ANTIBROWNING AGENTS ADDED IN POLYMERIC COATING APPLIED TO AVOCADO FRUIT SLICES

Mendoza-Gómez Violeta, Montserrat Calderón-Santoyo, Pedro Ulises Bautista-Rosales, Rosa Isela Ortiz-Basurto, Darvin Ervey Jimenez-Sánchez and Juan Arturo Ragazzo-Sánchez

SUMMARY

Enzymatic browning in fresh-cut products is an important problem in the food industry. These reactions are produced by the action of the polyphenol oxidase enzyme (PPO). The study evaluates the effect of 4-hexylresorcinol (4HSR) 0.025M, sodium D-isoascorbate (D-ISO) 0.094M, L-glutathione (LG) 0.05M, and sodium hexametaphosphate (HAS) 1% (w/v) in inhibiting the PPO activity on avocado slices, and their incorporation to a gellan gum coating in order to extend the shelf life of minimally processed avocados preserved by freezing. LG and the combination of LG and D-ISO reduced the in vitro *PPO activity. Kinetic studies indicate that inhibition of PPO by LG is of a competitive type, whereas inhibition by D-ISO is non-competitive. The addition of inhibitors to the polymeric matrix does not affect the film formation on the avocado slice surfaces. Finally, the addition of D-ISO and LG to the polymeric film, provides protection against enzymatic browning, delaying undesirable color changes in avocado slices during 8 hours.*

Introducction

Avocado is a fruit of high demand in Mexico and the world for its nutritional and sensory characteristics. Mexico is the main producer and global consumer of this fruit. However, only 16% of the total production is industrialized. Handling operations, processing and storage can induce undesirable changes in quality and appearance of fresh fruits and vegetables as a result of oxidative browning mediated by the enzyme polyphenol oxidase (PPO; EC1.14. 18.1). PPO catalyzes the oxidation of a wide variety of phenolic compounds, generating reactive quinones that participate in subsequent reactions producing colored pigments. Because of the potential health effects caused by the use of sulfites as antibrowning agents, alternative chemical treatments have been studied as an (Manolopoulou and Varzakas, 2011; Sulaiman *et al*., 2015; Ali *et al*., 2016). Biochemical treatments aimed at countering enzymatic browning in some fruits and vegetables has been studied (Ma *et al*., 2010; Bustos *et al*., 2015). Some biochemical agents act directly as inhibitors of PPO, while others induce a non-favorable environment for the browning reaction and, others reac with the reaction products of PPO before they become colored pigments (Chiabrando and Giacalone, 2012; Sulaiman *et al*., 2015).

Various inhibitor agents have been studied, not only individually but in binary mixtures, enhancing their action, with the idea of achieving a synergistic effect and better PPO inhibition results. The PPO inhibitor 4-hexylresorcinol (Martin-Belloso and Soliva-Fortuny, 2010) is known as GRAS; it is used in the prevention of shrimp melanosis (Nirmal and Benjakul, 2010) and it is also effective for the control of enzymatic browning in apple slices (Jokić *et al*., 2009). Compounds containing thiol (-SH) have been reported as effective reducing agents with potential to reduce enzymatic browning (Ioannou, 2013), L-Glutathione is the most abundant non-protein thiol compound present in living organisms and is used as an ingredient in pharmaceuticals, as well as a food additive and in cosmetics (Li *et al*., 2004). Ascorbic acid and its analogs, which are attributed acidifying and chelating properties (Holzwarth *et al*., 2013) have been widely used in the food industry, with acceptable microbial results and sensory preservation of mushrooms slices (Cliffe-Byrnes and O'berne, 2008). The industrial application of enzymatic inhibitors that preserve avocado slices requires a polymeric support with high water solubility and without off-flavor addition. Gellan gum (Rojas-Grau *et al*., 2006, 2008) was chosen as the polymeric matrix for the incorporation of chemicals antioxidants to browning inhibition in fresh-cut 'Fuji' apple slices.

