents for MP 'Royal Gala' apples.

Keywords: antioxidant activity, enzymes, minimal processing, phenolic compounds, sensorial analyses

1. INTRODUCTION

Apple (*Malus domestica* Borkh) is a widely consumed fruit in many Westernized diets due to its year-round market availability (VIEIRA *et al*., 2011). There is also a large retail demand for minimally processed (MP) apples due to practicality during consumption, transportation, and health benefits for those who consume. Nevertheless, apples are very sensitive to enzymatic browning, either by the high content of phenolic compounds and/or the activity of oxidative enzymes such as polyphenoloxidase (HOLDERBAUM *et al*., 2010) and peroxidase (ROJAS-GRAUE *et al*., 2008). Enzymatic browning in MP apple is a major problem for the food processing industry, and must be controlled to prevent undesirable changes in color, flavor and nutritional value (TOIVONEN and BRUMMELL, 2008; IOANNOU and GHOUL, 2013). An alternative to avoid the browning effect on minimally processed fruit pulps is the use of antioxidants (GALGANO *et al*., 2015). L-cysteine, erythorbic acid, sodium erythorbate, and kojic acid, used in combination with calcium chloride, are examples of antioxidants agents to preserve MP products quality. L-cysteine, commonly used to control/avoid pulp browning in MP products (ALI *et al*., 2016; CABEZAS-SERRANO *et al*., 2013; GHIDELLI *et al*., 2014), has been reported to have different mechanisms of action: a) inhibition of PPO (NI EIDHIN *et al*., 2006); b) adduct formation with *o*-quinones resulting in non-colored compounds during oxidation (RICHARD-FORGET *et al*., 1992); and c) reduction of *o*-quinones to their polyphenols precursors (CILLIERS and SINGLETON, 1990). Erythorbic acid (D-isoascorbic acid) (KALL and ANDERSEN, 1999), has also been used in MP products such as potatoes (CACACE *et al*., 2002), canned applesauce and beer (ANDERSEN, 1999). This antioxidant has been reported with similar antioxidants properties of its stereoisomer (ascorbic acid), however, it does not have the vitamin C activity, but it is at least five times cheaper (MARTIN-BELLOSO and SOLIVA-FORTUNY, 2011). Sodium erythorbate is a reducing agent (BUTA *et al*., 1999) commonly used in the food industry to reduce the superficial oxidation of MP fruits (GROSS *et al*., 2016; SAPERS and MILLER, 1998) and meat products (SOMMERS *et al*., 2002). Kojic acid has been used in Japan as food additive due to its inhibitory properties over different oxidase enzymes. This antioxidant has been reported in several food matrices such as syrup, flour, meet, flavoring and vegetables (SCPP, 2008). In addition, it has been reported that kojic acid significantly reduced the browning in Liberty apple slices (SON *et al*., 2001), Amasya apple juice (IYIDOGAN and BAYINDIRLI, 2004) and litchi pericarp (SHAH *et al*., 2017). Calcium chloride, when used in association with antioxidants, is responsible for the reduction of physiological imbalances and maintenance of color. In addition, it is recommended due to its firming action on a wide range of fruits (TECHAKANON and BARRETT, 2017). Despite the use of different antioxidants in MP apples, erythorbic acid, sodium erythorbate and kojic acid have never been tested as antibrowning agents in fresh-cut 'Royal Gala' apple, as far as we are concerned. In this context, the objective of this study was to evaluate the sensorial characteristics and physicochemical variables of MP 'Royal Gala' apples treated with erythorbic acid, sodium erythorbate and kojic acid associated with calcium chloride, under cold storage for 9 d, to simulate shelf-life conditions.

2. MATERIAL AND METHODS

2.1. Harvesting and storage of fruits

Royal Gala apples (*Malus domestica* Borkh), at commercial maturity stage, were harvested from a commercial orchard located in the Vacaria city (28º 30' 44" S, 50º 56' 02" W), Rio

Grande do Sul state, Brazil. The apples had the following average characteristics: starch content of 4.80, measured according to STREIF (1984); pulp firmness of 12.84 N; concentration of total soluble solids of 12.51ºBrix and titratable acidity of 0.23 g of malic acid 100 g^{-1} FW. The apples were selected by size (approximately 110 g) and for the absence of visible mechanical damage and rot and stored at 1.0°C ± 0.5°C and relative humidity of 90.0 % ± 5.0 %, at the Postharvest Physiology Laboratory from Embrapa Clima Temperado.

2.2. Treatments

Fruits were sanitized by dipping into a sodium hypochlorite solution (100 ppm, pH 6.5, at 6.5±1.5ºC) for 10 min, cut into eight wedge shape longitudinal slices with approximately the same size, with the removal of the central core and seeds, preserving the epidermis. The experimental treatments consisted of dipping the apple slices into different solutions for 1 min, as described below:

Treatments*	distilled water (W)	L-cysteine chloride 0.60 % (LC)	sodium erythorbate 5.00 % (SE)	erythorbic acid 3.00 % (EA)	kojic acid 0.07 % (KA)	$CaCl_2$ 1.00 % (CC)
W+CC (negative control)	X					X
LC+CC (positive control)		X				X
SE+CC			X			X
EA+CC				X		X
KA+CC					X	X

*The treatments W+CC (negative control) and LC+CC (positive control) were designed to be ineffective and highly effective against browning, respectively.

After dipping, the apple slices were drained for 5 min and placed onto expanded polystyrene trays, covered with PVC film (9 μm thickness) and stored for four different periods (0 d, 3 d, 6 d and 9 d), at 4±1.0°C and RH of 90.0±5.0 %, to simulate the shelf life of MP apples. The samples analyzed at 0 d had been exposed to each treatment for 8±1 h.

2.3. Reagents

Reagents were purchased from different suppliers: calcium chloride (≥ 99 %) from Synth (Diadema, SP, Brazil), L-cysteine (≥ 98 %) from Vetec (Duque de Caxias, RJ, Brazil), sodium erythorbate (≥ 98 %) and erythorbic acid (≥ 99 %) from Daxia Doce Aroma Ind. Com. Ltd. (São Paulo, SP, Brazil) and kojic acid (≥ 99 %) from Chengdu Jinkay Biology Engineering Co., Ltd. (São Paulo, SP, Brazil).

2.4. Physical and chemical analysis

2.4.1 Total soluble solids (TSS)

TSS was determined using a portable refractometer (ATAGO, model PAL-1) at 20ºC and the results were expressed in ºBrix.

