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Edible coating with Eugenia pyriformis leaf extract to control enzymatic browning in fresh-cut apples

Cobertura cosmestível com extrato da folha de Eugenia pyriformis para controle do escurecimento enzimático de maçã minimamente processada

Cobertura comestible con extracto de hoja de Eugenia pyriformis para el control del pardeamiento enzimático de manzanas minimamente procesadas

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Abstract

Fruits and vegetables are an important part of a healthy and balanced diet. Fresh cut fruits consumption is increasing, however keeping the food quality when processed is a challenge for the food industry. When the food is processed, some enzymatic changes can occur, being that enzymatic browning is one of this important degradation suffered by fresh cut apples. One alternative to reduce the enzymatic browning is the use of edible coating with anti browning components as vegetal extracts rich in phenolic compounds. The aim of this paper was characterize Uvaia (*Eugenia pyriformis* Cambess) leaf extract, evaluate the effect of sodium alginate edible coating formulated with uvaia leaf extract against enzymatic browning in fresh cut apples (cv. Golden Delicious and Royal Gala) during 8 days of storage. Phenolic compounds as chlorogenic acid, caffeic acid and *p-coumaric* acid were identified in Uvaia methanolic leaf extract. Uvaia aqueous leaf extract presented ABTS IC_{50} of 0.77 ± 0.002 mg/mL, increasing 40.66% the edible coating antioxidant activity. Uvaia aqueous leaf extract controlled 80% of polyphenol oxidase activity from Golden Delicious apple and edible coating with extract reduced enzymatic browning. Sodium alginate edible coating with Uvaia aqueous leaf extract is an alternative to reduced enzymatic browning of fresh cut apple (cv. Golden Delicious).

Keywords: Bioactive compounds; Enzymatic activity; Minimally processed; Polyphenol oxidase; Sodium alginate.

Resumo

Frutas e vegetais são partes importantes de uma dieta saudável e equilibrada. O consumo de frutas minimamente processadas é crescente, entretanto, manter a qualidade dos alimentos processados é um desafio para a indústria de alimentos. Ao processor os alimentos, diversas alterações enzimáticas podem ocorrer, o escurecimento enzimático é uma das principais degradações que maçãs minimamente processadas sofrem, sendo que uma das alternativas para redução desse escurecimento, é o uso de coberturas comestível com compostos antiescurecimento como por exemplo extratos vegetais ricos em compostos fenólicos. O objetivo do trabalho foi caracterizar o extrato da folha de Uvaia (*Eugenia pyriformis* Cambess) e avaliar o efeito da cobertura comestível de alginato de sódio formulada com extrato da folha de uvaia no escurecimento enzimático de maçãs minimamente processadas (cv. Golden Delicious e Royal Gala) durante 8 dias de armazenamento. Compostos fenólicos como o ácido clorogênico, ácido cafeíco e ácido p-coumárico foram identificados no extrato metanólico de folha de Uvaia. O extrato aquoso da folha de uvaia apresentou ABTS IC_{50} de 0.77 ± 0.002 mg/mL, aumentando 40.66% a atividade antioxidante da cobertura comestível quando adicionado. Para a maçã Golden Delicious o extrato aquoso de folha de uvaia controlou 80% da atividade da enzima polifenol oxidase e a cobertura comestível com extrato controlou o escurecimento enzimático. A cobertura comestível de alginato de sódio incoporada com extrato aquoso de Uvaia é uma alternativa para redução do escurecimento enzimático da maçã minimamente processada (cv. Golden Delicious).

Palavras-chave: Alginato de sódio; Atividade enzimática; Compostos bioativos; Minimamente processado; Polifenol oxidase.

Resumen

Las frutas y vegetales son partes importantes en una dieta balanceada y saludable. El consumo de frutas mínimamente procesadas es creciente, sin embargo, mantener la calidad de los alimentos procesados es un desafío para la industria alimentaria. Al procesar alimentos, diversas alteraciones enzimáticas pueden ocurrir, el pardeamiento enzimático es una de las principales degradaciones que manzanas mínimamente procesadas sufren, siendo que una de las alternativas para reducción del pardeamiento, es el uso de coberturas comestibles con compuestos anti-pardeamiento, como exemplo extractos vegetales ricos en compuestos