This study aimed to determine the kinetic behavior of the PPO enzyme in avocado (*Persea americana* Mill.) in the presence of 4-hexylresorcinol, L-glutathione, sodium D-isoascorbate and sodium hexametaphosphate, incorporated to a polymeric matrix based on gellan gum, in order

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AGENTES ANTI-PARDEAMIENTO AÑADIDOS EN UN RECUBRIMIENTO DE POLÍMEROS PARA REBANADAS DE AGUACATE

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RESUMEN

El pardeamiento enzimático en pre-cortados es una gran problemática en la industria alimentaria. Estas reacciones son causadas por la enzima polifenol oxidasa (PPO). En este estudio se evalúa el efecto de 4-hexilresorcinol (4HSR) 0,025M; D-isoascorbato de sodio (D-ISO) 0,094M; L-glutatión (LG) 0,05M y hexametafosfato de sodio (HAS) 1% (p/v) en inhibir la actividad de PPO en rebanadas de aguacate, y su incorporación en un recubrimiento a base de goma gellan, para incrementar la vida útil de los aguacates mínimamente procesados conservados por congelación. LG y la combinación de LG y D-ISO redujeron la actividad de PPO in vitro*. Los estudios cinéticos indican que la inhibición de PPO por LG es de tipo competitivo, mientras que la inhibición por D-ISO es no competitiva. La adición de inhibidores a la matriz polimérica no afecta la formación de película en la superficie de las rodajas de aguacate. Finalmente, la adición de D-ISO y LG en la película polimérica proporciona protección contra el pardeamiento enzimático, retrasando los cambios de color indeseables en las rebanadas de aguacate durante 8 horas.*

AGENTES ANTI-ESCURECIMENTO ADICIONADOS EM UM RECOBRIMENTO DE POLÍMEROS PARA FATIAS DE ABACATE

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RESUMO

O escurecimento enzimático em pré-cortados é uma grande problemática na indústria alimentaria. Estas reações são causadas pela enzima polifenol oxidase (PPO). Neste estudo é avaliado o efeito de 4-hexilresorcinol (4HSR) 0,025M; D-isoascorbato de sódio (D-ISO) 0,094M; L-glutationa (LG) 0,05M e hexametafosfato de sódio (HAS) 1% (p/v) em inibir a atividade de PPO em fatias de abacate, e sua incorporação em um recobrimento a base de goma de gelano, para incrementar a vida útil dos abacates minimamente processados conservados por congelamento. LG e a combinação de LG e D-ISO reduziram a atividade de PPO in vitro*. Os estudos cinéticos indicam que a inibição de PPO por LG é de tipo competitivo, enquanto que a inibição por D-ISO é não competitiva. A adição de inibidores à matriz polimérica não afeta a formação de película na superfície das rodelas de abacate. Finalmente, a adição de D-ISO e LG na película polimérica proporciona proteção contra o escurecimento enzimático, atrasando as mudanças de cor indesejáveis nas fatias de abacate durante 8 horas.*

to assess their effect on the visual quality and physicochemical properties preservation of avocado slices.

Materials and Methods

Biological material

Avocado fruits (*Persea americana Mill.*) cv. Hass, were purchased in Xalisco, Nayarit, Mexico (21º26'53"N, 104º54' 0"O) of consumption ripeness and uniform appearance and size (classification: premium, according to Mexican Standards NMX-FF-008 171-210).

Chemicals

The chemical agents used in the study were gellan gum (Gelzan™ Sigma, USA), sodium D-isoascorbate (D-ISO), 4-hexylresorcinol (4HSR) and L-glutathione (LG) from Sigma Aldrich, Germany, and sodium hexametophosphate (HAS) from Jalmek Scientific, Mexico.

PPO enzyme extraction

PPO extraction was performed according to a modified Soliva *et al.* (2001) method. An avocado purée (15g) was mixed with Mcllvaine buffer (1:1; pH adjusted to 6.5), and 0.1ml NaCl 1M and polyvinylpyrrolidone 5% (w/v) were added. Mixture was homogenized and subsequently centrifuged at 12000 rpm for 30min at 4ºC. The supernatant was collected and filtered through a paper filter Whatman # 1. The collected permeate was used immediately. Chemical agents were applied individually and mixed (Table I).