2.4.2 Titratable acidity (TA)

TA was determined on apple pulp juice. The titration endpoint was determined with a pH meter (Quimis model Q400A). Briefly, 10 mL of pulp juice (diluted in 90 mL distilled water) was titrated with 0.1 M NaOH to a pH 8.1 endpoint. The results were expressed as grams (g) of malic acid per 100 g^{-1} of fresh weight (FW).

2.4.3 TSS/TA ratio

TSS/TA ratio was calculated by dividing the TSS values by TA values.

2.4.4 Loss of mass

Mass loss was calculated by the equation 1.

$$Loss\ of\ mass\ (\%) = \frac{Initial\ mass - Final\ mass}{Final\ mass}\ x\ 100 \qquad \text{Equation 1}$$

2.4.5 Pulp firmness

Measured according to MELO *et al*. (2009) using a Texture Analyser (TA-XT plus 40855, Stable Microsystems, England) with a 2 mm diameter probe, penetration depth of 5 mm, pre-test velocity of 1.0 mm s^{-1}; 2.0 mm s^{-1} test; post-test of 10.0 mm s^{-1} and force of 5 kg. The readings were performed in the middle portion of the pieces and the results were expressed in Newton (N).

2.4.6 Color

Apple color of equatorial region of slices was measured using a Minolta CR-400 colorimeter with a CIE L*a*b* reading system, proposed by the *Comission Internacionale de I'Eclairage* (CIE). The Browning Index was calculated from L*, a* and b* values according to PALOU *et al.* (1999) and hue or chromatic hue, represented by the Hue (h°) angle, was calculated as the tangent arc of b*/a* quotient. The result was expressed in degrees.

2.4.7 Total phenolic compounds

Measured according to the Folin-Ciocalteu method adapted from SWAIN and HILLIS (1959). Briefly, 250 µL aliquot of the extracts (the same used for DPPH· analysis) was combined with 250 µL of 0.25 M Folin-Ciocalteu reagent and 4000 µL ultrapure water. After 3 min of reaction, 500 µL of 0.5 M Na_2CO_3 was added, following incubation for 2 h at room temperature and absorbance reading at 725 nm. The results were expressed as grams of chlorogenic acid equivalents (CAE) per 100 g^{-1} of FW. Chlorogenic acid standard curve (0.0 mg mL^{-1} to 0.5 mg mL^{-1}) was used.

2.4.8 Antioxidant activity (DPPH)

The antioxidant activity was evaluated using the method described by BRAND-WILLIAMS *et al.* (1995) with some modifications. First, 10 mL of methanol was added to 2.5 g of fresh apple and homogenized during 1 min (ultra-turrax homogenizer, IKA, Artur Nogueira, SP). Extracts were centrifuged (Eppendorf - Centrifuge 5810 R) at 3050 g for 30 min at 1.0ºC. The supernatant was collected and stored at –18ºC until analysis. Apple extracts (100 µL) were added to 3900 µL DPPH solution (in methanol), and the reaction

mixture was kept in the dark for 24 h. After this period, the absorbances were spectrophotometrically read at 515 nm, and results expressed as mg of Trolox equivalent per 100 g^{-1} of FW.

2.4.9 Peroxidase (POD) enzyme activity

The POD enzyme activity was determined according to the methodology described by COELHO and SALAS-MELLADO (2014), adapted for micro quantities. Five grams (5.0 g) of apple pulp were homogenized (ultra-turrax homogenizer, IKA, Artur Nogueira, SP) with 0.2 g of polyvinylpyrrolidone (diluted in 20 mL of 0.2 M phosphate buffer pH 6.0) for 3 min. The homogenate was centrifuged (Eppendorf - Centrifuge 5810 R) at 3050 g, for 30 min at 1ºC. The supernatant (apple extract) was collected and maintained at ±4ºC. An aliquot of 45 μL of apple extract was mixed with 230 μL of a mixture of phosphate buffer (1.5 mL, pH 6.5, 0.05 M), distilled water (2.0 mL), hydrogen peroxide (1.0 mL, 0.08 %, v/v) and guaiacol (0.5 mL, 1 %). Samples were incubated at 37°C with readings (Molecular Devices - Spectramax 190 spectrophotometer) at 470 nm at time zero and after 15 minutes of reaction. The enzymatic activity was calculated based on the amount of protein, where a unit of enzyme activity was defined as the amount of enzyme causing the increase in absorbance of 0.01per min^{-1}. The results were expressed in units of enzyme/μg of protein.

2.4.10 Polyphenoloxidase (PPO) enzyme activity

PPO enzyme activity was measured by following the methodology described by RAI *et al.* (2011), with some adaptation. Briefly, 55 μL of the above apple pulp extract was added to 220 μL of the mixture consisting of phosphate buffer (1.5 mL, pH 6.5, 0.05 M), catechol (0.5 mL, 0.05 M) and water (2.0 mL). The reaction occurred at 37°C with reading (Molecular Devices - Spectramax 190 spectrophotometer) at 425 nm at time zero and 30 min. The enzymatic activity was calculated based on the amount of protein where a unit of enzyme activity was defined as the amount of enzyme, which causes an increase in absorbance of 0.01 per min^{-1}. The results were expressed in units of enzyme / μg of protein.

2.4.11 Protein

Protein concentration was determined according to BRADFORD (1976), using the reagents from Sigma-Aldrich® (Bradford Reagent, #B6916 and BSA Protein Standard, #P0834) and the corresponding protocol to assay the protein samples on 96 well plate. The absorbance was read at 595 nm in a spectrophotometer (Molecular Devices - Spectramax 190), and the results were expressed in μg of protein per μL.

2.5. Respiratory gas analysis

The O_2 and CO_2 concentration in the headspace of the hermetically sealed packages were determined every 3 d using Oxybaby 6.0 - Witt-Gasetechnik gauge analyzer. The results were expressed as a percentage (%).

2.6. Sensory analysis of minimally processed apple slices

A set of possible sensory judges, comprising male and female participants (between 20 and 40 years old) who consumed apple frequently, were trained for the evaluation of apple attributes under analysis during 30 days (2 weekly meetings of 15 minutes). The top 17 judges, those who showed better results, were selected. For each period of storage (0 d,

3 d, 6 d and 9 d), five samples (12.0±1.0 g each) were identified with three-digit code and randomly provided to the judges. A glass of water was provided for cleaning the palate between one sample and another. Flavor, odor, darkening, succulence, firmness and overall quality acceptability were the parameters evaluated using structured scales of 9 cm labelled "bad" to "excellent". This information was converted into scores from 0 to 9, respectively. The evaluation was performed at room temperature and in uniform laboratory conditions. The analysis was submitted to the evaluation by the Research Ethics Committee of the Faculty of Medicine of the Federal University of Pelotas and approved for being in accordance with Resolution 196/96 of the National Health Council (CAAE - 48625015.1.0000.5317).

