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A COMPARATIVE EVALUATION OF INTEGRITY AND COLOUR PRESERVATION OF SLICED APPLES PROTECTED BY CHITOSAN AND ZEIN EDIBLE COATINGS

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Key words: edible coatings, chitosan, zein, minimally processed apples, colorimetric analysis.

ABSTRACT

Chitosan (chitin deacetylated derivate) and zein (storage proteins of maize) are promising materials for use as protective edible coatings on several fruits and, in particular, for post-harvest conservation, increasing self-life of intact fruits. In the present study, two basic coating formulations, one based on chitosan (hydrophilic) and one based on zein (hydrophobic), reported as efficient in covering intact apples, were evaluated on sliced samples. Chitosan gel was prepared in a concentration of 2.0 gL^{-1} dissolved in 1 % acetic acid aqueous solution and the zein formulation with 4.0 % (w/w) with 0.25 % wt of oleic acid as plasticiser dissolved in 70 % ethanol aqueous solution. Sliced apples (cv. Gala) were separately coated and evaluated with respect to mass loss, firmness and colour alteration when storage at room temperature. The results show that both formulations have some positive action in preserving mass and firmness, with better results to zein-coated samples. Nevertheless, both coatings introduce significant variations on mesocarp colour and may accelerate the surface's browning instead of inhibiting it, not being indicated for use as protection on processed apples. Increasing in coating thickness and the association of antioxidant agents are discussed.

UNA EVALUACIÓN COMPARATIVA DE LA INTEGRIDAD Y DE LA PRESERVACIÓN DEL COLOR DE MANZANAS EN RODAJAS RECUBIERTAS POR QUITOSANO Y ZEÍNAS.

Palabras claves: recubrimientos comestibles, quitosano, zeínas, manzanas minimamente processadas, análisis colorimétricas.

RESUMEN

Quitosano (derivado desacetilado de la quitina) y zeínas (proteínas de reservas del maíz) son materiales promisores para uso en la formación de coberturas comestibles sobre frutos y, en particular, en la conservación post cosecha de frutos intactos. En este estudio, dos formulaciones básicas, una fundamentada en quitosano (hidrofílica) y otras en zeínas (hidrofóbica), reportada como eficientes en el recubierto de manzanas intactas, fueron evaluadas sobre las amuestras cortadas. Gel de quitosano fue preparado en la concentración de 2g/L disueltas en solución acuosa de 1% de ácido acético y formulación con zeínas en la concentración de 4,0 % (w/w) con 0,25% de ácido oleico como plastificante disuelto en etanol a 70%. Rodajas de manzanas fueron separadamente recubiertas y evaluadas con respecto a la pérdida de la masa, firmeza y alteración del color cuando almacenadas en temperatura ambiente. Los resultados indican que ambas formulaciones tienen alguna acción positiva en la preservación de la masa y de la firmeza, con mejores resultados para las coberturas con zeínas. Sin embargo, las coberturas introducen variaciones significativas en el color del mesocarpio y puede acelerar el obscurecimiento de la superficie en lugar de inhibirlo, no siendo indicada en la protección de las manzanas procesadas. Aumento en la espesura y asociación con agentes antioxidantes son discutidos.

INTRODUCTION

Fruits and vegetables have been emerging as one of the rapidly growing segment within the food market, experiencing exponential sales growth in the later decades. In particular the acceptance of lightly processed fruits is inherently increasing reflecting a general consumer tendency to purchase pre-packaged ready-to-eat fresh-cut fruits in retail, street shops and market places.

Projection for worldwide sales of "lightly processed" fruits is expected to increase by an average of 30 % yearly (Cadwell, 2005). In USA, for instance, cut fruits and vegetables already account for nearly 15 % of all fruit and vegetable market (Tojas-Graü et al., 2011), and in Europe, Spain figures as one of the most pre-prepared convenience food consumer, where, according to data from 2005, minimally processed products represented 5 % of all fruits and vegetables consumed (Fruittoday, 2011). Actually, this figure is not different in the whole European territory showing an on-going general trend.

Minimally processed fruits undoubtedly increase the economic values of fresh products, benefiting from their convenience and broad array of possibilities and diversity of flavour and variety. Cut fruits however, present many technical problems since the wounded tissues spoil faster. During peeling, cutting and shredding, the production of ethylene and the respiration rate increase and the fruit's surface suffers rapid enzymatic browning and the exposure to microbial contamination is facilitated.