PPO enzymatic activity

In order to determine enzyme activity, 3 ml of catechol 0.05M, 75µl of enzyme extract and 75µl of the chemical inhibitor were placed into a 4ml quartz cell. The tests were performed at 25ºC, absorbance was monitored for 3min and data recorded every 25s, at 410nm wavelength (UV-visible spectrophotometer Varian Cary 50 Bio). Slopes were obtained from the linear portion of the resulting curves. One activity unit was considered as the amount of enzyme required for the formation of 1µmol of benzoquinone/min. A completely randomized experimental design was used and the data were analyzed using SAS software system 2015.

Kinetics of PPO inhibition

Catechol (Sigma-Aldrich, Germany) as substrate in concentrations of 0.0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.10, 0.11 and 0.120M was prepared, and 3ml of each concentration was placed into a quartz cell along with 75µl of each inhibitor or their combinations. Reaction was started by adding 75µl of enzyme extract and absorbance at 410nm was followed through 2min with data recorded every 10sec for each individual reaction. The kinetic parameters Km, Vmax and inhibition coefficients (Ki) were determined through Michaelis-Menten and Lineweaver-Burk plots (Dogan, 2004).

In vivo *inhibitory effects of the agents added to gellan gum*

Chemical agents that exhibited significant effect on PPO activity were incorporated into a gellan gum matrix 0.5% w/v; glycerol 1% (v/v) was added as a plasticizer and polyoxyethylene (20) sorbitan monooleate 0.5% v/v as a surfactant, in order to formulate edible coatings (Lin and Zhao, 2007). Avocado fruits were washed by immersion in water with so-

TABLE I
AVOCADO CV. HASS PPO ENZYMATIC ACTIVITY IN PRESENCE OF INHIBITORS (SINGLE AND BINARY COMBINATIONS) AND KINETIC PARAMETERS

Inhibitor agent	Concentration	Activity units (μmol/min)	Vmax	Km	Ki (mM)	Inhibition type
PPO (control)		148.45 ±57.25 a	175.43	0.0175	nd	nd
4-hexylresorcinol (4HSR)	0.025M	112.63 ±58.33 a	nd	nd	nd	nd
D-sodium isoascorbate (D-ISO)	0.094M	2.86 ±1.22 a	0	0	-	Non-competitive (lineal)
L-glutathione (LG)	0.05M	1.74 ±0.17 a	175.43	1.2982	0.07	Competitive
Sodium hexametaphosphate (HAS)	1% w/v	208.3 ±63.84 a				
4-hexylresorcinol (4HSR) + D-sodium isoascorbate (D-ISO)	0.025M + 0.094M	7 ±4.1 a	175.43	0.7192	1.48	Competitive
4-hexylresorcinol (4HSR) + L-glutathione (LG)	0.025M + 0.05M	7.71 ±2.21 a	172.41	0.431	3	Competitive
4-hexylresorcinol (4HSR) + sodium hexametaphosphate (HAS)	0.025M + 1% w/v	201.15 ±77.63 a	nd	nd	nd	nd
D-sodium isoascorbate (D-ISO) + L-glutathione (LG)	0.094M + 0.05M	0.99 ±0.22 a	175.43	3.49	0.94	Competitive
D-sodium isoascorbate (DI) M + sodium hexametaphosphate (HAS)	0.094 M + 1% w/v	1.74 ±0.11 a	nd	nd	nd	nd
L-glutathione (LG) + sodium hexametaphosphate (HAS)	0.05 M +1% w/v	2.58 ±1.47 a	nd	nd	nd	nd

dium hypochlorite (100ppm) during 3min. Subsequently, the peel and seed were removed and 1cm thick longitudinal slices were obtained. The edible coating formulations were applied by immersion during 1min and excess solution was drained in a sieve for 5min.

Six hundred avocado slices with and without coating formulations were manually packaged in bags Cryovac® Europe (Grace S.A., Sant Boi de Llobregat, Spain), of low oxygen permeability ($15cm^3 \cdot m^{-2}$/24h at 23ºC and 0% RH). Air was removed with nitrogen; then, the bags were subjected to vacuum and passed through a food freezer tunnel (CAS Function ABI Co., Japan) at -45ºC during 40min. Finally, samples were stored at -20ºC until analysis. Analyses were carried out in triplicate.

Quality assessment of slices exposed to environmental conditions

Samples were taken out of the freezer and the avocado slices were exposed to room environment conditions (25ºC and 70% RH). This moment was considered as time zero.