2.7. Statistical analysis

The experimental design was completely randomized, in a 5 x 4 two-factorial scheme (five treatments x four storage periods) with three replicates. Data were analyzed for variance ($p \leq 0.05$) using the Statistica 7.0 program. For significant results, the means were compared by the LSD - Least Significant Difference ($p \leq 0.05$) test. The multivariate factorial analysis technique was also applied, using the Varimax rotation to improve the interpretation of the factors, using Statgraphics Centurion XVII software.

3. RESULTS AND DISCUSSION

It is important to notice that the samples analyzed at 0 d had been exposed to the corresponding treatment for about eight hours before analysis, causing the treatments to affect the results even at 0 d. Changes in the *ratio* (TSS/TA), commonly used to evaluate the fruit quality, can impact apple flavor (PIAGENTINI and PIROVANI, 2017). Treatments with W+CC, LC+CC, SE+CC and KA+CC resulted in a *ratio* increase (Fig. 1a) over the 9 days of storage. This *ratio* increase can be explained by the conversion of organic acids in non-acid molecules during the respiratory metabolism (PECH *et al.*, 2008), or due to the sugar accumulation by loss of humidity. Apples treated with EA+CC showed a decrease on *ratio*, probably due to the higher acidity (1.18 g of citric acid/100 mL, pH 2.39) of this antioxidant agent.

Mass loss can compromise the shelf-life of MP products (SANCHIS *et al.*, 2016). Minimal processing exposes fruits and vegetables tissues to a lower water pressure environment and can cause substantial loss of mass. W+CC and LC+CC were the treatments with higher mass loss (Fig. 1b). However, these values were lower than 1 %, not enough to change the fruit quality. In a similar study, PIZATO *et al.* (2013) and QI *et al.* (2011) obtained higher values for mass loss. Significant mass loss has physiological effect and can compromise fruits and vegetables appearance, texture and nutritional quality (SANCHIS *et al.*, 2016).

Results for pulp firmness (Fig. 1c) were not affected by the loss of mass, although slight variations occurred over the 9 days of storage. According to GANG *et al.* (2015), the use of $CaCl_2$ has an essential role in the maintenance of pulp integrity and firmness due to its action as bio-membrane stabilizer, supporting the integrity of the membrane cell wall. Besides, appropriated processing and storage conditions and use of antioxidants contribute to prevent pulp softening.