fenólicos. El objetivo del trabajo fue caracterizar el extracto de hoja de Uvaia (*Eugenia pyriformis* Cambess) y evaluar el efecto de la cobertura comestible de alginato de sodio formulada con extracto de hoja de Uvaia en el pardeamiento enzimático de manzanas mínimamente procesadas (cv. Golden Delicious y Royal Gala) durante 8 días de almacenamiento. Compuestos fenólicos como el ácido clorogénico, ácido cafeico y ácido *p-coumaric*o fueron identificados en el extracto metanólico de hoja de Uvaia. El extracto acuoso de hoja de Uvaia presentó ABTS IC50 de 0.77 mg/mL, aumentando 40.66% la actividad antioxidante de la cobertura comestible cuando añadido. Para la manzana Golden Delicious, el extracto acuoso de hoja de Uvaia he controlado 80% de la actividad de la enzima polifenol oxidasa y la cobertura comestible con extracto he controlado el pardeamiento enzimático. La cobertura comestible de alginato de sodio incorporada con extracto acuoso de Uvaia es una alternativa para reducción del pardeamiento enzimático de manzana mínimamente procesada (cv. Golden Delicious).

Palabras clave: Alginato de sodio; Actividad enzimática; Compuestos bioactivos; Mínimamente procesado; Polifenol oxidase.

1. Introduction

Fruits and vegetables are an important part of healthier diets and the consumption of fresh-cut products has been growing because their nutritional value, convenience, flavor and practicality (Alves et al., 2017; Ma et al., 2017; Yousuf et al., 2018).

Keep the quality of processed foods is a challenge for the food industry, fresh-cut fruits for example, pass through some kind of process and deteriorate faster than unprocessed fruits. During fresh-cut fruit processing, when are peeled and sliced, occurs the increase of respiration rate and production of ethylene, affecting the quality and accelerating the degradation of fruit. Fresh-cut apples for example, present enzymatic browning when are processed (Ma et al., 2017; Sukhonthara et al., 2016).

Enzymatic browning is a recurrent problem in many different processed fruits and vegetables. This browning can occur due to the presence of enzyme polyphenol oxidase (PPO) and peroxidase (POD). When the fruit suffers an injury and exposes a surface to oxygen, the phenolic compounds get in contact with enzymes that can result in the formation of dark compounds, decreasing the sensorial quality and depreciating the product for the consumer market (Guo et al., 2018; Tinello & Lante, 2018; Waleed et al., 2009).

Different strategies can be used to keep the fresh-cut fruits quality. Between them are the use of packaging with or without modified atmosphere, cold storage, use of natural or synthetic food additives and edible coatings (Alves et al., 2017; Ma et al., 2017). Different ways can be used to control the PPO activity during post-processing of products: change of pH or storage temperature, thermal treatment for enzyme inactivation, use of synthetic substances as copper chelating agents and sulfites that act as bleaching and antioxidants agents, but they can bring a negative impact for the consumers that seek for products more natural and with less synthetic substances (Guo et al., 2018; Tinello & Lante, 2018).

The natural anti-browning agent is a strategy that can help in fresh-cut fruit conservation and keep the natural aspect without synthetic additives. This kind of control can be done applying in the fruit an aqueous solution with a natural substance or with utilization of edible coating as carrier for the substance that also can work as a physical barrier against oxygen (Guo et al., 2018; Sepulcre et al., 2015; Tinello & Lante, 2018).

Different matrix as polysaccharides, protein, lipids or a combination between these materials can be used to formulate an edible coating. According to formulation, the edible coating properties will change. It is also possible to incorporate other substances like antioxidants, antimicrobials and amino acids that can improve the fresh-cut fruits quality and extend the shelf-life. For example a formulation with whey protein concentrate, beeswax and citric acid or cysteine as anti-browning agent presented a good control of browning in fresh-cut apples, but did not control weight loss (Alves et al., 2017; Perez-Gago et al., 2006; Sepulcre et al., 2015).

Some plants can be a natural source of bioactive compounds that can act as anti browning agents. *Eugenia pyriformis* Cambess is a native species of the south region of Brazil, where it is popularly known as “Uvaia”. Belongs to the Myrtaceae family, that has other species rich in bioactive compounds as *Eugenia uniflora* and *Psidium guajava*. Different bioactive compounds as: gallic acid, *p-coumaric* acid, quercetin, and chlorogenic acid are found in *E. pyriformis* fruit methanolic extract. Few papers about bioactive compounds present in the leaf of *E. pyriformis* were found, specially using water as solvent (Armstrong et al., 2012; Haminiuk et al., 2014; Klein et al., 2018).