Color changes evaluation

The color changes were registered by a colorimeter (Konica Minolta CR-400 Chroma Meter), in terms of the parameters L, a and b. Five random measurements were performed on the surface of avocado slices at 1h intervals after time zero. The total color change was calculated by Eq. 1.

$$\Delta E^* = \sqrt{\left(\Delta L^*\right)^2 \left(\Delta a^*\right)^2 \left(\Delta b^*\right)^2} \quad (1)$$

Sensory evaluation

A duo-trio sensory test by comparison with 15 untrained judges (each judge had extensive experience in the production line and supervision of finished avocado products) was performed. Three sets of coded samples were presented to the judges, one of the samples pertains to a reference or standard (product line for export), identified as R, and judges were asked to respond which of the two samples was equal to the reference sample (R). The data were treated using χ^2 for determining the presence or absence of significant taste difference between the samples at time zero (Pedrero, 1996; Olivas *et al.*, 2009).

Microbiological analysis

Samples with and without coating formulations were analyzed for total mesophiles and coliforms (mesophilic aerobic count, ISO 4833, 2003; total coliform count plate, Standard ISO 4832, 1991). The microbiological analyses were performed immediately after defrosting (to) and after 8h (t8) and reported as UFC/g.

Statistical analysis

All experiments were done in triplicate and the results were expressed as mean values ±standard deviation. Analysis of variance ($P<0.05$) and Tukey's range test ($\alpha= 0.05$) were used to assess significant differences between samples. The statistical analysis was carried out using Statistica v.10 from StatSoft, Inc.

Results and Discussion

Inhibitory effect of chemical agents on avocado PPO extract

The individual effect of 4-hexylresorcinol (4HSR) and sodium hexametophosphate (HAS) did not reduce significantly ($p<0.05$) the avocado PPO activity *in vitro* in the concentrations evaluated in this study. Similar results were obtained by Ghidelli *et al.* (2013), who evaluated the effect of 4HSR on the activity of artichoke PPO precipitates; they found that 4HSR in concentrations of 0.002% and 0.005%, were the least effective among the treatments applied; they also noted that increasing the concentration of 4HSR was accompanied with a decrease of the color L values, in conjunction with an increase of a, indicating tissue damage at concentrations >0.005%. On the other hand, HAS was not effective against enzymatic browning even at $50mol \cdot ml^{-1}$. The effect of HAS as anti-browning agent has been reported as effective in mixtures with other agents such as citric and ascorbic acid in acid pH, on PPO activity in apple slices (Pilizota and Sapers, 2004).

The agents sodium D-isoascorbate (D-ISO; 0.094M) and L-glutathione (LG; 0.05M) showed effect on avocado PPO activity individually, achieving a reduction from $148.45\mu mol \cdot min^{-1}$ to 2.86 and $1.74\mu mol \cdot min^{-1}$, respectively. The combination of these chemicals affects the enzymatic activity of PPO in a similar manner (Table I); it reduced the reaction rate to just $0.99\mu mol \cdot min^{-1}$, but also showed a Km value of 3.49, the highest observed in this study. Results suggest that there is a difficulty in the formation of the enzyme-substrate complex, and also a synergistic effect of this combination is exhibited on the enzymatic activity of PPO avocado cv. Hass.

Similar results of the effect of LG on the inhibition of PPO

activity in apple have been reported (Billaud *et al*., 2004). Treatments using D-ISO also showed a significant reduction of PPO activity in peach (Nogueira *et al*., 2011). No reports with regard to the combined effect of D-ISO (0.094M) and LG (0.05M) were found; however, the synergistic effect of other combinations of anti-browning agents has been reported. Rojas-Grau *et al*. (2006) studied the effect of four anti-browning agents (4HSR, GL, N-acetylcysteine and D-ISO) on minimally processed Fuji apples. Synergistic effects of antioxidant combinations (4HSR/N-acetylcysteine and N-acetylcysteine/GL) were reported by these authors, who proposed that the synergism is probably due to the combined effect of the ability of the competitive agents 4HSR and GL to bind with the available enzyme at the active site, in conjunction with substrate blockade through N-acetylcysteine (Altunkaya and Gökme, 2009).