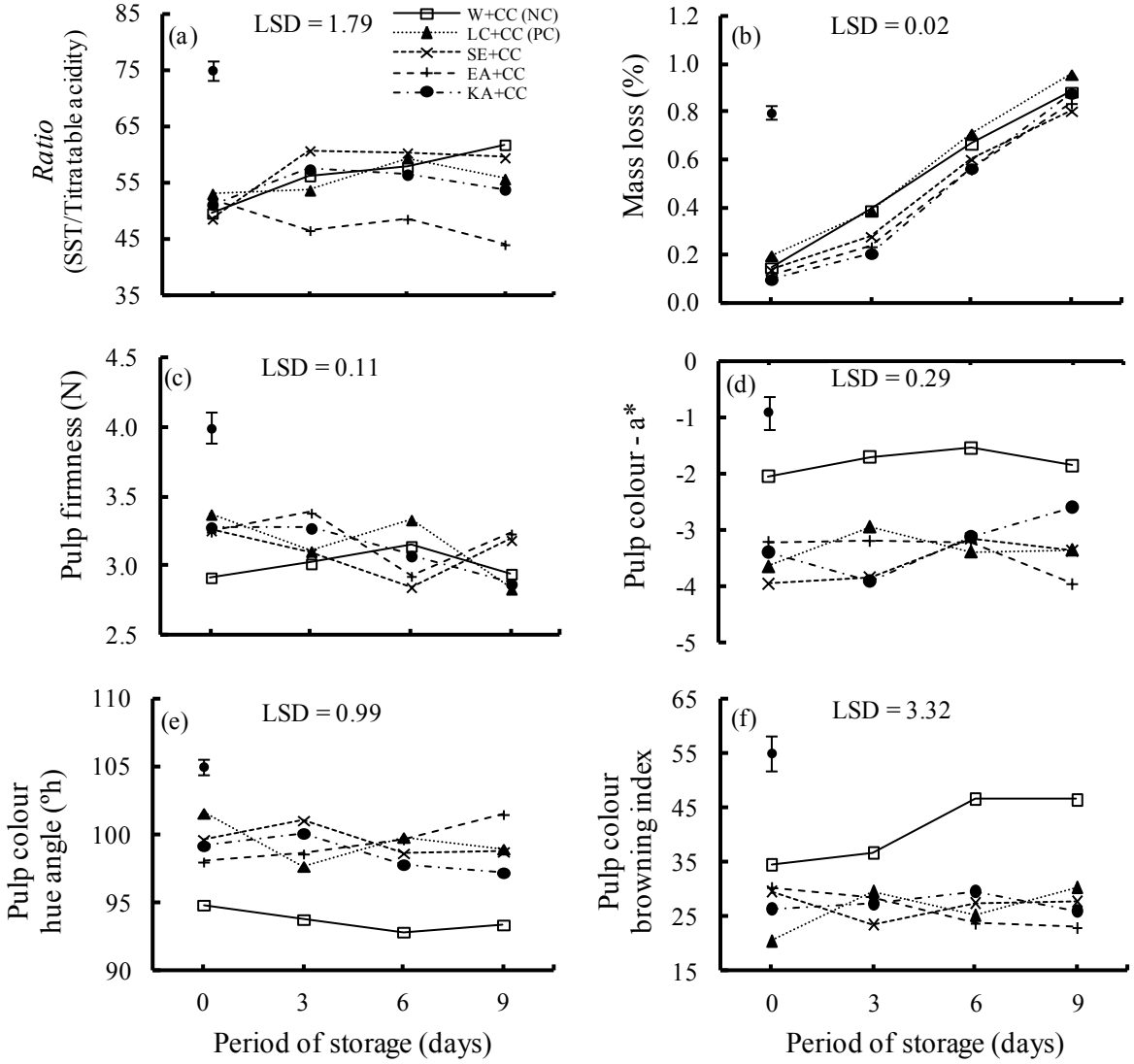


Figure 1. Effect of sodium erythorbate 5.0 % (SE) + calcium chloride 1.0 % (CC), erythorbic acid 3.0 % (EA) + CC and kojic acid 0.07 % (KA) + CC on physicochemical variables: mean values for *Ratio* (**a**), mass loss (**b**), pulp firmness (**c**) and pulp color variables: a* (**d**), hue angle (**e**) and browning index (**f**) for minimally processed 'Royal Gala' apple recorded at 0 d, 3 d, 6 d and 9 d, at 4.0ºC. Negative control (NC) consisted of distilled water (W) + CC, and positive control (PC) consisted of L-cysteine chloride 0.6 % (LC) + CC. The vertical bar indicates the Least Significant Difference (LSD) with ($p \leq 0.05$).

Color change in the pulp of the MP apples is associated with the oxidative processes due to the rupture of the cell membranes during product preparation. Green-red chromatic coordinate (a*) is an important parameter for the study of browning since brown color represents a combination between green (-a *) and red (+a *) (KIM and LEE, 2008). The green-red chromatic coordinate (a*) values were high for W+CC treatment in all periods of storage (Fig. 1d), corresponding to a more reddish apple pulp. Lower values of a* coordinate (yellow to green fruits) were observed for treatments LC+CC, SE+CC, EA+CC and KA+CC until day 6. However, the result of a* coordinate value for KA+CC treatment increased between day 6 and day 9. This increase on reddish color of apples treated with KA+CC can be due to the reversible linkage of kojic acid and PPO enzyme (CHEN *et al.*, 1991). The process of oxidative coloration is triggered by the cell membrane rupture and

the resulting mixture of polyphenol substrates with PPO (TOIVONEN and BRUMMELL, 2008). PPO enzyme when in contact with oxygen catalyzes two reactions: (1) hydroxylation of monophenols in diphenols and (2) oxidation of diphenols in quinones. The first is relatively slow and results in colorless products. The second one is relatively fast, and the resulting quinones are colored. Subsequent reactions of the quinones lead to the accumulation of melanoidin, which is the brown pigment (CORTELLINO *et al.*, 2015).
Fig. 1e displays the hue angle values obtained for the different antioxidant treatments. The treatments LC+CC, SE+CC, EA+CC and KA+CC resulted in a yellow-green hue throughout the nine days of storage. On the other hand, apples treated only with water and calcium chloride (W+CC), showed a tendency to brown, from the beginning of storage period, intensifying slightly over the 9 days of storage. These antagonistic results point to the importance of the antioxidant to inhibit the darkening of the pulp. Calcium chloride, besides maintaining pulp firmness, can also be responsible for the maintenance of color. This effect is related to its action on the prevention of the cellular membrane degradation, with consequent reduction in substrates release for PPO activity (TOIVONEN and BRUMMELL, 2008), protecting, therefore, the color of MP products (PEREZ-CABRERA *et al.*, 2011). Regarding the browning index (Fig. 1f), the treatments LC+CC, SE+CC, EA+CC and KA+CC maintained the pulp color, without statistical differences on the 6-day and 9-day of evaluation. Overall results for the color measurements revealed that all treatments maintained the apple pulp color satisfactorily, despite the differences between the mechanism of action of the antioxidants. L-cysteine has the ability to inhibit the enzymatic browning caused by PPO, through a competitive mechanism that captures *o*-quinones through the formation of cysteinyl adducts (RICHARD-FORGET *et al.*, 1992). The subgroup erythorbates comprise two compounds, erythorbic acid and sodium erythorbate. Erythorbic acid is a stereoisomer of ascorbic acid, with strong reducing properties, differing only from the relative position of the hydroxyl groups and of the hydrogen in the fifth carbon atom in the molecule (CAROCHO *et al.*, 2018). This acid reacts with oxygen and can thus remove it from a closed system (LEE *et al.*, 2012). Sodium erythorbate antioxidant activity is due to the quenching of singlet oxygen, hydrogen donation and as a reducing agent (CAROCHO *et al.*, 2018). Erythorbates have essentially the same function (FIDLER *et al.*, 2004) and are widely used as alternatives to ascorbic acid and its salts in products that the action of vitamin C is not necessary (MARTIN-BELLOSO and SOLIVA-FORTUNY, 2011). The antioxidant properties of kojic acid can be attributed to its ability to inhibit the thyroxinase and polyphenoloxidase enzymes, and its chelating activity (MITANI *et al.*, 2001). The mechanisms of action of kojic acid are similar to those of L-cysteine and ascorbic acid since they occur through interference with the absorption of O_2 required for the enzymatic reaction, reduction of the quinones to diphenols, or the combination of both (CHEN *et al.*, 1991).
The antioxidant activity (DPPH scavenging) of SE+CC treatment was higher than all the other treatments during the entire period of storage (Fig. 2a), with significant decrease (24.3 %) from day 0 to day 9. This result can be attributed to sodium erythorbate ability to prevent oxidation (FIGUEIREDO *et al.*, 2014; REISCHE *et al.*, 2002); or capture of the oxygen present in the medium through stable chemical reactions, preventing this oxygen of acting as propagator of auto-oxidation or as synergist in the regeneration of primary antioxidants (RAMALHO and JORGE, 2006). On the other hand, the EA+CC treatment showed a very similar activity to SE+CC treatment during the first six days of storage. However, a marked drop of DPPH activity (35.8 %) was observed between day 6 and day 9. This decrease can be attributed to the non-enzymatic degradation of erythorbic acid in aerobic conditions (CORZO-MARTÍNEZ *et al.*, 2012; CROPOTOVA *et al.*, 2016). Other treatments, W+CC, LC+CC and KA+CC, although oscillating and presenting statistical

difference between them, maintained a mean DPPH activity of 91.9 mg Trolox per 100 g of FW.

