

RESEARCH ARTICLE

Modified atmosphere using propolis in post-harvested 'Eva' organic apple

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SUMMARY

The objective of this study was to evaluate the efficacy of different propolis extracts in post harvesting 'EVA' organic apple, in order to prolong storage and fruit quality maintenance. After selection, fruits were sanitized with vinegar (6%) solution, dried in the air and immersed in propolis solution for 1 minute when it has been possible. Treatments were T1: Control without application; T2: 1.5% propolis alcoholic extract (1.5% EEP); T3: 2.5% propolis alcoholic extract (2.5% EEP); T4: 1.5% aqueous propolis extract (1.5% EAqP) and T5: 2.5% aqueous propolis extract (2.5% EAqP). Fruits were packed in rigid plastic boxes and stored in a cold room with temperature of $5 \pm 1^\circ\text{C}$ and $85 \pm 1\%$ RH for 80 days. Every 10 days some traits were evaluated as fruit weight loss (%), respiratory activity ($\text{mL of CO}_2 \text{ kg}^{-1} \text{ of fruit}^{-1} \text{ hour}^{-1}$), luminosity, chroma, Hue angle, pH, soluble solids ($^\circ\text{Brix}$), titratable acidity ($\text{g of malic acid } 100\text{g}^{-1}$) and reducing and non-reducing sugars. The experimental design was completely randomized in a factorial scheme and data were submitted to F test ($p < 0.05$) and significant interactions were deployed via regression analysis. The application of propolis extract (aqueous and alcoholic) in 'Eva' organic apple post-harvest does not prolong refrigerated storage ($5 \pm 1^\circ\text{C}$ and $85 \pm 1\%$ RH) and does not influence in fruit quality conservation.

Keywords: Coating; *Malus domestica*; Quality; Storage

INTRODUCTION

Storage is one of the most important factors in maintaining fruit quality and making it possible to supply market for long periods. Apple culture, for example, has made good progress over the years with investments in chambers and new technologies such as storage under a controlled modified atmosphere (Petri et al., 2011).

However, more accessible techniques may be used in addition to refrigeration. Among known methods which may be used to extend fruit storage, it is possible to highlight the use of passive modified atmosphere, which may be by plastic films (packaging) use or coating with special waxes (Daiuto et al., 2012), among them propolis.

Propolis is a natural resinous compound produced by *Apis mellifera* bees from which they extract from various

plant exudates and behave as hives protection layer against microorganisms such as fungi and bacteria proliferation (Meneses et al., 2009; Silva et al., 2006). The composition of propolis is directly related to region vegetation from it is extracted, being composed of resins (phenolic and sterile compounds), essential oils, balsams, waxes, vitamins and pollen (Zahid et al., 2013; Konishi et al., 2004).

It is used medicinally for having many biological properties such as: antitumor effect, antioxidant, antimicrobial, anti-inflammatory and immune modulatory, among others. These biological activities are attributed mainly to phenolic compounds in its composition, such as flavonoids. The concentration of it, in its extract, is directly dependent of used extractor, as in its composition there are soluble substances in water and oil (Mello et al., 2010; Viuda-Martos et al., 2008).

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Some studies have shown positive results when propolis extract was used in Pita fruits post-harvest quality (Zahid et al., 2013), strawberry (Minarelli et al., 2014), avocado (Daiuto et al., 2012; Santos et al., 2015), 'Ponkan' tangerine (Cantillano et al., 2011), 'Moscatel' grape (Pastor et al., 2011) and 'Prata' banana (Passos et al., 2016). However, in apple, few studies have been carried out analyzing post-harvest propolis extract.

The objective of this study was to evaluate the efficacy of different propolis extracts in organic 'Eva' apple post-harvested fruits, in order to prolong storage and maintenance of fruit quality.

MATERIALS AND METHODS

The organic 'Eva' apple fruits used in study were produced in commercial production area at Mitsuo Hino Farm, located in Botucatu, São Paulo State, Brazil (22° 95' 97" S and 48° 45' 02" W), in 2015/2016 crop season. They were harvested at physiological maturation stage and then stored in a cold room at 5°C, in the rural property itself.

Afterwards fruits were transported in plastic boxes to São Paulo State University (UNESP), School of Agriculture, in municipality of Botucatu, and conducted at Laboratory of Fruits and Vegetables of Department of Horticulture (Fig. 1). After receiving the fruits, they were submitted to visual selection aiming lot uniformity. Then, they were washed in running water and sanitized by immersion in vinegar solution (6%) for 10 minutes, hygienizing allowed for organic products (Brasil, 2009). After this process they were placed on a laboratory bench lined with pink semi Kraft® paper to drain water and dry fruits.

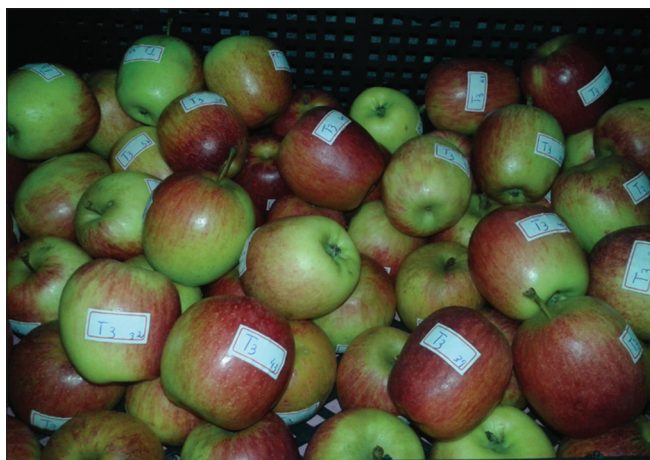


Fig 1. organic 'Eva' apple fruits used in study were produced in commercial production area at Mitsuo Hino Farm, located in Botucatu, São Paulo State, Brazil.