In addition, the synergistic combinations of RC promoting a modified atmosphere with the PPO inhibitors have been reported in various pre-cut products (Laurila *et al*., 1998; Gorny *et al*., 2002; He and Luo, 2007, Rojas Grau *et al*., 2008).

Inhibition mechanisms

The combinations of LG (0.05M) + D-ISO (0.094M), D-ISO (0.094M) + 4HSR (0.025M) and LG (0.05M) + 4HSR (0.025M) and LG (0.05M) led to an increase in the reaction constant Km, which reveals a difficulty in forming the enzyme-substrate complex, while Vmax was not affected (Table I). A Km increase accompanied with unmodified Vmax is characteristic of competitive inhibition, which usually occurs when the inhibitor has a chemical similarity with the substrate of the enzyme (catechol in this case) and competes with the enzyme for the active site. Vmax is not modified, as its value indicates the moment in which all the active sites of the enzyme are busy, situation that occurs in competitive inhibition because active sites are occupied by substrate but also by the inhibitor. Moreover, the Lineweaver-Burk and Eadie-Hofstee graphs confirm a pattern of competitive inhibition (Figure 1).

The combination of LG (0.05M) + D-ISO (0.094M) allowed a greater increase of Km to 3.45, which indicated a high difficulty to form the enzyme-substrate complex, probably due to the ability of L-glutathione to act as a competitive inhibitor, as it is a strong reducing and chelating agent (Witschi *et al*. 1992; Jiang and Fu, 1998). It may interact with the two copper atoms located at the active site of the enzyme, and thus inhibit PPO activity. The kinetics of PPO in the presence of D-ISO does not fit a typical Michaelis-Menten kinetics, since it shows an asymptotic behavior on the abscissa, corresponding to a total or linear inhibition (Fersh, 1985), because the slope is equal to zero, as well as Km/Vmax.

Moreover, the intersection of the ordinate is zero then 1/Vmax= 0, since Vmax corresponds to the time of the reaction when all the active sites of the enzyme are saturated forming enzyme-substrate complexes (Voet and Voet, 1990; Bell and Bell, 1998). The results suggest that the reaction rate at any point of the reaction is zero. It is known that sodium D-isoascorbate is an isomer of ascorbic acid and it reduces the O-quinones formed in the reaction catalyzed by PPO, returning them to its form of O-diphenols, avoiding in this manner, the formation of colored pigments (Golan-Goldhirsh and Whitaker, 1984). Since the absorbance measured corresponds to the product of the reaction catalyzed by PPO, the speed of the O-quinones synthesis could be equal to the rate at which these were returned to their O-diphenols form by the reducing action of D-sodium isoascorbate (Janovitz-Klapp *et al*., 1990), then it is possible that Vmax values were not detected in the reaction of PPO when it acts in the presence of D-ISO. Additionally, the inhibition coefficient Ki obtained in this study (0.07 to 3mM, Table I) is similar to that obtained by Serap *et al*. (2006), who studied the kinetics of inhibition using PPO extract from mushrooms with cinnamic acid as an inhibitor, obtaining Ki in the range of 0.064 to 14.09mM.

Effect of inhibitor agents incorporation to the edible coating on visual quality and color

Avocado slices exposed at room temperature (Figures 2 and 3) treated with formulations of gellan gum matrix containing 0.05M LG, and the combination of inhibitors LG 0.05M + D-M ISO 0.094M and D-ISO 0.094M + 0.025M 4HSR, showed L values of 55 units that were maintained through 8h, whereas slices treated with formulations of gellan gum containing combination of agents D-ISO 0.094M + 4HR 0.025M and the control, showed an important reduction of L values, decreasing below 50 units after a 3h exposure at room temperature. These results show the correlation between the values of L and visual quality observed in surfaces of avocado slices.

The analysis of the color parameters a (Figure 4) and b (Figure 5) showed that during the initial two hours, the avocado slices treated with all formulations still possess the characteristic yellow and green avocado colors, which correspond to negative values of a (-7 to -8) and positive values of b (27-30).