The aim of this paper was characterize the extract of *Eugenia pyriformis* Cambes leaf and evaluate the effect of sodium alginate edible coating formulated with leaf extract on the enzymatic browning of fresh-cut apples (cv. Golden Delicious and Royal Gala).

2. Methodology

Apple cultivars were obtained from the local market (Maringá, state of Paraná, Brazil). A Royal Gala (RG) was produced by Fisher Group (Fraiburgo, state of Santa Catarina, Brazil) and Golden Delicious (GD) was produced by Val Venosta Apples (Val Venosta, Italy). The leaves of *E. pyriformis* was collected in rural property located in Sarandi (state of Paraná, Brazil) (23° 27' 8" S, 51° 51' 10" W).

Folin-Ciocalteu reagent, gallic acid, cathecol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,20-Azinobis-3-ethylbenzotiazoline-6-sulfonic acid (ABTS), sodium carbonate, potassium persulfate and calcium chloride were from Sigma Aldrich, sodium alginate, methyl alcohol, ethyl alcohol, sodium acetate buffer and acetone were of analytical grade.

2.1 Characterization of *E. pyriformis* leaf extract

The *E. pyriformis* leaf extract was prepared according to Daniel & Krishnakumari (2015) with modification. Leaves of *E. pyriformis* were sanitized with sodium hypochlorite solution (200ppm) during 15 min and dried at 50°C with air circulation for 24 hours. The material was grind and screened through 48 mesh sieves. Was prepared the *E. pyriformis* aqueous leaf extract (EPE) and methanolic leaf extract (EPM). To prepare the extracts was used ultrapure water (25 °C) or methanol (1:10 w/v), homogenized (15 min), centrifuged at 3000 rpm (10 min) and filtered.

For a high performance liquid chromatography was followed the procedure described by Haminiuk et al. (2012), using a HPLC coupled to a multi λ fluorescence detector (waters 2475) and photodiode array detector (waters 2998), with a column C18. Two different mobile phases were used, methanol (A) and acidified water with phosphoric acid 1% (B) with solvent gradient: A 15-0 % , A 25-15 %, A 30-25 %, A 35-30 %, A 50-35 %, A 60-50 %, A 80-60 %, A 100-80 %, A 5-100 % in 2, 5, 10, 15, 25, 30, 35, 45 and 60 min respectively. The flow rate was 1 mL/min and the detection was recorded at 280 nm. The peak retention times were compared with the literature for the identification of compounds. Was used the *E. pyriformis* leaf extract in a concentration 1:100 using methanol as solvent.

Total phenolic content (TPC), was determined for EPA and EPM in a concentration 1:100. TPC was assessed by the method described by Singleton & Rossi (1965) with modifications, EPA or EPM (125 µL) was mixed with Folin-Ciocalteu reagent (1:1 deionized water) (125 µL) and sodium carbonate (28 g/L) (2250 µL), incubated in a dark room at 25 °C

for 30 min, absorbance was measured with spectrophotometer (Evolution 300 UV Vis Thermo Scientific) at 725 nm. The results were expressed as mg gallic acid equivalent (GAE)/g of dried weight using a standard curve of gallic acid ranging from 0 to 100 mg/L.

Total flavonoids content was determined using the aluminum chloride method (Buriol et al., 2009) with modifications (Vital et al., 2018). EPA or EPM (300 µL) (1:1000 w/v) was mixed with 150 µL aluminum chloride (5% w/v in methanol), and 2550 µL methanol. The mixture was left at 25°C in the dark for 30 min. The absorbance was measured at 425 nm. A quercetin standard curve was prepared ranging from 0 to 300 mg/L, and the results were expressed as mg quercetin equivalents QE/g dw.

For ABTS assay was used the methodology described by Re et al. (1999) with modification (Vinet & Zhedanov, 2011). ABTS·+ radical was prepared with 5 mL ABTS solution (7 mM) with 88 µL potassium persulfate solution (120 mM) and then kept in the dark room for 16 hours. The radical ABTS·+ solution was diluted in ethanol until an absorbance of 0.70 ± 0.02 measured at 734 nm. A sample of EPA or EPM (40 µL) was added with ABTS·+ solution (1960 µL), after 6 min the absorbances were measured. The absorbance was read using different concentrations (0.25 - 0.0312%) of extract. With the chart plotted (absorbance x concentration) was obtained the curve equation used to measure the ABTS IC_{50} was expressed in mg/mL dw of *E. pyriformis* leaf.