'Royal Gala' apple treated with SE+CC and EA+CC showed the highest values of total phenolic content (Fig. 2b), which was maintained throughout the storage period. Other treatments (W+CC, LC+CC and KA+CC) showed total phenolic compounds contents within the values reported in the literature for apples, 50 mg per 100 g FW to 380 mg per 100 g FW (CEYMANN *et al.*, 2012). Sodium erythorbate was reported to preserve the phenolic compounds in MP oyster mushroom (VENTURA-AGUILAR *et al.*, 2017). Ascorbic acid is a well-known antioxidant that prevents the polyphenol degradation due to its reducing action (GIL *et al.*, 1998). Thus, it can be inferred that its stereoisomer, erythorbic acid, has a similar capacity, effectively preserving the polyphenols content as observed in this experiment. Results of phenolic compounds and antioxidant activity in apples were correlated. Treatments that preserved the largest amount of phenolics were the same ones that maintained the highest antioxidant activity. Besides the antioxidant capacity, phenolic compounds are also responsible for apples flavor (ZHANG *et al.*, 2017). In addition, they have biological functions such as immunomodulatory and anti-inflammatory properties in humans and animals (ZHANG *et al.*, 2017). Therefore, the preservation of these properties is important to maintain the quality of the fruit and, consequently, the health benefit for consumers.

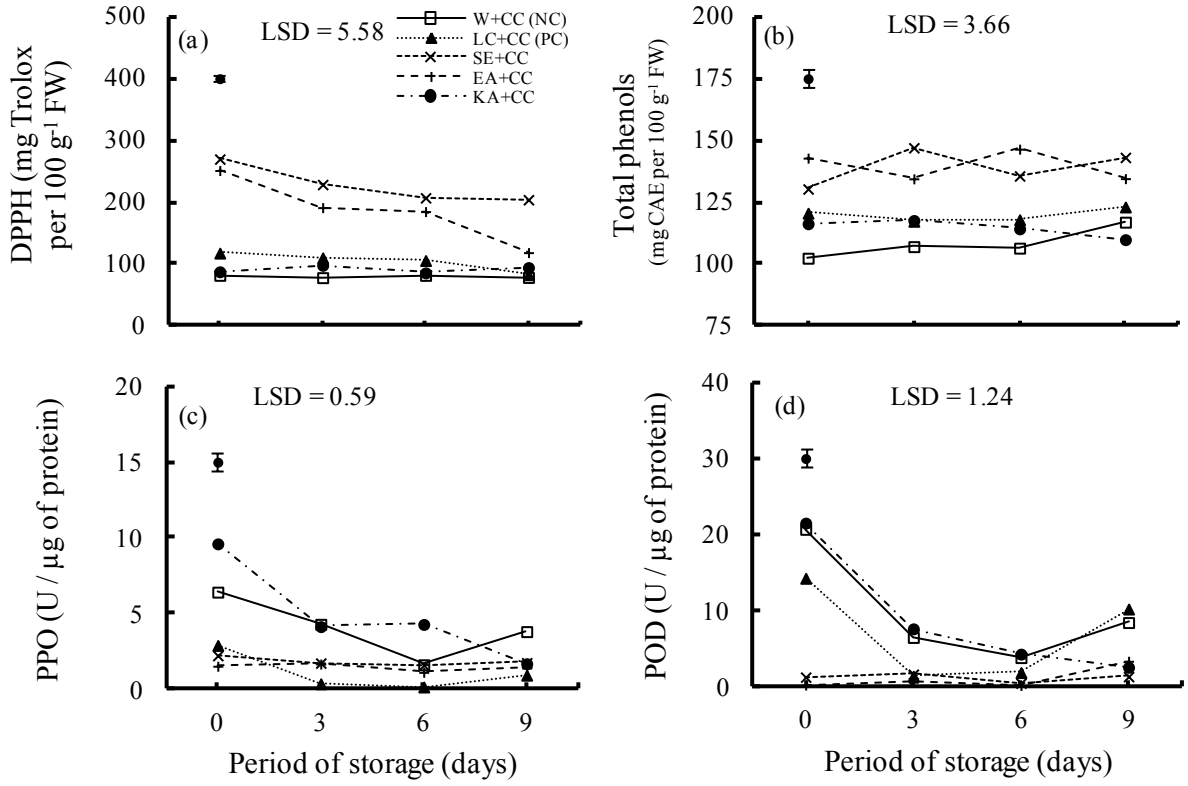


Figure 2. Effect of sodium erythorbate 5.0 % (SE) + calcium chloride 1.0 % (CC), erythorbic acid 3.0 % (EA) + CC and kojic acid 0.07 % (KA) + CC on DPPH activity (**a**), total phenolic content (**b**), PPO (**c**) and POD (**d**) activities for minimally processed 'Royal Gala' apple recorded at 0 d, 3 d, 6 d and 9 d, at 4.0ºC. Negative control (NC) consisted of distilled water (W) + CC, and positive control (PC) consisted of L-cysteine chloride 0.6 % (LC) + CC. The vertical bar indicates the Least Significant Difference (LSD) with ($p \leq 0.05$).

Regarding the enzymatic activity, changes in PPO activity (Fig. 2c) were treatment-dependent. Apples treated with SE+CC, EA+CC and LC+CC showed the lowest enzymatic activity. This result was reflected in the preservation of total phenolics, antioxidant activity, and maintenance of MP fruit color. Inactivation of PPO by EA+CC and LC+CC can also be attributed to the low pH of these solutions (2.22 and 1.62 respectively). As the ideal pH for PPO activity is 7.0, by reducing the pH its activity decreases rapidly, suggesting that the effect of pH is very important for most solutions that aim to inhibit the browning of the MP pulp. Similar results were obtained by TSOUVALTZIS and BRECHT (2017) studying "Russet Burbank" potatoes where the immersion of MP tubers in H_2SO_4 (< 0,04 %), pH 2.39, reduced the PPO activity in comparison to control samples. They attributed the reduction of PPO activity to the deviation from the ideal pH (5 to 7). Another mechanism for browning inhibition of L-cysteine is attributed to its conjugation with *o*-quinones, forming colorless compounds, or by reducing *o*-quinones to the precursor phenolic compounds (CILLIERS and SINGLETON, 1990). According to RICHARD-FORGET *et al.* (1992), these conjugated compounds may act as competitive inhibitors of PPO. However, they advert that in the presence of an excess of quinones, after all cysteine is consumed, the quinones can react with the cysteine-quinone addition compounds, forming violet pigments (RICHARD-FORGET *et al.*, 1992), which were not observed in our experiment. Nevertheless, SE+CC (pH 7.0) inactivated almost completely the enzymatic activity regardless of the pH value of the antioxidant solution. This antioxidant agent has no direct effect on PPO activity, but rather on browning since this compound acts as a free radical scavenger and changes the redox potential, reducing *o*-quinones to diphenols (MOSNEAGUTA *et al.*, 2012). KA+CC treatment did not completely inhibit the enzymatic activity, probably because this acid could not act satisfactorily in one of these mechanisms: prevent the absorption of the O_2 required for the enzymatic reaction; to reduce the quinones to diphenols; or to the combination of the two previously mentioned (CHEN *et al.*, 1991). Besides the positive effect provided by antioxidants, calcium chloride, which has similar properties to sodium chloride, generates chlorine dioxide under acidic conditions, which is involved in the PPO inhibition (GOMES *et al.*, 2014). This observation suggests that the enzymatic inactivation results from synergism between antioxidants agents and $CaCl_2$.