According to Garg et al. (1990), peeled and sliced fruits have been shown a six to seven-fold increase in microbial numbers along time when compared to intact products. Although a wide range of different sanitizers is available for disinfecting/sanitizing fresh produce, their efficacy is variable and none are able to ensure the total elimination of pathogens. Problems relative to sanitation of fresh-cut products and wash disinfection were reviewed by Gil et al. in 2009.

Nevertheless, fresh-cut produce has a great consumption appeal, due to convenience, functionality and attractive appearance. As a matter of fact, according to surveys carried out by Nassu et al. (2001) and Kader (1993), not only the nutritional properties, but also a combination of sensorial factors (appearance, aroma and flavour), strongly influence sales and determines the value of processed products to consumers. Colour particularly has been considered to have a key aesthetic role in processed fruit choice (Clydesdale, 1993). According to Heber (2002), an array of produces with vivid colours, uniformly cut and absent of any visual signs of diseases is more enticing of their health promoting benefits. In this sense, the development of novel approaches for preserving the aspect and quality are very desirable.

The current and most usual preservative procedure however, has been the simple sealing within semi-permeable polymer packages and cold storage. The use of modified atmosphere and more recently, protective applications such as edible coatings has been evaluated as potential technology feasible for enhance sliced fruits shelf-life (Janjarasskul & Krochta, 2010). Edible coatings can be applied or formed directly on the surface generating an internal modified atmosphere (McHugh & Senesi, 2000) and providing a barrier to pathogens and water loss.

Several types of materials have been successfully tested for preserving fresh products. Formulations based on lipids, polysaccharides, and proteins, alone or in combinations, have been used to form edible coatings (Bourtoom, 2008). Among them, attention has been given to the use of the polysaccharide chitosan and the maize protein zein to prolong the storage life mainly of whole fresh fruits, as apples (Baldwin et al., 2002; Bai et al., 2003; Hsu et al., 2005).

Chitosan is a natural polymer soluble in acidic medium (pH < 5.5) and has antibacterial and antifungal activities well documented (Rabea et al., 2003; Goy et al., 2009). Chitosan has good film-forming property and for most applications no plasticiser is needed.

Zein is the main storage protein in the corn endosperm and contributes to more than half the total mass of the seed proteins. Pure zein is brittle and soluble in alcohol and, when blended with plasticiser, can be transformed into flexible, heat-sealable films, suitable for applications as gas and moisture-barrier. Common zein plasticisers include glycerol, poly(ethylene glycol), sorbitol, etc., and recently the preparation of edible zein films involving plasticisation with oleic acid for food purpose has been considered (Wang and Padua, 2006; de Almeida et al., 2010; Scramin et al., 2011).

The present study evaluates one basic chitosan (hydrophilic) and one basic zein (hydrophobic) formulation as edible coatings, (regularly referred as efficient in covering intact fruits), on sliced apples, comparing their protective features concerning mass loss, firmness and mainly the mesocarp colour alteration.

MATERIALS AND METHODS

Preparation of Film-Forming Solutions

The starting chitosan was purchased from Sigma-Aldrich (Brazil) and used as received (medium molar mass; degree of deacetilation around 89 %). Coating solution was prepared by dispersing 2.0 g/L of the polymer in 1 % acetic acid (v/v) aqueous solution under moderate stirring, until complete dissolution (around 4 hours). The initial pH was adjusted to 4.5. No plasticiser was added to chitosan formulation. The 2.0 g/L concentration was adopted based upon satisfactory results (homogeneity, stability) previously attained by this formulation as edible coating forming (Moreira et al., 2009; Assis, 2008).

The zein (maize protein) used for gel preparation, was previously extracted from the corn gluten meal (CGM), supplied by Brazil Corn Products. Briefly, the extraction of zein consisted in an initial oil removal from CGM carried out in a soxlhet apparatus using hexane (1:5 v/v). The residual mass is then mixed with 70 % (v/v) ethanol and left overnight with stirring at room temperature. Zein is than obtained after solvent evaporation. Details of extraction sequence and protein characterisation are available in the literature (Forato et al., 2003). For zein coating the formulation was 4.0 % w/w (34.0 g/L) of protein with 0.25 % (w/w) of oleic acid, as plasticizer, dissolved in 70 % ethanol (v/v) aqueous solution. These proportions are similar to those which good results as reported in wrapping intact apples (Baldwin & Baker, 2002; Scramin et al., 2007).