Determination of polyphenol oxidase activity inhibition by *E. pyriformis* leaf aqueous extract was made using two steps, first was prepared the enzymatic extract of both cultivars (Ansari et al., 2017) and after, was determined the enzyme activity for each cultivar and the inhibition by aqueous and methanolic extract of each enzymatic extract (Yang & Wang, 2008). To prepare the apple enzymatic extract, 50 g of macerated apple was added in 100 mL of potassium phosphate buffer (0.2 M, pH 7.0), filtered through gauze after 5 min and centrifuged at 5.000 g (10 min). The supernatant was recovery, mixed with cold acetone (-5°C) in a proportion 1:1.5 and stirred (30 min). The mixture was centrifuged at 10.000 g (15 min) and the precipitate was dissolved in 50 mL of potassium phosphate buffer. To determine the PPO activity was mixed 2 mL of catechol (20 mM), 900 µL of sodium acetate buffer (0.2 M, pH 4.0), 100 µL of enzymatic extract. The absorbance was read during 5 min at 420 nm in spectrophotometer, PPO activity was measured through the increase of absorbance, an increase of 0.001/min in absorbance represents one enzymatic unit (EU) of PPO. The final absorbance minus initial absorbance divided by min expressed the enzyme activity in EU/min. The same method was followed to determine the inhibition by extract, but was added 200 µL of *E. pyriformis* leaf extract (0,075%) in the bucket with the same amounts of

cathecol, sodium acetate buffer and enzymatic extract previously described. The results were expressed in % of inhibition using the follow equation:

$$\% \text{ inhibition} = ((TA - TAE)/TA)*100$$

TA = Total activity without leaf extract

TAE = Total activity with leaf extract

2.2 Edible coating characterization

The edible coating was prepared with sodium alginate (1.5%) and glycerin (1%) as plasticity agent. The sodium alginate powder and glycerin was mixed in deionized water (80°C) and stirred until it was homogenized. The edible coating control (ECC) was prepared with sodium alginate and glycerin, an edible coating with extract (ECE) used the same proportion and was added 2% of EPA (37.6 mg/mL dw of *E. pyriformis* leaf), so the edible coating with extract had an EPA final concentration of 0.75 mg/mL that is the ABTS IC_{50} concentration of *E. pyriformis* leaf.

The edible coating microstructure analysis was performed according to Matumoto-Pintro, Rabiey, Robitaille, & Britten (2011), apple samples with one day of storage were frozen with liquid nitrogen, and lyophilized. Samples were mounted on aluminum stubs and coated with a gold layer (sputter coater, Bal-Tec, SCD 050). Observations were made on a scanning electron microscope (Quanta 250, FEI company, Oregon, EUA) at 15 kV.

The edible coating control and edible coating with EPA was mixed with methanol 1:10 (v/v), homogenized during 15 min, centrifuged at 3000 rpm for 10 min, filtered and stored in opaque packaging. To determine the antioxidant activity, was followed the ABTS assay method used for EPA, the radical scavenging activity (%) was obtained with the follow equation:

$$\text{Radical scavenging activity (\%)} = (1-(\text{Abs sample}_{t=0} / \text{Abs sample}_{t}))*100$$

Abs sample $_{t=0}$: absorbance of sample.

Abs sample $_{t}$: absorbance of ABTS solution

The X-ray diffraction analysis of edible coating was made following Santiago et al. (2018). XRD patterns have been recorded with a XRD-7000 diffractometer (Shimadzu), with Cu-Kα radiation source (λ = 1.54439 Å). XRD data were collected with a 2θ range of 10 – 60° at a scanning rate 5° min^{-1}.

2.3 Effect of edible coating in fresh-cut apple

Apples were sanitized, sliced in cube and immediately dipped in a coating solution (30 s), the excess was removed and dipped again in a calcium chloride solution (2% w/v) (15 s) for polysaccharide cross-linking. The slices were placed in polypropylene pots and capped. Control treatment was dipped in deionized water (45 s). All pots were stored at 4 ± 1 °C during 8 days.