Although PPO is recognized as the main enzyme related to enzymatic browning in apples, it is also necessary to study the changes in POD enzymes activity, since these enzymes may also contribute to change the color of this fruit (JANG and MOON, 2011). Results obtained for POD activity (Fig. 2d) and PPO activity (Fig. 2c) were similar. Apples treated with LC+CC showed the highest enzymatic activity at day zero, which decreased at day 3 and day 6, increasing at day 9. POD activity was close to zero for SE+CC and EA+CC treatments. Since these antioxidants are structurally similar to ascorbic acid (AA), this effect is in accordance with those of JANG and MOON (2011), who reported that the presence of AA effectively reduced POD activity in MP apples. Reduction of POD activity in fruits treated with AA could be the result of lower oxidative stress on their surface due to the antioxidant nature of AA or the POD-enzyme-hydrogen-donor complex formation (SABA and SOGVAR, 2016). These results suggest that treatments containing sodium erythorbate and erythorbic acid may be an efficient way to maintain the quality of MP apples without pulp browning during storage.

Concerning the respiratory gases, oxygen (O_2) and carbon dioxide (CO_2) in the headspace of the packages, all the treatments showed a similar pattern, with a decrease in O_2 (Fig. 3a) and increase in CO_2 (Fig. 3b) over the 9 d of storage. The O_2 concentration for all the treatments (LC+CC, SE+CC, EA+CC, KA+CC) remained lower than the control treatment (W+CC), possibly due to the higher metabolism of apples not treated with antioxidants.

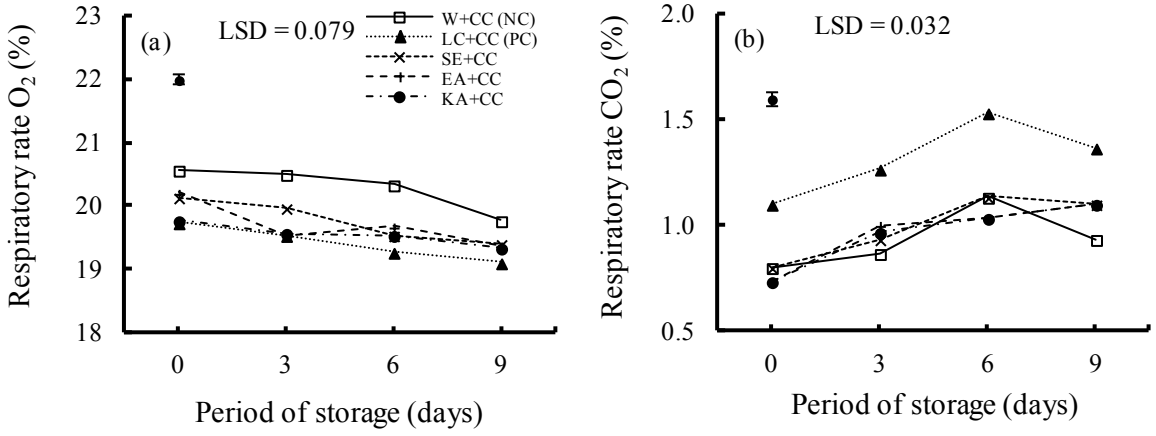


Figure 3. Respiratory rate of minimally processed 'Royal Gala' apples treated with sodium erythorbate 5.0 % (SE) + calcium chloride 1.0 % (CC), erythorbic acid 3.0 % (EA) + CC and kojic acid 0.07 % (KA) + CC based on concentration of O_2 (**a**) and concentration of CO_2 (**b**) in the headspace of the pack, recorded at 0 d, 3 d, 6 d and 9 d, at 4.0ºC. Negative control (NC) consisted of distilled water (W) + CC and positive control (PC) consisted of L-cysteine chloride 0.6 % (LC) + CC. The vertical bar indicates the Least Significant Difference (LSD) with ($p \leq 0.05$).

The respiratory process consumes O_2 and releases CO_2 because of the oxidation of carbohydrates, lipids and proteins, which are present in the product. In addition, higher respiration rates indicate faster overall metabolism and deterioration (VENTURA-AGUILAR *et al.*, 2017). According to MANURAKCHINAKORN *et al.* (2012), the reduction of O_2 and the increase of CO_2, a consequence of the respiratory process, is detectable in MP fruits even if stored in closed containers permeable for gas exchange. Interestingly, for unknown reasons, apples treated with LC+CC accumulated in the packs an average of 26.9 % more CO_2 than the apples treated with other antioxidants.

Sensory analysis showed that apples treated with the antioxidants LC+CC, SE+CC and EA+CC had no noticeable darkening in the pulp (Fig. 4a), being classified as very good and excellent. On the other hand, apples treated with W+CC presented a considerable increase in browning during the nine days of evaluation, reaching the marketing limit from the sixth day. The KA+CC also showed increased browning, however with lower intensity. Flavor parameter (Fig. 4b) was only affected for apples treated with LC+CC. Acceptability of apples treated with LC+CC was lower than for other treatments, already on the first day of evaluation. This behavior was intensified along the storage periods to the point of being between the marketing and inedible limits. The presence of calcium chloride did not significantly modify the flavor of the samples, which corroborates that this salt does not modify the flavor of the fruits. Due to the high acidity of the EA+CC treatment, it was necessary to carefully reduce the droplets of this antioxidant on the epidermis of the apple slices, since when this happened, the evaluators noticed and reported the unpleasant sensation caused by this antioxidant solution. This peculiarity makes this treatment a secondary option of using this product. Considering the odor attribute, apples treated with LC+CC also showed low acceptability (Fig. 4c). Evaluators were able to perceive the sulfur-containing compounds in the samples containing L-cysteine, which agrees with the results obtained by PEREZ-GAGO *et al.* (2006). Other treatments, although with lower intensity, also had a decrease in the odor attribute along the storage. This decrease can be attributed to the volatilization of compounds responsible for the aroma of the fruit due to damages caused in the pulp during the minimal processing. The attributes succulence and firmness (data not presented) did not present

statistical difference between the treatments, being in the range between very good and excellent. When including all evaluated attributes and score the overall quality, apples treated with SE+CC, EA+CC and KA+CC presented high acceptability since all evaluated attributes were satisfactory (Fig. 4d).