Samples

Gala apples (*Malus domestica* Borkh.) were acquired at local market from a same lot and presumably at similar maturity stage. After sanitization (immersion in aqueous solution of sodium hypochlorite at 200 ppm for 10 minutes), the samples were sliced into two halves and then individually immersed in the coating solutions. Excess of gel was allowed to drain off and the film formed by spontaneous drying at room temperature. Twenty samples were prepared for each formulation and a same number was kept uncoated as reference. The apples slices (coated and uncoated) were stored in air under non-controlled conditions (an ambient temperature of between 25-28 ^{o}C and RH of 76 %).

Quality Evaluation

Several variables can currently be measured to determine quality in cut fruits. Since this study has focus on physical integrity and visual aspects that influence the acceptability to the consumer, the attributes

here evaluated were: (*i*) the loss in mass, which occurs mainly due to transpiration process through the cut surface; (*ii*) the firmness, expressed by the force required to produce a deformation, and (*iii*) the colour alteration on cut surfaces (mesocarp) over storage period. The first two are related to texture breakdown.

The loss of mass was recorded daily and values estimated as the average of individual weights and measured in triplicate. Samples were weighed daily in a digital Gehaka AG 2000 analytical scale (Gehaka Ltd., São Paulo, Brazil). Relationships with the storage time were established by a linear regression model along with the R^2 value. The average gradients (slope) of each line were calculated by means of the first derivative expressing indirectly the rate of mass loss.

The firmness was assesses using a hand penetrometer with an 8 mm diameter plunger. The penetration speed was around 50 mm/min and two readings were made at the equatorial region of each sample, on peeled faces, every other day and the measurements expressed in Newtons (1 N = 0.1 KgF).

Colour on cut surfaces was evaluated using a colorimeter Chroma Meter CR-400 (Konica Minolta Sensing Inc, Sakai, Japan), and measurements made at 2 locations for each sample up to seven storage days. The CIE - L*a*b* colour system was used to evaluate colour changes, where L* defines the lightness/darkness (L* = 0 yields black and L* = 100 indicates diffuse white), and a* and b* define the chromatic characteristics, i.e., a* negative values indicate green while positive values indicate magenta, and b*, negative values indicate blue and positive values indicate yellow.

The total colour differences (ΔE) were estimated as:

$$\Delta E = [(L^* - L_o^*)2 + (a^* - a_o^*)2 + (b^* - b_o^*)2]^{1/2} \qquad /1/$$

Where L_o, a_o e b_o correspond to the initial values. The browning index (BI) was calculated and used as an indicator of intensity of brown colour following the Palou et al. (1999) relation:

$$BI = [100 (X - 0.31)] / 0.172;$$
$$X = (a^* + 1.75L^*) / (5.645L^* + a^* - 3.021b^*) \qquad /2/$$

For ΔE and IE calculation, only the medium values of a*, b* and L* were considered.

Firmness and colorimetric data were subjected to statistical evaluation by one-way analysis of variance (ANOVA), considered significant for $P \leq 0.05$ using a Microcal Origin 8.0 software (OriginLab Co., Northampton, MA, USA).

RESULTS AND DISCUSSION

When deposited on fruit cut surfaces, both formulations result in a highly transparent coating almost imperceptible to the naked eyes. After superficial evaporation of the solvent, (around 20 min in air at room temperature), by a simple visual assessment one can say that the chitosan-coated samples appear slightly bright, while zein surfaces resulted in relatively dull less shiny appearance. Nevertheless, no significant changes on visual aspect were easy to be observed amongst lots in the first day of evaluation.

In Figure 1 is shown the evolution of weight decay, in percentage with respect to initial mass. The mass loss takes place by water vapour permeation and for coated samples the barrier effect is better distinguishable after the first week of storage. Both chitosan and zein-based coatings reduce the rate of loss. Zein-coatings however, protect better mainly after the 10th day of storage. For these, during the first week the preservation is 7 % superior to those measured to uncoated samples, retaining around 13 % more weight by the end of the 20th day. Chitosan coatings were less efficient in retarding loss of mass, what in some extent is related to chitosan hydrophilic

characteristics, in which the water vapour transference is favoured across the hydrophilic parts of the coating (Hernandez, 1994).