Apple samples were evaluated for total titratable acidity (TTA) and total soluble solids (TSS). The TTA was determined using the method described by AOAC (2000), results were expressed in percentage of malic acid. Total soluble solids content were measured using a digital refractometer (model HI96801, Hanna Instruments, São Paulo, Brazil) and were expressed in °Brix.

The enzymatic browning was measured using the browning index (Perez-Gago et al., 2006) Color measurements were obtained at day 1, 2, 4 and 8 using a colorimeter (Chroma Meter CR-400; Konica Minolta, Ramsey, New Jersey, USA) with the standard illumination C. Chromaticity coordinates X, Y, and Z were used to calculated the browning index (BI) expressed in % and determined using the following equation (Perez-Gago et al., 2006):

$$BI = (x - 0.31/0.172)*100$$

x is the chromaticity coordinate calculated from X, Y and Z.

$x = X/(X + Y + Z)$

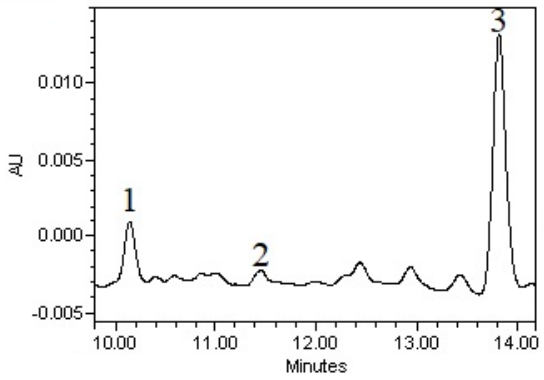
2.4 Statistical analysis

Three treatments were prepared for each cultivar (Golden Delicious and Royal Gala): one control without edible coating, one with edible coating without extract and one with edible coating incorporated with extract. The experiment used a completely randomized design in triplicate for each day (day 1, 2, 4 and 8). For each pot was used six apple slices (2 for each treatment) and the experiment was repeated three times to confirm repeatability. All analysis was made in triplicate. Statistical analysis was made using the software SAS Studio (SAS Institute Inc., Cary, NC, USA) for windows. Analysis of variance (ANOVA) was performed and variances were tested for homogeneity and statistical significant differences with the Fisher's Least Significant Differences (LSD) test. The significance level was defined as $p \leq 0.05$.

3. Results and discussion

Phenolic compounds were identified through HPLC analysis. The chromatograms of EPM are presented in Figure 1. All peaks founded was compared with the literature (Haminiuk et al., 2014) and three common peaks with similar retention time were identified, which probably are chlorogenic acid (10.14 min), caffeic acid (11.45 min) and *p-coumaric* acid (13.80 min).

Figure 1. Chromatogram of *Eugenia pyriformis* Cambess leaf methanolic extract (EPM) at 280 nm.



Peak	Retention Time (min)	Area (µV*sec)	Compound
1	10.143	44091	Chlorogenic acid
2	11.451	6692	Caffeic acid
3	13.813	153814	*p-coumaric* acid

Source: Elaborated by authors.

Antioxidant activity, TPC, TFC and PPO activity inhibition were shown at Table 1. Methanolic leaf extract presented higher values for TPC and TFC than aqueous leaf extract due to the difference of solvent used, usually methanol extract more bioactive compounds than water. Haminiuk et al. (2014) quantified polyphenols extracted from *E. pyriformis* fruits with methanol and water and obtained results according to this experiment, the difference occurs because of the polarity difference between the solvents. Generally polyphenols are more soluble in organic solvents less polar than water. Each type of polyphenol present a different antioxidant activity, according Badanai et al. (2015), *p-coumaric* acid present higher ABTS IC_{50} (0.83 mg/mL) than caffeic acid (17.89 mg/mL) and gallic acid (1.13 mg/mL)(Badanai et al., 2015). Haminiuk et al. (2014) showed that methanol extracted

different concentrations of polyphenols than water. The water extracted more *p-coumaric* acid which has higher antioxidant activity, this difference can explain how the methanolic leaf extract presented a lower antioxidant capacity when compared with aqueous leaf extract.