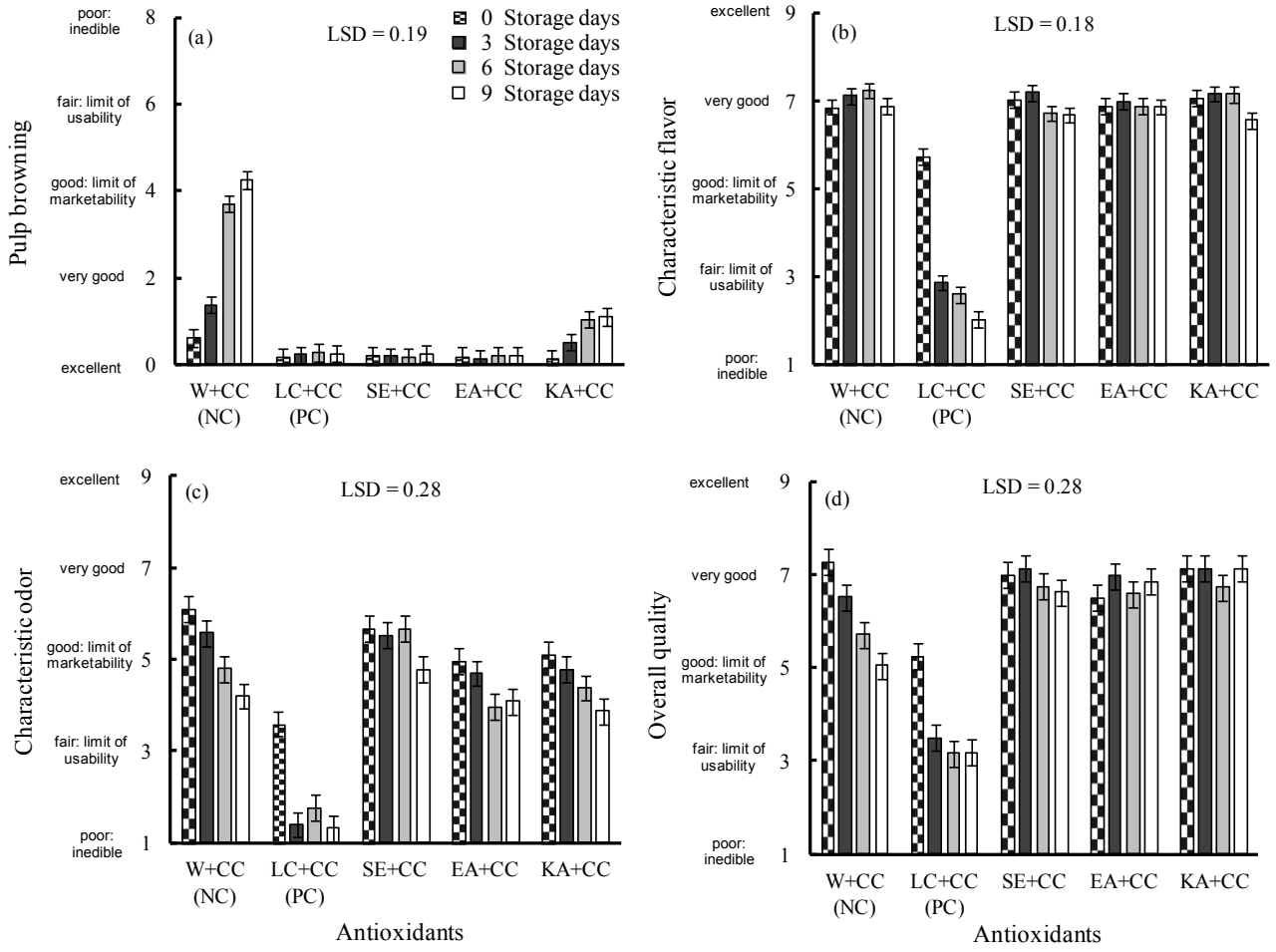


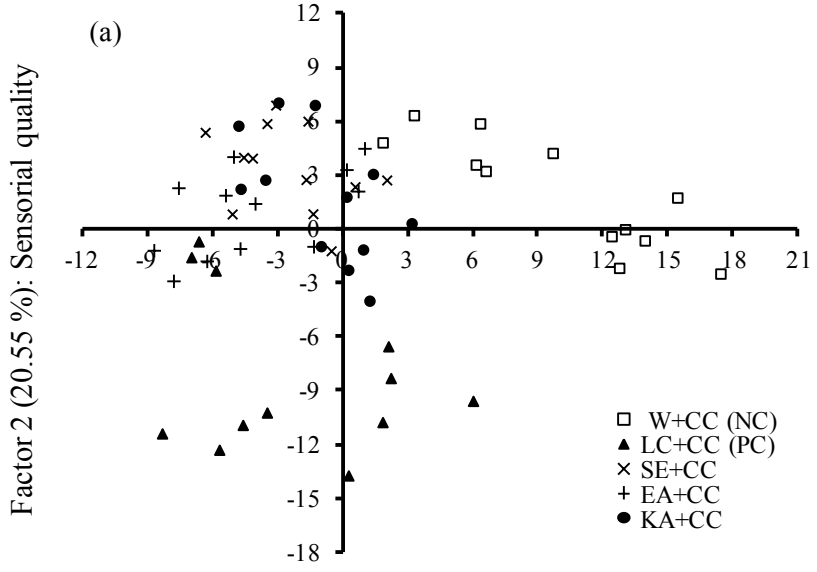
Figure 4. Sensorial characteristics of minimally processed 'Royal Gala' apple treated with sodium erythorbate 5.0 % (SE) + calcium chloride 1.0 % (CC), erythorbic acid 3.0 % (EA) + CC and kojic acid 0.07 % (KA) + CC, and recorded at 0 d, 3 d, 6 d and 9 d; at 4.0ºC: pulp browning (**a**); characteristic flavor (**b**), characteristic odor (**c**) and overall quality (**d**). Negative control (NC) consisted of distilled water (W) + CC, and positive control (PC) consisted of L-cysteine chloride 0.6 % (LC) + CC. The vertical bar indicates the Least Significant Difference (LSD) with ($p \leq 0.05$).