After a 3h exposure to ambient temperature, a values of avocado slices treated with formulations D: Gellan gum, D-ISO 0.094M, 0.5% v/v polysorbate 80, glycerol 1.0% v/v and E (control) change drastically, reaching positive values. A significant decrease in the b values was also observed, corresponding to the loss of green and gain of brown color, a decrease in the visual quality of the product. Avocado slices treated with formulations A: Gellan gum, LG 0.05M, 0.5% v/v polysorbate 80, glycerol 1.0% v/v; B: Gellan gum, LG 0.05M, D-ISO 0.094, 0.5% v/v polysorbate 80, glycerol 1.0% v/v; and C: Gellan gum, D-ISO0.094M + 4HSR 0.025M, 0.5% v/v

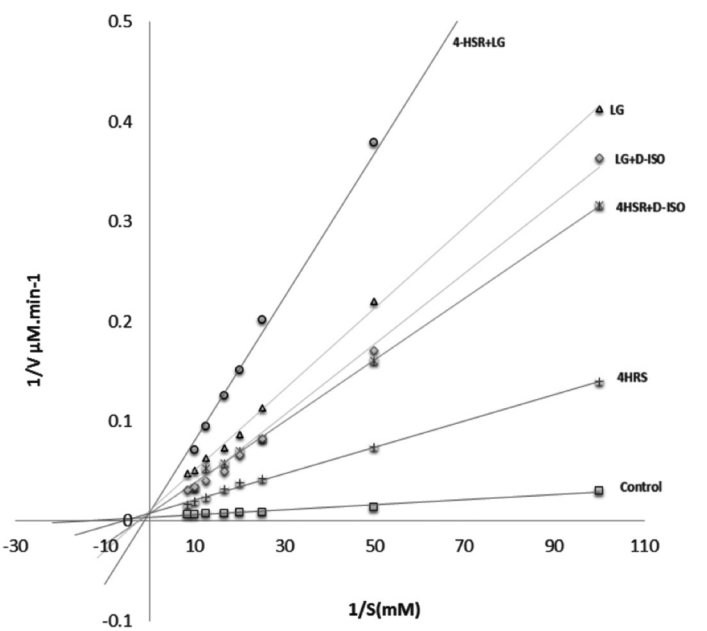

Figure 1. Kinetic behavior of the browning reaction mediated PPO in presence of L-glutathione (LG) 0.05M, 4-hexylresorcinol (4HSR) 0.025M, D-isoascorbate (D-ISO) 0.094M catechol (0.0 to 100mM) and 75µl of enzyme extract in McLlvaine buffer (pH 6.5), total volume of 3ml.

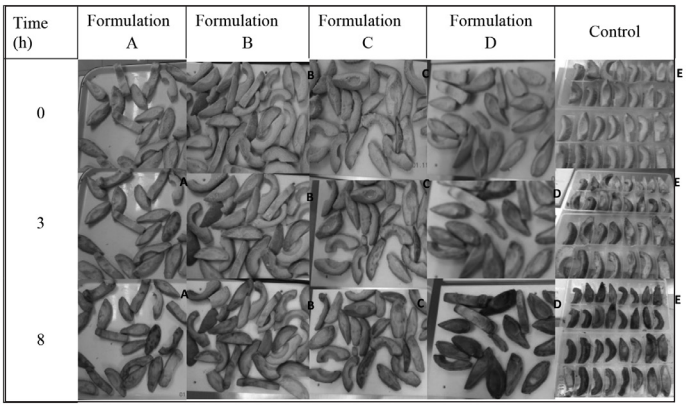

Figure 2. Visual quality of avocado slices treated with inhibitors incorporated into gellan gum matrix at 0h, and after 3 and 8h at room temperature. Formulation A: Gellan gum, L-glutathion 0.05M, polysorbate 80 al 0.5% v/v, glycerol 1.0% v/v); Formulation B: Gellan gum, L-glutathion 0.05M, D-isoascorbate 0.094M, 0.5% v/v polysorbate 80, glycerol 1.0% v/v; Formula C: Gellan gum, D-isoascorbate 0.094M + 4hexylresorcinol 0.025M, 0.5% v/v polysorbate 80, glycerol 1.0% v/v; Formulation D: Gellan gum, D-isoascorbate 0.094M, 0.5% v/v polysorbate 80, glycerol 1.0% v/v; Formulation E: control.