The propolis, produced by *Apis mellifera* L. bees was collected at Apiculture area of School of Veterinary Medicine and Animal Science from the same university, located at Edgárdia Farm (22° 82' S and 48° 39' W).

For alcoholic extract (EAP) the propolis was crushed transferring 30 g to a Griffin's cup adding ethyl alcohol (70%) until 100mL. Immediately the solution was stored in amber glass shaking daily for one minute during seven days for ethanolic propolis extract (EEP) preparation. After this period solution was purified in analytical filter and stored in amber glass (Orsi et al., 2000). For aqueous extract (EAQp) ethyl alcohol was replaced by mineral water. to facilitate preparation by producers, and following the same methodology of alcoholic extract. From the 30% extract, dilutions were performed until extracts reached concentrations of 1.5% and 2.5%.

The treatments were: T1 – Control, without application; T2 – 1.5% alcoholic propolis extract (1.5% EEP); T3 – 2.5% alcoholic propolis extract (2.5% EEP); T4 – 1.5% aqueous propolis extract (1.5% EAQp) and T5 – 2.5% aqueous propolis extract (2.5% EAQp). The extracts application was by immersion for one minute, according to methodology described by Minarelli et al. (2014).

After treatments application, apples were placed to dry in the air. Fruits were packed in rigid plastic boxes (dimension: 30 x 36 x 55 cm) and stored in cold room at temperature of $5 \pm 1^\circ\text{C}$ and $85 \pm 1\%$ RH for 80 days.

Fruit quality determinations were obtained through following evaluations: mass loss (%), respiratory activity ($\text{mL of CO}_2 \text{ kg}^{-1} \text{ of fruit}^{-1} \text{ hour}^{-1}$) following guidelines of Bleinroth et al. (1976), luminosity, chroma, Hue angle (Minolta, 1998). The pH, soluble solids ($^\circ\text{Brix}$) and titratable acidity ($\text{g of malic acid } 100\text{g}^{-1}$) were analysed according to methodology cited by Brasil (2008), maturation index (Tressler and Joslyn, 1961) and reducing and non-reducing sugars (Somogy, 1945 adapted by Nelson, 1944).

The experimental design was completely randomized in a 5 x 9 factorial scheme (five treatments x 9 storage periods) with three replications. Data were submitted to F test ($p < 0.05$) and significant interactions were deployed via regression analysis and means compared by Tukey test when the regression was not significant.

RESULTS AND DISCUSSION

Climacteric behavior (Fig 2) was observed in the study as reported by Vieites et al. (2014) and Fante et al. (2013). The climacteric peak of the control, 1.5% propolis alcoholic

extract (EAP), 2.5% propolis alcoholic extract (EAP) and 1.5% aqueous propolis extract (EAqP) occurred within 70 days of storage. However, 1.5% EAP treatment and 1.5% EAqP presented the lowest peaks (36.2 mL and 39.5 mL of $\text{CO}_2 \text{ kg}^{-1} \text{ fruit}^{-1} \text{ hour}^{-1}$, respectively) when compared to control treatment (56.9 mL $\text{CO}_2 \text{ kg}^{-1} \text{ fruit}^{-1} \text{ hour}^{-1}$). Possibly the extracts 1.5% EAP and 1.5% EAqP worked as fruits coating, reducing gas exchange to external environment. Daiuto et al. (2012), researching 'Hass' avocado cited delay in climacteric peak in fruits treated with propolis alcoholic extract (2%) and wax. In contrast, treatment with 2.5% EAqP advanced fruits climacteric peak for tenth day, with respiratory rate of 69.6 mL of $\text{CO}_2 \text{ Kg}^{-1} \text{ fruit}^{-1} \text{ hour}^{-1}$, possibly advancing fruits senescence.

For mass loss (Table 1) significant difference was observed at treatments means ($p < 0.05$) and storage period ($p < 0.01$). The lowest fresh mass losses occurred in fruits submitted to 1.5% EAP (6.4%), not differing statistically from control (6.5%), 2.5% EAP (6.7%) and 2.5% EAqP (6.9%), evidencing that treatment with propolis in organic 'Eva' apples was not effective to reduce mass loss, as treatment with less mass loss have not differed from control (Fig. 3). Opposite results were obtained in avocado treated with propolis alcoholic extract (2%) (Daiuto et al., 2012), strawberry treated with propolis aqueous extract (1%) (Minarelli et al., 2014)

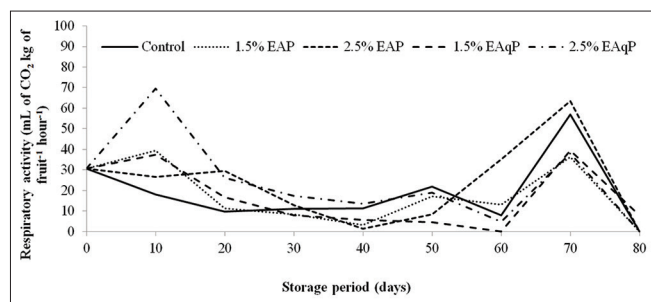


Fig 2. Respiratory activity (mL of $\text{CO}_2 \text{ kg}^{-1} \text{ fruit}^{-1} \text{ hour}^{-1}$) in organic 'Eva' apple with application of different propolis extracts over 80 days of storage. EAP: alcoholic propolis extract, EAqP: aqueous propolis extract.

and agroecological avocado coated by propolis alcoholic extract (30%) (Santos et al., 2015).