Opposite situation occurred with W+CC treatment, mainly due to the appearance of undesirable brown pigments on the surface of the slices due to the oxidation processes. LC+CC treatment was able to inhibit pulp browning completely, however, the apple taste and odor significantly changed, leading to low acceptability of apples treated with this antioxidant. The changes caused by L-cysteine in the odor are attributed to enzymatic reactions, mainly the degradation of methionine, cysteine and derivatives. These enzymatic degradations lead to the formation of volatile compounds containing aliphatic sulfur, such as thiols and polysulfides (VARLET and FERNANDEZ, 2010). In view of these results, the application of sodium erythorbate and erythorbic acid proved to be viable alternatives for the maintenance of the sensorial and physical-chemical attributes of

apples evaluated in this study. In addition, erythorbic acid has a relatively low cost because calcium 2-ketogluconate is easily converted during glucose fermentation (LEE *et al.*, 2012). This antioxidant is also reported to potentiate the non-heme iron uptake (FIDLER *et al.*, 2004), helping to provide the nutritional needs of iron (SINGH *et al.*, 2016). According to HALAGARDA and SUWALA (2018), the importance of sensory analysis at every stage of the product development or improvement process is due to significant cost and failure reduction after product launch. Moreover, the fulfilment of expectations, especially the sensorial ones, leads to consumer satisfaction, increasing the likelihood of commercialization of the product (GRUNERT, 2002).

The factorial analysis allowed us to verify the most important factors for fruit quality. In this analysis, five factors explained 70.86 % of the total variability of the experiment (data not shown). The Factor 1 (termed pulp color) explained 26.25 % of total variability (Fig. 5a), and showed higher correlation coefficients with the variables index of darkening (0.963), b* (0.946), chroma (0.942) and ºHue (-0.875). On the positive axis of the abscissa are the variables with the higher positive correlation coefficients values (index of darkening, b*, sensorial darkening and chroma) and on the negative axis is the variable with the higher negative correlation coefficient value (Hue).

It appears that these variables evolved together, being classified in the same factor, which indicates that the differences were detected by the sensory panel of judges. Regarding the distribution of the treatments in the factors, it can be observed that the treatment W+CC (negative control), as expected, resulted in slices of apples with higher levels of darkening, being grouped in the positive axis of the abscissa on the graph. The other treatments, with a low level of darkening, were grouped predominantly on the negative axis of the abscissa. The Factor 2 (termed sensorial quality), which explains 20.55 % of total variability, showed higher correlation coefficients with the variables odor (0.909), taste characteristics (0.882) and overall quality (0.865). All of them were located on the positive axis of the ordinate. It can be observed that the values of sensory quality in apples treated with SE+CC, EA+CC and KA+CC, resulted in a good level of acceptability by the sensory panel of judges. On the other hand, the application of LC+CC presented lower sensory quality. In relation to the storage periods (0 d, 3 d, 6 d and 9 d) it can be observed (Fig. 5b) that periods in which the MP fruit presented the best quality are located on the positive axis of the ordinate axis, and MP fruits of inferior quality are located on the negative axis of the ordinate.



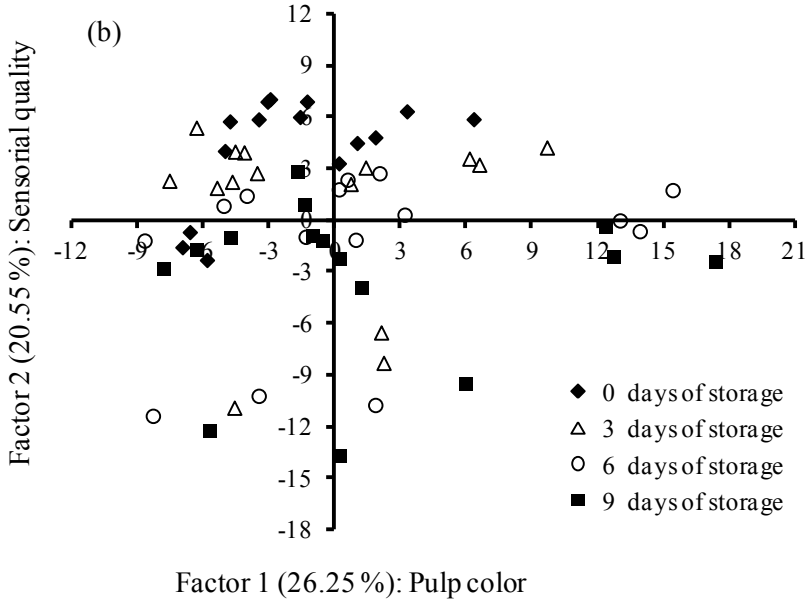


Figure 5. Factorial analysis of minimally processed 'Royal Gala' apples treated with sodium erythorbate 5.0 % (SE) + calcium chloride 1.0 % (CC), erythorbic acid 3.0 % (EA) + CC and kojic acid 0.07 % (KA) + CC (a) recorded at 0 d, 3 d, 6 d and 9 d (b); at 4.0ºC. Factor 1: color and Factor 2: sensorial quality after Varimax orthogonal rotation. Negative control (NC) consisted of distilled water (W) + CC, and positive control (PC) consisted of L-cysteine chloride 0.6 % (LC) + CC. The vertical bar indicates the Least Significant Difference (LSD) with ($p \leq 0.05$).

This result indicates that MP apples slices lose quality as the refrigerated storage period increased. However, this reduction in quality is a normal process, as many compounds are metabolized, resulting in senescence and cell death.

4. CONCLUSIONS

Apple browning is of high concern for the industry of minimally processed fruits since it has a high impact on color, flavor and nutritional value. Our results showed that treatment with either 5 % sodium erythorbate or 3 % erythorbic acid, associated with 1 % calcium chloride, controlled the apple pulp browning and preserved the phenolic compounds and the antioxidant activity for up to 9 days under cold storage. Besides, both antioxidants significantly inhibited the peroxidase and polyphenoloxidase enzymes and preserved the sensorial quality, which was found to be equivalent to the fresh fruit. Thus, the treatment of MP 'Royal Gala' apples with 5 % sodium erythorbate or 3 % erythorbic acid, associated with 1 % calcium chloride, can be suitable to extend the product shelf-life.

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