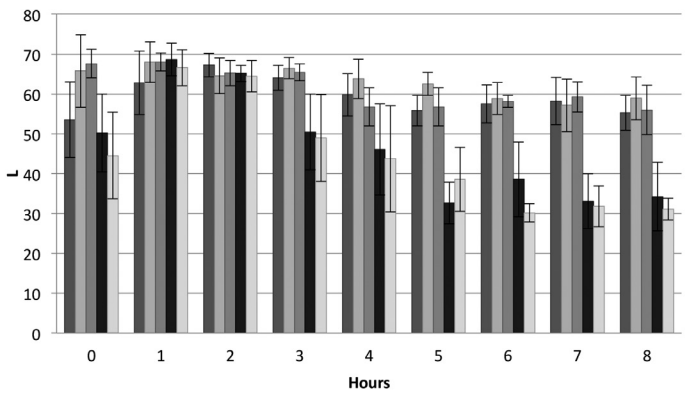

Figure 3. Brightness as L values of avocado cv. Hass slices through 8h exposed to room temperature. ■ Formulation A: Gellan gum, L-glutatión 0.05M, 0.5% v/v polysorbate 80, glicerol 1.0% v/v); ■ Formulation B: Gellan gum, L-glutatión 0.05 M, D-isoascorbate 0.09 M, 0.5% v/v polysorbate 80, glycerol 1.0% v/v; ■ Formulation C: Gellan gum, D-isoascorbate de sodio 0.094M + 4hexylresorcinol 0.025M, 0.5% v/v polysorbate 80, glycerol 1.0% v/v, ■ Formulation D: Gellan gum, D-isoascorbate 0.094M, 0.5% v/v polysorbate 80, glycerol 1.0% v/v; ■ E: control.

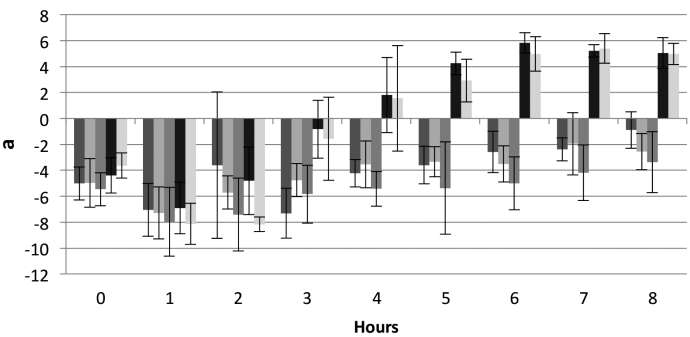

Figure 4. a values of avocado cv. Hass slices through 8h exposed to room temperature. ■ Formulation A: Gellan gum, L-glutatión 0.05M, 0.5% v/v polysorbate 80, glicerol 1.0% v/v); ■ Formulation B: Gellan gum, L-glutatión 0.05 M, D-isoascorbate 0.09 M, 0.5% v/v polysorbate 80, glycerol 1.0% v/v; ■ Formulation C: Gellan gum, D-isoascorbate de sodio 0.094M + 4hexylresorcinol 0.025M, 0.5% v/v polysorbate 80, glycerol 1.0% v/v, ■ Formulation D: Gellan gum, D-isoascorbate 0.094M, 0.5% v/v polysorbate 80, glycerol 1.0% v/v; ■ E: control.

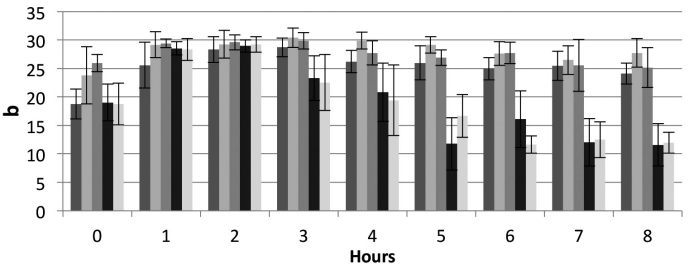

Figure 5. b values of avocado cv. Hass slices through 8 h exposed to room temperature. ■ Formulation A: Gellan gum, L-glutatión 0.05M, 0.5% v/v polysorbate 80, glicerol 1.0% v/v); ■ Formulation B: Gellan gum, L-glutatión 0.05 M, D-isoascorbate 0.09 M, 0.5% v/v polysorbate 80, glycerol 1.0% v/v; ■ Formulation C: Gellan gum, D-isoascorbate de sodio 0.094M + 4hexylresorcinol 0.025M, 0.5% v/v polysorbate 80, glycerol 1.0% v/v, ■ Formulation D: Gellan gum, D-isoascorbate 0.094M, 0.5% v/v polysorbate 80, glycerol 1.0% v/v; ■ E: control.