In relation to period of storage it is possible to notice that mass loss behaved in an increasing linear way (Fig 4). At eightieth day of storage it has lost 13.5% of mass. This behavior is probably directly related to water loss through transpiration and solutes (carbohydrates) via glycolysis during respiration. Climacteric fruit has an intrinsic increase in respiration rate at a certain stage of its life cycle (Chitarra and Chitarra, 2005). The same authors mention that losses from 3 to 6% are capable of causing loss of quality in stored fruits. In the storage conditions of current research ($5^\circ\text{C} \pm 1^\circ\text{C}/80\% \pm 1\% \text{ RH}$) organic 'Eva' apple fruits have lost 6% of mass at 35 days of storage.

Soluble solids (SS) contents were not influenced by propolis extracts application and even for the interaction between them ($p > 0.05$) (Table 2). Santos et al. (2015), reported as current research, there was no influence of propolis extracts on SS contents in agroecological avocado. Passos et al. (2016), researching about propolis extracts and despite finding influence on levels of SS between treatments, mentioned that treatments that better conserved the contents (aqueous, wild and green) did not statistically differentiate from control.

Contents from 13.3 °Brix to 16.7 °Brix were observed in 'Eva' organic apple fruits, with a general average of 14.9 °Brix. Similar values were found by Vieites et al. (2014) working with organic 'Eva' apple from the same production area, 13.9 °Brix to 15.8 °Brix. Chagas et al. (2012) reported levels of 15.2 °Brix to grow conventional 'Eva'. Oliveira et al. (2014) observed 9.2 °Brix to 13.6 °Brix in apple 'Eva' harvested in five seasons and Fante et al. (2013) found levels of 13.2 °Brix to 14.8 °Brix in apples of same cultivar produced in a conventional system, both works performed in Minas Gerais State. The fruits have a biological character and they present numerous changes in their composition according to each variety characteristics, climatic conditions, soil, management and harvesting point.

Table 1: Mass loss (%) in organic 'Eva' apple with application of different propolis extracts over 80 days of storage

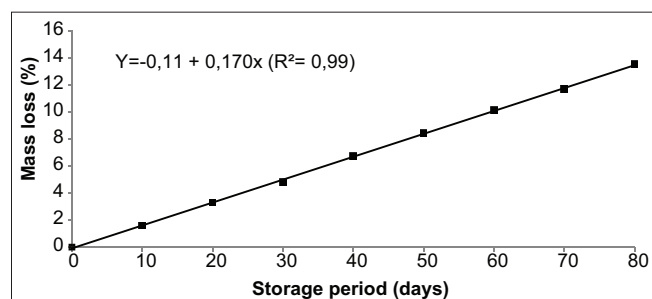
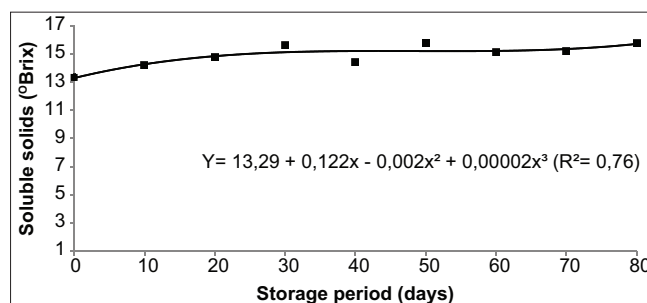
Treatments	Storage (days)									Mean
	0	10	20	30	40	50	60	70	80	
Control	0.00	1.50	3.20	4.70	6.50	8.20	9.70	11.50	13.10	6.50 ^{ab}
1.5% EAP	0.00	1.50	2.90	4.60	6.20	8.00	9.70	11.20	12.90	6.40 ^b
2.5% EAP	0.00	1.70	3.10	4.80	6.80	8.40	10.10	11.70	13.40	6.70 ^{ab}
1.5% EAqP	0.00	1.70	3.30	5.20	7.10	8.90	10.70	12.30	14.20	7.10 ^a
2.5% EAqP	0.00	1.70	3.10	4.90	7.10	8.80	10.40	12.10	13.90	6.90 ^{ab}
D.M.S.						0.70				
C.V. (%)						17.4				

Means followed by the same lowercase letter in the column do not differ from each other by Tukey test at 95% probability. EAP: alcoholic propolis extract, EAqP: aqueous propolis extract

Table 2: Soluble solid (°Brix) in organic 'Eva' apple with application of different propolis extracts over 80 days of storage

Treatments	Storage (days)									Mean
	0	10	20	30	40	50	60	70	80	
Control	13.30	14.30	14.30	16.00	14.10	14.80	15.30	15.8	16.70	15.00
1.5% EAP	13.30	14.40	15.60	14.90	14.60	16.20	14.60	14.90	16.40	15.00
2.5% EAP	13.30	14.60	14.60	16.00	12.90	16.00	14.90	15.10	15.30	14.70
1.5% EAqP	13.30	13.80	14.60	15.40	15.70	16.40	15.70	15.20	15.60	15.10
2.5% EAqP	13.30	13.80	14.60	15.80	14.80	15.70	15.10	15.10	15.00	14.80
D.M.S.						0.82				
C.V. (%)						7.20				

EAP: alcoholic propolis extract, EAqP: aqueous propolis extract.

**Fig 3.** Organic 'Eva' apple with application of different propolis extracts at 30 days of storage.**Fig 4.** Mass loss (%) in organic 'Eva' apple with application of different propolis extracts over 80 days of storage.**Fig 5.** Soluble solids (°Brix) in organic 'Eva' apple with application of different propolis extracts over 80 days of storage.

For storage period, soluble solids contents increased (Fig 5), starting from 13.3 °Brix at experiment assembly time, achieving 15.78 °Brix at the end of experiment time. This soluble solids increase tends to occur during ripening due to polysaccharides (starch) degradation reaching maximum value at the end of ripening, conferring quality to the product (Chitarra and Chitarra, 2005). The same behavior was described by Fante *et al.* (2013) in 'Eva' apple with 135 days of storage. Authors justify this increase to fruit maturation and/or concentration of these solutes due to the loss of fresh mass.