Table 1. Bioactive characterization of *Eugenia pyriformis* Cambess leaf extract

Extract	TPC (mg GAE/g dw)	TFC (mg QE/g dw)	ABTS IC_{50} (mg/mL)	PPO activity inhibition (%)	
				GD	RG
EPA	76.03[b]	3.53[b]	0.77[b]	80.00[a]	92.91[a]
EPM	173.00[a]	11.44[a]	1.05[a]	73.27[b]	82.40[b]
SEM	27.99	1.78	0.08	2.08	3.10
P-VALUE	< 0.0001	< 0.0001	0.0113	0.0611	0.0219

EPA = leaf aqueous extract, EPM = leaf methanolic extract, TPC = total polyphenol content, TFC = total flavonoids content, PPO = polyphenol oxidase, GD = Golden Delicious, RG = Royal Gala, GAE = gallic acid equivalent, QE = quercetin equivalent. Means with different lowercase letter, in the same column, present significant difference at $p \leq 0.05$, according to the Fisher test.
Source: Elaborated by the authors.

The inhibition of PPO activity is demonstrated at Table 2. The aqueous and methanolic extract presented an inhibition for the enzyme PPO from both cultivars. The aqueous leaf extract presented a higher inhibition for cultivar Royal Gala than methanolic leaf extract, but for enzyme from Golden Delicious the both extracts didn't present significant difference. The inhibition was caused by the possible presence of *p-coumaric* acid in extract. This polyphenol had a good response in the control of apple and potato PPO activity, showing as an anti-browning agent option (Sukhonthara et al., 2016). The action of chlorogenic acid and caffeic acid against the PPO activity is less effective than *p-coumaric* acid (Son et al., 2001).

Table 2. Characterization of apple cultivars Golden Delicous (GD) and Royal Gala (RG)

Cultivar	Titratable acidity (% acid malic)	Total soluble solids (° Brix)	PPO Activity (EU/min)
GD	0.17[a]	13.40[a]	5.60[a]
RG	0.11[b]	10.53[b]	3.50[b]
SEM	0.02	0.62	0.63
P-VALUE	0.0006	0.0003	0.0365

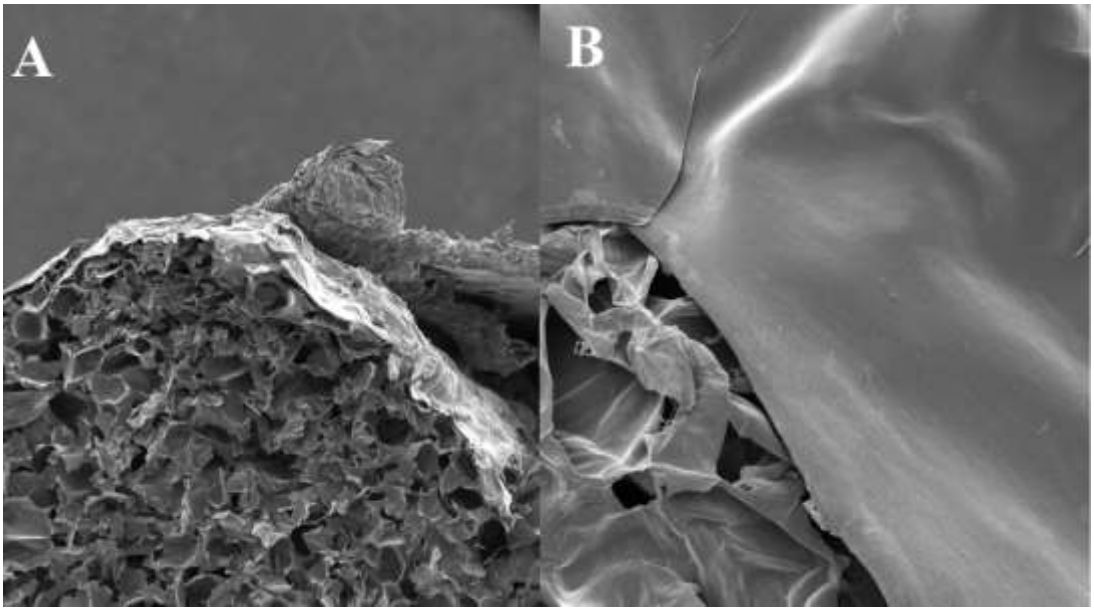
PPO = polyphenol oxidase, GD = Golden Delicious, RG = Royal Gala EU = enzymatic unit. Means with different lowercase letter, in the same column, present significant difference at $p \leq 0.05$, according to the Fisher test.
Source: Elaborated by the authors.