polysorbate 80, glycerol 1.0% v/v, despite a slight increase in the a values, this parameter did not change to positive values upon 4h of exposure to room temperature, maintaining the green-yellow pigments during the 8h of evaluation, preserving the visual quality of the treated samples during the period of evaluation (Mendoza-Gómez *et al.*, 2016). This behavior was evidenced with the total color change (ΔE) for each treatment from 0h to 8h exposure (Figure 6). Formulations A and C were those with greater effectiveness in color conservation, which indicates a decrease in PPO activity (Campas-Ríos *et al.*, 2012).

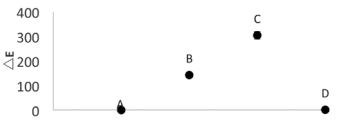

Figure 6. Total color change (ΔE) in avocado cv. Hass slices through 8h exposed to room temperature. Formulation A: Gellan gum, L-glutathion 0.05M, polysorbate 80 al 0.5% v/v, glycerol 1.0% v/v); Formulation B: Gellan gum, L-glutathion 0.05M, D-isoascorbate 0.094M, 0.5% v/v polysorbate 80, glycerol 1.0% v/v; Formula C: Gellan gum, D-isoascorbate 0.094M + 4hexylresorcinol 0.025M, 0.5% v/v polysorbate 80, glycerol 1.0% v/v; Formulation D: Gellan gum, D-isoascorbate 0.094M, 0.5% v/v polysorbate 80, glycerol 1.0% v/v; Formulation E: control.

Microbiological and sensory evaluation

Avocado slices treated with polymeric formulations or without it showed a low microbial count. For mesophilic aerobic and total coliform were found values of 180 ±30 and values <100 UFC/g, respectively. These values are lower than those allowed by international standards (ISO 4833, 2003 for mesophilic aerobic and ISO 4832, 1991 for total coliform).

For samples treated with the formulations A: Gellan gum, LG 0.05M, 0.5% v/v polysorbate 80, glycerol 1.0% v/v; B: Gellan gum, LG 0.05M, D-ISO 0.094, 0.5% v/v polysorbate 80, glycerol 1.0% v/v; and D: Gellan gum, D-ISO 0.094M, 0.5% v/v polysorbate 80, glycerol 1.0% v/v, the data collected from the panelists do not shown significant differences ($p<0.05$) in taste, compared against the reference at time zero. The individual components of these formulations have no dominant sensory characteristics that could generate significant differences in treated avocado slices. The samples treated with formulation C: gellan gum, D-ISO 0.094M + 4HSR 0.025M, 0.5% v/v polysorbate 80 and glycerol 1.0% v/v showed statistically significant difference ($p>0.05$) in flavor compared against the reference. This is probably due to the presence of 4-hexylresorcinol, since Mendoza-Gómez *et al.* (1993) found that concentrations greater than 0.03% of this compound

influenced the taste of apple slices cv. Fuji.

Conclusions

The edible coating formulation with gellan gum, added with the mixture of L-glutathione 0.05M, D-Isoascorbate 0.094M, 0.5% v/v polysorbate 80 and glycerol 1.0% v/v proved effectiveness in inhibiting PPO, preventing enzymatic browning and preserving the visual quality along color parameters (L, a and b) and total color change (ΔE*) in avocado slices through 8h at room temperature. This represents an increase in shelf life of 5h compared to the control, which only lasts 3h, or 2.6 times the increase in the commercial time compared to untreated avocado slices. Additionally, the incorporation of the formulation (edible coating) to the avocado slices does not alter their sensory properties. Finally, this edible coating, is highly likely to be effective if it is applied in other fruits sensitive to enzymatic browning caused by the PPO enzyme.

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