In relation to pH values (Table 3) there was significant difference at treatments means ($p < 0.01$) and storage period ($p < 0.01$) with values from 3.91 to 4.16. Vieites *et al.* (2014) reported lower values between 3.73 and 3.84 in fruits from the same growing site. Among treatments, the lowest value was observed in the fruits covered with 2.5% EAqP (3.88) when compared to control (4.05). However, pH value of control fruits have not statistically differed from treatment 2.5% EAP and 1.5% EAqP (3.93 and 3.90, respectively). This lower pH value in 'Eva' for 2.5% EAqP treatment may be related to the greater conservation in storage and/

or decrease of microbial contamination due to propolis action, as according to Jacxsens *et al.* (2003) the microbiota (bacteria) actively participates in plants degradation, resulting the increase of pH, result of protein breakdown and compounds release. These results corroborate with Izumi *et al.* (1996), who associated pH increase in minimally processed zucchini with microbiological contamination.

The pH values during storage period (Fig 6) presented small increase (3.91 to 3.95). The pH tends to increase with fruit ripening due to the use of organic acids during respiration process and/or increase of their microbiota. Similar behavior was described by Passos *et al.* (2016) in 'Prata' banana using propolis hydro alcoholic extract, increasing pH values in storage. However, Minarelli *et al.* (2014) observed little pH interference during storage period of strawberries treated with aqueous propolis extract.

The titratable acidity (TA) of organic 'Eva' apples (Table 4) was not influenced by propolis extract application, storage time and the interaction between them ($p > 0.05$). Similarly, Passos *et al.* (2016) did not find differences between treatments in 'Prata' banana, Santos *et al.* (2015)

Table 3: Values of pH in organic 'Eva' apple with application of different propolis extracts over 80 days of storage

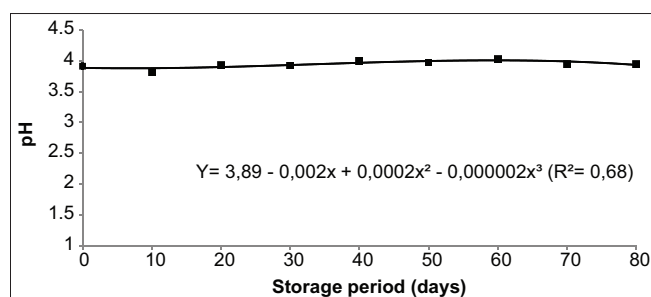
Treatments	Storage (days)									Mean
	0	10	20	30	40	50	60	70	80	
Control	3.91	3.98	4.08	4.01	4.08	4.06	4.16	4.09	4.13	4.05 ^a
1.5% EAP	3.91	3.86	3.95	3.93	4.00	3.99	4.00	3.99	3.98	3.95 ^b
2.5% EAP	3.91	3.78	3.89	3.94	4.00	3.97	4.01	3.99	3.89	3.93 ^{ab}
1.5% EAqP	3.91	3.73	3.88	3.88	3.99	3.96	4.00	3.91	3.87	3.90 ^{ab}
2.5% EAqP	3.91	3.78	3.85	3.86	3.96	3.89	3.98	3.81	3.89	3.88 ^c
D.M.S.	0.06									
C.V. (%)	2.00									

Means followed by the same lowercase letter in the column do not differ from each other by Tukey test at 95% probability. EAP: alcoholic propolis extract, EAqP: aqueous propolis extract.

Table 4: Titratable acidity (g of malic acid 100g⁻¹ of pulp) in organic 'Eva' apple with application of different propolis extracts over 80 days of storage

Treatments	Storage (days)									Mean
	0	10	20	30	40	50	60	70	80	
Control	0.40	0.40	0.40	0.42	0.39	0.40	0.36	0.40	0.41	0.40
1.5% EAP	0.40	0.42	0.41	0.37	0.60	0.48	0.42	0.37	0.39	0.43
2.5% EAP	0.40	0.45	0.42	0.34	0.38	0.44	0.37	0.38	0.41	0.40
1.5% EAqP	0.40	0.39	0.41	0.33	0.41	0.44	0.38	0.36	0.39	0.39
2.5% EAqP	0.40	0.36	0.40	0.35	0.43	0.45	0.44	0.37	0.39	0.40
D.M.S.	0.042									
C.V. (%)	13.70									

EAP: alcoholic propolis extract, EAqP: aqueous propolis extract.

**Fig 6.** Values of pH in organic 'Eva' apple with application of different propolis extracts over 80 days of storage.

in agroecological avocado and Minarelli et al. (2014) in strawberry. Titratable acidity contents of 0.33 to 0.60 g of malic acid 100g⁻¹ were observed in apple fruits regardless of treatment; similar contents, 0.32 to 0.43 g of malic acid 100g⁻¹ were found by Oliveira et al. (2014) and Paganini et al. (2004) and higher by Vieites et al. (2014), 0.69 to 0.96 g of malic acid 100g⁻¹ and Chagas et al. (2012), 0.63 g of malic acid 100g⁻¹.

For maturation index (SS/TA) (Table 5), there was no statistical difference for application of propolis extract and for interaction ($p > 0.05$). It was observed only influence for storage time at maturation index ($p < 0.01$), evidencing that propolis extract did not interfere in apple maturation, as behavior of treatments did not differ from control.

Values of 22.2 were observed at the beginning of the experiment and 26.8 at the end for maturation index.