The apple properties (TA, SST and PPO activity) were also presented at Table 2. Comparing the values obtained between two cultivars, Golden Delicious (GD) presented values 64.71, 78.36 and 60.34 % higher than Royal Gala (RG) for TA, SST and PPO activity respectively. Hutabarat & Halbwirth (2019) shows a difference in PPO enzyme activity between cultivars linked to the genetics difference between the different cultivars. This difference of PPO activity influences directly on the browning index of each cultivar.

The aqueous extract was used to formulate the edible coating because it showed better antioxidant activity and a similar inhibition of the enzymatic activity when compared with the methanolic extract. The edible coating with aqueous leaf extract presented an antioxidant activity of 18.27% and edible coating control (without extract) 7.43%. The extract addition showed an antioxidant activity increment of 40.66% when compared with edible coating control. Scanning electron microscopy was performed to observe how the formation of edible coating occurred (Figure 2). The edible coating formation occurred homogeneously and uniformly on the fresh-cut apple slice surface (Figure 2a), in a transversal section of fresh-cut

apple slice (Figure 2b) was also possible to see the edible coating formation for all over the surface.

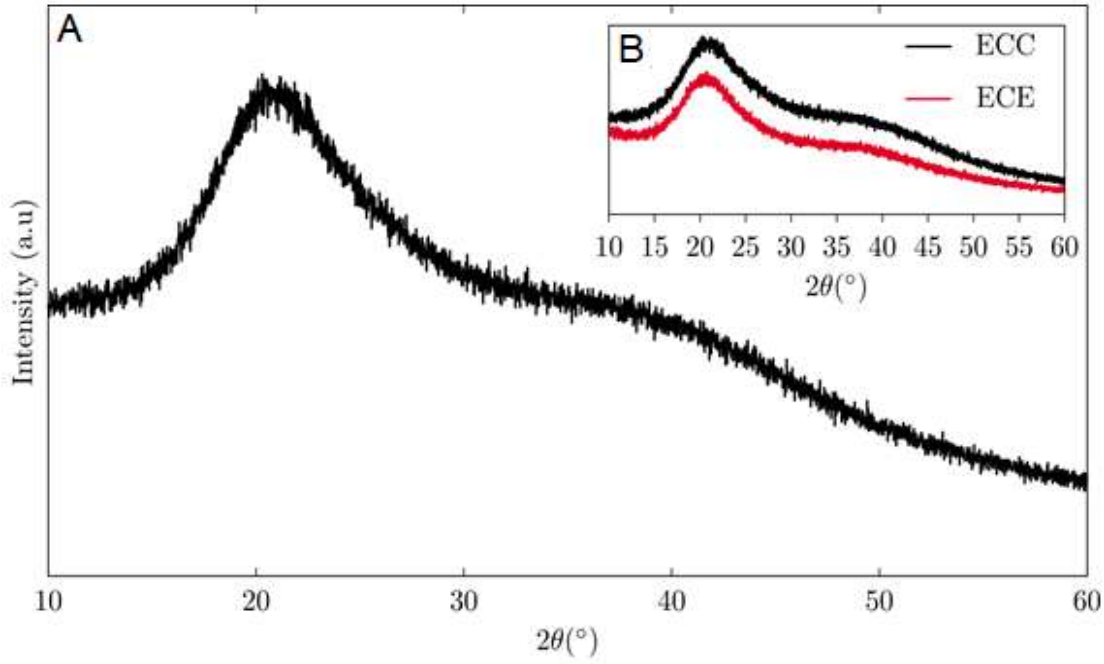
Figure 2. Scanning electron micrographs of the fresh-cut apple with sodium alginate edible coating. (A) Transversal section of apple slice (Magnification of 100x) (B) and homogeneity of the film formation on fresh-cut apple (Magnification 500x).



Source: Elaborated by the authors.

The XRD standard for edible coating control (without extract) and edible coating with extract was presented in Figure 3. The diffractograms present a broad peak at 21°, which confirms the presence of the amorphous structure of the sodium alginate (Lu et al., 2005; Salama et al., 2018). There was no apparent change in XRD patterns of edible coating control when compared with edible coating with extract, as shown in Figure 3b. This can be explained by the fact that aqueous leaf extract was used at low concentrations, therefore, according to x-ray diffraction analyzes, there was no structural change in the material.

Figure 3. (A) X-ray diffraction scatter pattern of edible coating control (ECC) and (B) a comparative between ECC and edible coating with extract (ECE).