Varieties of apples with maturation index lower than 20 are more suitable for agroindustry for juices and citrons production, while fruits with higher SS/TA are classified as sweet and are indicated for table purpose (Chagas et al., 2012; Czelusniak et al., 2003). Using this classification organic 'Eva' apples from current research would be destined for in natura consumption.

At storage period (Fig 7) the values of maturation index showed increase in shelf life. This behavior indicates fruit ripening, expected behavior for climacteric fruits. Fante et al. (2013) also found the same behavior, increased SS/TA values, from 22.8 to 28.4 at 135 days of storage for the same apple cultivar.

Table 6 shows the values of reducing sugar (RS) contents. There was no statistical difference between treatments and for interaction between studied factors ($p > 0.05$), there was influence only at storage period ($p < 0.01$). Reducing sugar contents were observed from 6.4% to 8.8% during all period of evaluation. Fante (2011) cited similar contents, 6.4% in conventional 'Eva' apple fruits, while higher values were found by Paganini et al. (2004) of 13.2% of glucose. Sugars have an effect on sensorial properties, on nutritional value and are considered quality indicators. In apples which present high levels of fructose they are considered as functional food, because this reducing sugar is absorbed in intestine and metabolized in epithelial cells, being thus classified as dietary and can be consumed by people with metabolic diseases (Wu et al., 2007; Czelusniak et al., 2003).

Table 5: Maturation index (SS/TA) in organic 'Eva' apple with application of different propolis extracts over 80 days of storage

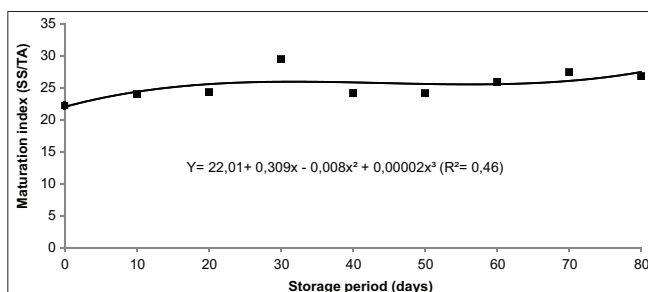
Treatments	Storage (days)									Mean
	0	10	20	30	40	50	60	70	80	
Control	22.2	24.3	24.1	25.8	23.9	25.0	29.2	26.8	27.7	25.4
1.5% EAP	22.2	23.0	25.9	28.1	24.7	23.2	22.9	27.3	28.0	25.0
2.5% EAP	22.2	22.7	23.5	32.5	23.5	24.3	26.8	26.5	25.2	25.2
1.5% EAqP	22.2	24.0	23.8	31.0	25.7	24.8	27.5	28.8	27.0	26.1
2.5% EAqP	22.2	26.0	24.6	29.9	23.5	23.2	23.2	28.0	26.1	25.2
D.M.S.						2.1				
C.V. (%)						11.1				

EAP: alcoholic propolis extract, EAqP: aqueous propolis extract

Table 6: Maturation index (SS/TA) in organic 'Eva' apple with application of different propolis extracts over 80 days of storage

Treatments	Storage (days)									Mean
	0	10	20	30	40	50	60	70	80	
Control	6.4	7.5	6.5	7.3	6.8	7.4	7.8	7.7	8.0	7.3
1.5% EAP	6.4	7.5	6.8	6.5	6.5	7.3	7.5	7.5	8.8	7.2
2.5% EAP	6.4	7.1	6.2	6.5	6.7	7.0	7.3	7.5	8.1	7.0
1.5% EAqP	6.4	7.5	6.6	7.0	7.1	7.4	7.8	7.3	8.2	7.2
2.5% EAqP	6.4	7.9	6.0	6.6	6.9	7.0	7.9	7.8	7.2	7.1
D.M.S.						0.36				
C.V. (%)						6.60				

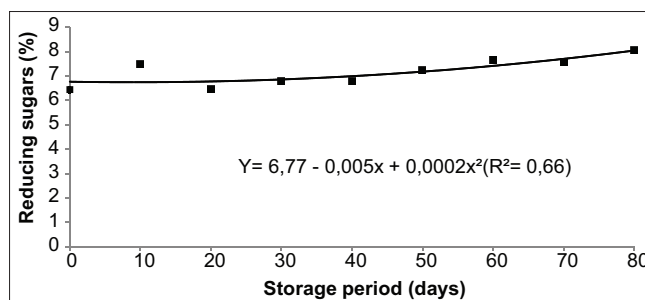
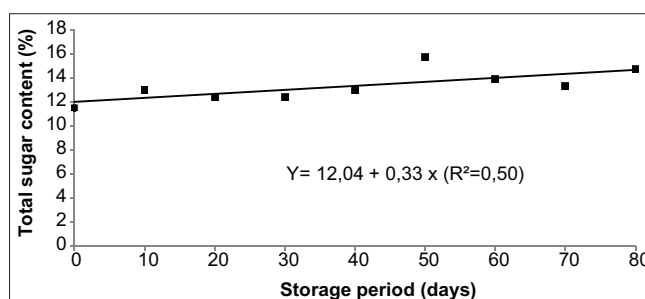
EAP: alcoholic propolis extract, EAqP: aqueous propolis extract

**Fig 7.** Maturation index (SS/TA) 'Eva' apple with application of different propolis extracts over 80 days of storage.

For evaluated period of time there was an increase in RS contents from 6.44% to 8.05% evidencing organic 'Eva' apple fruits maturation (Fig 8). In this aspect, there is an increase in simple sugars (fructose and glucose) content until the complete maturation. This is due to hydrolysis of long-chain carbohydrates and a consequent increase in sucrose, fructose and glucose contents (Chitarra and Chitarra, 2005; Oliveira et al., 2001).

For total sugar contents (Table 7) no significant differences were observed for treatment and their interactions ($p > 0.05$), with influence only for storage period ($p < 0.05$). Total sugar values were observed from 11.5% to 23%, with an overall mean of 13.3%. Similar values in apple 'Eva' were reported in Fante (2011) survey, 10.51%.

Fig 9 also shows total sugar contents during the storage period. Small contents increase of 11.5 to 14.7% is observed, Santos et al. (2006) also observed an increase in

**Fig 8.** Reducing sugars (%) in 'Eva' apple with application of different propolis extracts over 80 days of storage.**Fig 9.** Total sugar contents (%) in 'Eva' apple with application of different propolis extracts over 80 days of storage.

pitanga contents, and justified this because of the loss of fresh mass. Behavior that may be confirmed in the present work, as mass loss during evaluation was similar to total sugar contents. Another hypothesis may be polysaccharides conversion from cell wall of fruits into soluble sugars (Chitarra and Chitarra, 2005).

In sucrose contents (Table 8) there was no statistical difference between treatments, storage period and interaction between factors ($p > 0.05$). Organic 'Eva' apple fruit ranged from 4.5 to 14.9%, with an overall mean of 5.9% sucrose. Fante (2011) cited levels of 6.5 g 100g⁻¹ corroborating with this research.

The coloration is directly related to perceived appearance by consumer, being important that fruit presents color intensity and uniformity, being able to be evaluated at shell and pulp (Chitarra and Chitarra, 2005). The luminosity is an attribute that may vary from zero (black) to 100 (white) (Trigo *et al.*, 2012). For organic 'Eva' apple pulp luminosity (Table 9), there was significant difference only for treatments ($p < 0.01$). The highest values of luminosity were observed in treatments with 1.5% EAqP (88.0); 2.5% EAqP (87.8%) and 1.5% EAP (87.9), not statistically different from fruits treated with 2.5% EAP (87.5), being more enlightened (close to color white). The control had the lowest brightness value (86.8) with less brightness.

For the chroma values (Table 10) there was no statistical difference between treatments and interaction ($p > 0.05$). The pulp chroma was influenced by storage period ($p < 0.01$). There were values of 19.0 to 28.8 in pulp, showing little color saturation, and colors close to gray. Results close to zero express neutral colors (ashes) and close to 60, strong colors (Mendonça *et al.*, 2003).

For Hue angle values (Table 11), there was statistical difference only between treatments ($p < 0.01$). The highest values were found in fruits treated with 2.5% EAqP (102.1), not statistically differing from treatments 1.5% EAP (101.4); 2.5% EAP (101.0) and 1.5% EAqP (101.1) being the lowest value found in the control (100.2).

In spite of statistical difference, all fruits presented yellow flesh pulp, but with low color saturation (chroma = 23.3 – general mean) and high luminosity ($L = 87.6$ - mean overall), resulting in pale yellow tint. Similar values were reported by Chagas *et al.* (2012) in work with apple varieties

Table 7: Total sugar contents (%) in organic 'Eva' apple with application of different propolis extracts over 80 days of storage

Treatments	Storage (days)									Mean
	0	10	20	30	40	50	60	70	80	
Control	11.5	13.1	12.4	13.3	13.4	23.1	14.0	13.9	15.4	14.5
1.5% EAP	11.5	12.8	13.5	11.6	13.2	13.9	13.5	13.3	15.0	13.1
2.5% EAP	11.5	12.8	12.1	11.7	13.1	13.9	13.7	13.3	14.6	13.0
1.5% EAqP	11.5	13.3	11.6	12.0	13.3	14.1	14.6	13.4	14.9	13.2
2.5% EAqP	11.5	13.3	12.5	13.3	12.3	13.3	13.8	12.6	13.4	12.9
D.M.S.	2.29									
C.V. (%)	22.6									

EAP: alcoholic propolis extract, EAqP: aqueous propolis extract

Table 8 : Sucrose contents (%) in organic 'Eva' apple with application of different propolis extracts over 80 days of storage

Treatments	Storage (days)									Mean
	0	10	20	30	40	50	60	70	80	
Control	4.8	5.3	5.6	5.7	6.2	14.9	5.9	5.9	7.1	6.8
1.5% EAP	4.8	5.0	6.4	4.9	6.4	6.2	5.7	5.5	5.9	5.6
2.5% EAP	4.8	5.4	5.5	5.0	6.1	6.5	6.1	5.5	6.2	5.7
1.5% EAqP	4.8	5.5	4.7	4.8	5.9	6.4	6.5	5.8	6.4	5.6
2.5% EAqP	4.8	5.1	6.1	6.4	5.1	6.1	5.6	4.5	5.9	5.5
D.M.S.	2.19									
C.V. (%)	49.3									

EAP: alcoholic propolis extract, EAqP: aqueous propolis extract

Table 9: Luminosity values in organic 'Eva' apple with application of different propolis extracts over 80 days of storage

Treatments	Storage (days)									Mean
	0	10	20	30	40	50	60	70	80	
Control	87.6	89.2	83.8	87.0	87.6	88.0	84.6	87.4	87.0	86.8 ^b
1.5% EAP	87.6	87.5	87.6	87.8	88.4	88.0	88.6	88.2	87.6	87.9 ^a
2.5% EAP	87.6	87.2	86.7	88.2	88.2	88.0	86.4	87.4	88.4	87.5 ^{ab}
1.5% EAqP	87.6	88.5	88.0	88.6	89.2	87.4	88.0	86.8	88.4	88.0 ^a
2.5% EAqP	87.6	88.7	87.7	87.6	87.4	86.6	88.2	88.6	88.2	87.8 ^a
D.M.S.	1.01									
C.V. (%)	1.9									

Means followed by the same lowercase letter in the column do not differ from each other by Tukey test at 95% probability. EAP: alcoholic propolis extract, EAqP: aqueous propolis extract