Source: Elaborated by the authors.

The browning index (BI) is expressed at Table 3. For cultivar Golden Delicious, the control sample (GDC), suffered the biggest browning all the days and the lowest browning index was presented for the samples that had the edible coating with extract (GDFE) applied. The aqueous leaf extract act as anti-browning agent and improving the ability of sodium alginate coating to control the enzymatic browning, the reducing of fresh-cut apple browning using sodium alginate edible coating with anti-browning agent it has been previously reported (Rojas-Graü et al., 2008). The samples with edible coating without extract (GDF) presented a lower browning than GDC, but larger than GDFE, this effect occurred because the calcium chloride can be considered an anti-browning agent by the interaction of chloride ion with copper at the PPO active site, besides the capacity of the edible coating can act as a semipermeable physical barrier that reduce the amount of oxygen in contact with the surface, necessary for browning reactions (Olivas et al., 2007).

Table 3. Effect of sodium alginate edible coating on browning index (%) of fresh-cut apples

	Day 1	Day 2	Day 4	Day 8	SEM	P-VALUE
GDC	32.15^{a}	33.29^{a}	32.85^{a}	34.15^{a}	0.39	0.3146
GDF	28.75^{Bb}	31.76^{Ab}	30.44^{ABb}	30.40^{ABb}	0.38	0.0546
GDFE	26.28^{Bc}	26.05^{Bc}	26.90^{ABc}	27.98^{Ac}	0.26	0.0319
SEM	0.57	0.59	0.47	0.54		
P-VALUE	<0.0001	<0.0001	<0.0001	<0.0001		
RGC	34.26^{a}	33.36^{a}	34.33^{a}	33.81^{a}	0.32	0.6811
RGF	28.02^{b}	27.78^{b}	29.01^{b}	28.65^{b}	0.35	0.5846
RGFE	27.19^{b}	26.50^{b}	27.17^{b}	28.59^{b}	0.35	0.2672
SEM	0.62	0.54	0.59	0.51		
P-VALUE	<0.0001	<0.0001	<0.0001	<0.0001		

GDC = Golden Delicious control, GDF = Golden Delicious with edible coating, GDFE = Golden Delicious with edible coating and EPA. RGC = Royal Gala control, RGF = Royal Gala with edible coating, RGFE = Royal Gala with edible coating and EPA. Means with different uppercase letter, in the same row for the same cultivar, and different lowercase letter, in the same column for the same cultivar, present significant difference at $p \leq 0.05$, according to the Fisher test.
Source: Elaborated by the authors.

For cultivar Royal Gala, the control samples (RGC) presented the highest browning index in all days. The samples with edible coating (RGF) and edible coating with extract (RGFE) presented a reducing of browning index, but didn't present significant difference among them, this can be explained by the RG PPO activity (Table 2) that was lower than the GD PPO activity. Only the effect of the edible coating and calcium chloride were enough to reduce the RG PPO activity, while in the GD cultivar, the aqueous leaf extract as anti-

browning agent was necessary due the high GD PPO activity, showing significant difference between GDF and GDFE.

For the both cultivars the browning index of the control sample (GDC and RGC) didn't increase over the days, reaching maximum browning and consuming all the substrate even on the first day. For GD that presents a higher PPO activity, the GDF had an increase of browning index between the day 1 and day 2, this can occur because the edible coating can retard the enzyme action consuming all the substrate at the day 2, and between the day 4 and 8, the GDFE also present an increase of browning index, due to the action of the enzyme that is only reduced and not totally eliminated by the edible coating and the aqueous leaf extract. All RG treatments kept the browning index constant over the days possible because of the lower PPO activity.

4. Conclusion

The extract of *Eugenia pyriformis* Cambess leaf presented as a source of bioactive compounds, presenting antioxidant capacity and inhibition of apple polyphenol oxidase activity. The edible coating with *E. pyriformis* leaf extract caused the higher reduction of browning for Golden Delicious. For cultivar Royal Gala the edible coating without extract was sufficient to control the enzymatic browning. Sodium alginate edible coating did not control the weight loss, but the results obtained demonstrate a lower loss. For Golden Delicious fresh-cut apple the sodium alginate edible coating with *E. pyriformis* leaf extract is an option to reduce the enzymatic browning. It is necessary more researches to identify the compounds responsible for enzyme inhbition.

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