Table 10: Chroma values in organic 'Eva' apple with application of different propolis extracts over 80 days of storage

Treatments	Storage (days)									Mean
	0	10	20	30	40	50	60	70	80	
Control	23.0	20.5	25.3	21.2	22.6	24.4	28.8	24.0	28.2	24.3
1.5% EAP	23.0	25.7	22.0	22.2	24.4	22.8	23.0	22.0	26.4	23.4
2.5% EAP	23.0	24.7	23.3	21.2	23.2	22.8	28.2	23.2	21.4	23.4
1.5% EAqP	23.0	25.0	23.7	22.2	24.0	21.6	23.0	24.6	23.0	23.3
2.5% EAqP	23.0	19.0	20.5	22.8	22.0	22.2	23.4	22.8	23.2	22.1
D.M.S.	2.17									
C.V. (%)	16.0									

EAP: alcoholic propolis extract, EAqP: aqueous propolis extract

Table 11: Hue angle values in organic 'Eva' apple with application of different propolis extracts over 80 days of storage

Treatments	Storage (days)									Mean
	0	10	20	30	40	50	60	70	80	
Control	100.8	102.7	100.0	100.2	100.0	100.6	99.2	101.4	97.4	100. ^{2b}
1.5% EAP	100.8	101.5	101.7	102.6	101.2	102.6	101.2	100.4	100.8	101.4 ^{ab}
2.5% EAP	100.8	100.5	100.7	102.4	101.8	100.2	99.6	101.2	102.0	101.0 ^{ab}
1.5% EAqP	100.8	101.5	100.7	103.0	99.8	101.4	100.6	101.0	101.8	101.1 ^{ab}
2.5% EAqP	100.8	104.5	102.3	102.2	102.0	101.4	101.0	102.6	102.2	102.1 ^a
D.M.S.	1.43									
C.V. (%)	2.4									

Means followed by the same lowercase letter in the column do not differ from each other by Tukey test at 95% probability. EAP: alcoholic propolis extract, EAqP: aqueous propolis extract

characterization of Hue angle in apple 'Eva' of 83.7 and luminosity of 85.2.

CONCLUSIONS

The application of propolis extract (aqueous and alcoholic) in 'Eva' organic apple post-harvest does not prolong refrigerated storage ($5 \pm 1^\circ\text{C}$ and $85 \pm 1\%$ RH) and does not influence in fruit quality conservation.

Author's contributions

Flávia Aparecida de Carvalho Mariano-Nasser: experiment conduction, experiment design, data analysis and paper writing; Mauricio Dominguez Nasser: paper writing; Juliana Arruda Ramos: experiment conduction and paper writing; Karina Aparecida Furlaneto: experiment conduction; Giovanna Alencar Lundgren: experiment conduction and literature review; Maximiliano Kawahata Pagliarini: paper writing; Rogério Lopes Vieites: experiment design, data analysis and paper writing; Ricardo de Oliveira Orsi: experiment conduction and experiment design.

REFERENCES

- Bleinroth, E. W., A. G. Zuchini and R. M. Pompeo. 1976. Determination of characteristics and mechanics of avocado varieties and their conservation by cold. *Coletânea ITAL*. 7:29-81.
- Brasil – Ministério da Saúde. 2008. Instituto Adolfo Lutz. Physical and chemical methods for food analysis. 4. ed. São Paulo: Instituto Adolfo Lutz. 1020 p.
- Brasil. Ministry of Agriculture. Livestock and Supply. Normative Instruction n.46. Technical regulation for processing, storage and transport of organic products. Brasília. DF. 2009. Available from: <http://sistemasweb.agricultura.gov.br/sislegis/action/detalhaAto.do?method=consultarLegislacaoFederal>. [Last accessed on 2019 May 21].
- Cantillano, R. F. F., S. P. Galarça, R. O. Treptow and L. A. S. Castro. 2011. Effect of the modified atmosphere on the postharvest quality of 'Ponkan' mandarins during cold storage. *Boletim técnico. Embrapa. Pelotas*. 36 p.
- Chagas, E. A., P. C. Chagas, R. Pio, J. E. Bettiol Neto, J. Sanches, S. A. Carmo, P. Cia, M. Pasqual and A. S. Carvalho. 2012. Production and quality attributes of apple tree cultivars in subtropical conditions at eastern of São Paulo state. *Ciência Rural*. 42:1764-1769.
- Chitarra, M. I. F. and A. B. Chitarra. 2005. Postharvest fruits and vegetables: physiology and handling. 2. ed. Lavras. UFLA. 785p.
- Czelusniak, C., M. C. S. Oliveira, A. Nogueira, N. C. C. Silva and G. Wosiack. 2003. The quality of apples produced in Brazil: physical and chemical aspects. *Braz J of Food Tech*. 6:25-31.
- Daiuto, E. R., P. H. Minarelli, R. L. Vieites and R. O. Orsi. 2012. Propolis and vegetable wax in the conservation of Hass avocado. *Semina: Ciênc Agr*. 33:1463-1474.
- Fante, C. A. 2011. Characterization, quality and postharvest conservation of 'Eva' apple (*Malus* sp.). Tese Ciências de Alimentos (área de concentração em Ciências de Alimentos) - Universidade Federal Lavras. Lavras. 105p.
- Fante, C. A., A. C. V. Boas, A. C. Costa, E. P. Silva, M. C. Oliveira and L. C. O. Lima. 2013. 1-MCP on physiological and postharvest quality of apples Eva during refrigerated stored. *Ciência Rural*. 43: 2142-2147.
- Izumi, H., A. E. Watada and W. Douglas. 1996. Low O₂ atmospheres affect storage quality of zucchini squash slices treated with calcium. *J of Food Sci*. 61:317-321.
- Jacxsens, L., F. Devlieghere, P. Ragaert, E. Vanneste and J. Debevere. 2003. Relation between microbiological quality.

- metabolite production and sensory quality of equilibrium modified atmosphere packaged fresh-cut produce. *Internat J of Food Microb.* 8:263-280.
- Konishi, S., A. C. H. F. Sawaya, A. R. Custodio, I. B. S. Cunha and M. T. Shimizu. 2004. Analysis of the influence of solubilizing agents on antimicrobial activity of propolis extracts and a hydroalcoholic spray formulation. *Mensagem Doce* 75:22-25.
- Mello, B. C. B. S., J. C. C. Petrus and M. D. Hubinger. 2010. Performance of nanofiltration concentration process in propolis extracts. *Food Sci and Tech.* 30:166-172.
- Mendonça, K., A. P. Jacomino, T. X. Melhem and R. A. Kluge. 2003. Ethylene concentration and exposure time for greening of lemon 'Sicilian'. *Braz J of Food Tech.* 6:179-183.
- Meneses, E. A., D. L. Durango and C. M. Garcia. 2009. Antifungal activity against postharvest fungi by extracts from Colombian propolis. *Quím Nova.* 32:2011-2017.
- Minarelli, P. H., E. R. Daiuto and R. L. Vieites. 2014. Postharvest behavior of strawberry of fruits subjected to the application to the hydroxyethylcellulose and aqueous propolis. *Rev Iberoamerica de Tec.* 15: 69-76.
- Minolta, K. 1998. Precise color communication: quality control from perception to instrumentation. Osaka. Konika Minolta Sensing. INC. 59 p.
- Nelson, N. A. 1944. A photometric adaptation of Somogy method for the determination of Glucose. *J Biolog Chem.* 153:375-380.
- Oliveira, D. L., A. A. Alvarenga, E. D. Gonçalves and M. R. Malta. 2014. Quality of Eva apples produced in two regions of Minas Gerais-Brazil. *Braz J of Food Tech.* 17:269-272.
- Oliveira, M. A., M. P. Cereda, C. Cabello and L. H. Urbano. 2001. Quantification of sugars in 'Biuti' variety peaches storage at environment conditions and refrigeration. *Rev Bras de Frut.* 23:424-427.
- Orsi, R. O., S. R. C. Funari, A. M. V. C. Soares, S. A. Calvi, J. M. Sforzin and V. Bankova. 2000. Immuno modulatory action of propolis on macrophage activation. *J of Ven Anim and Tox including Trop Dis.* 6:205-219.
- Paganini, C., A. Nogueira, F. Denardi and G. Wosiacki. 2004. Industrial fitness analysis of six apple cultivars, considering their physico-chemical evaluation. *C e Agrotec.* 28:1336-1343.
- Passos, F. R., F. M. Mendes, M. C. Cunha, M. T. Pigozzi, A. M. X. Carvalho. 2016. Propolis extract in postharvest conservation banana 'Prata'. *Rev Bras de Frut.* 38:1-11.
- Pastor, C., L. Sánchez-González, A. Marcilla, A. Chiralt, M. Cháfer and C. González-Martínez. 2011. Quality and safety of table grapes coated with hydroxypropylmethylcellulose edible coatings containing propolis extract. *Postharvest Biol and Techn.* 60:64-70.
- Petri, J. L., G. B. Leite, M. Couto and P. Francescatto. 2011. Advances of the apple crop in Brazil. *Rev Bras de Frut.* 33:48-56.
- Santos, A. F., S. M. Silva and R. E. Alves. 2006. Storage of Suriname cherry under modified atmosphere and refrigeration: I - postharvest chemical changes. *Rev Bras de Frut.* 28:36-41.
- Santos, J. L. F., E. M. Ataíde, A. K. E. Santos and M. S. Silva. 2015. Edible coatings in avocado post-harvest conservation. *Scientia Plena.* 11:1-7.
- Silva, R. A., A. E. Rodrigues, M. C. M. Ribeiro, A. R. Custódio, N. E. D. Andrade and W. E. Pereira. 2006. Physicochemical characteristics and antimicrobial activity of the extracts propolis of the Paraíba, Brazil. *Ciência Rural.* 36:1842-1848.
- Somogy, M. 1945. Determination of blooded sugar. *J Biolog Chem.* 160:69-73.
- Tressler, D. K. and M. A. Joslyn. 1961. Fruits and vegetables juice processing technology. Westport: AVI. 1028 p.
- Trigo, J. M., S. Albertini, M. H. F. Spoto, S. B. S. Sarmiento, A. E. Lai Reyes and G. A. Sarriés. 2012. Effect of edible coatings on the preservation of fresh cut papayas. *Braz J Food Tech.* 15:125-133.
- Vieites, R. L., L. P. R. Soares, E. R. Daiuto, V. Z. Mendonça, K. A. Furlaneto and E. Fujita. 2014. Organic apple 'Eva' subjected to the calcium chloride application in postharvest. *Nativa.* 02: 87-93.
- Viuda-Martos, M., Y. Ruiz-Navajas, J. Fernández-López and J. A. Pérez-Alvarez. 2008. Functional properties of honey, propolis and royal Jelly. *J of Food Sci.* 73:117-125.
- Wu, J., H. Gao, L. Zhao, X. Liao, F. Chen, Z. Wang and X. Hu. 2007. Chemical compositional characterization of some apple cultivars. *Food Chem.* 103:88-93.
- Zahid, N., A. Ali, Y. Siddiqui, M. Maqbool. 2013. Efficacy of ethanolic extract of propolis in maintaining postharvest quality of dragon fruit during storage. *Postharv Biol and Techn.* 79:69-72.