The lines which fits the points were adjusted to a linear model, such as $y = a + bx$ for simple mathematical consideration. In Figure 1 are inserted the correspondent squared linear coefficients (R^2) and the slope values (s). For all set of samples the loss of mass can be adequately adjusted to a linear model pointing to a loss of mass by a same mechanism. The higher the slope (s) greater will be the rate of mass loss and the better adjustment (R^2) can be interpreted as a continuous behaviour in a predictable sequence. The uncoated samples resulted in higher R^2 (0.978) and s values (3.06), indicating a strong linear association with a more intense loss of mass over time. Conversely, for the zein-coated samples the slope is inferior, meaning a reduction on the rate and consequently a slight delay in senescence.

An increasing in the coating efficiency, as measured after the first week, could be used strategically since some studies have shown that some nutrients, as vitamin C and carotene, degrade very little during the first week under refrigerated condition in some fresh-cut fruits (Wright and Kader, 1997). So a combination of controlled cold environment and edible coatings, can contribute to minimize overall fruit deterioration.

Tissue softening is also a very serious problem with processed products and can be monitored via puncture tests as an indicator of firmness, as plotted in Figure 2. The results also suggested that both coats have action on softening, marked for zein-formulation which presents the higher average firmness values. All samples however, behave similarly in function of time with a gradual decreasing, at a similar rate with stabilization after the 8th day. As the samples weight loss, the slices become dried which lead to and stabilization and a subsequent increase in the firmness of the slices along time. It is worth mentioning that associated to the increasing in the respiratory CO_2 and C_2H_4 production due cutting, apple is a climacteric fruit and a ripening stage may occur during storage also contributing to initial firmness decay.

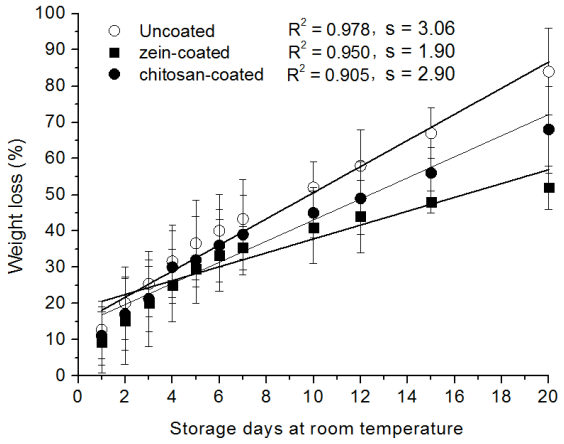

Figure 1 - Weight loss in function of storage time, for coated and uncoated samples. Data adjusted by linear regression.

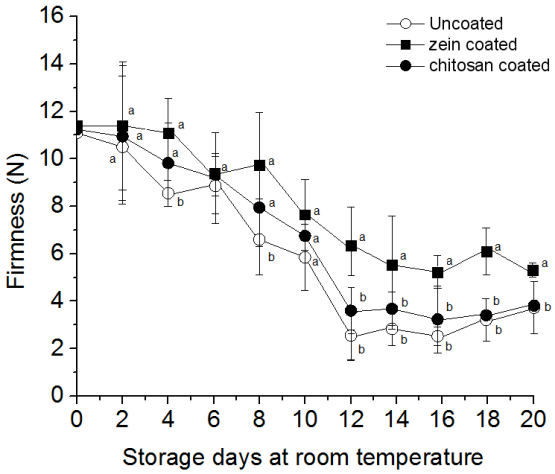

Figure 2 - Firmness of (uncoated and coated) cut apples during storage. Points with different letters are statistically different at p< 0.05.

Concerning colorimetric measurements, Figure 3 shows the variation in colours and lightness. The main change in colour on the cut apple surfaces is attributed to the enzymatic oxidation of endogenous phenols

into quinones, which polymerize into brown products. Thereby, an increasing in the stimulus a* indicates an increasing towards reddish brown color, i.e., an increase of polymerized quinones. In the tested samples, the a* values (Fig. 3(a)) increases daily with stabilization after 4 days of storage. There are important differences between a* values for coated and uncoated apples, mainly in the first days. Though, by visual naked eyes, a reliable differentiation of colour changes could not be positively confirmed among samples. Instrumental measurements, however, demonstrate a trend opposite to expected, i.e., browning predominates on the coated apples instead to uncoated, mainly on those under zein coated formulation.

Surface reactions due to the elevated concentration of alcohol in the zein solvent formulation (70 % ethanol) could play an important role in this result. The functional group for alcohols is the hydroxyl -OH radical which imparts polar characteristics to the molecule. In aqueous solution, the ethanol can transfer a proton to water producing free hydronium ion (H3O+) and an alkoxide or a stable phenoxide ion. These compounds can share a bonding pair of electrons, favouring the solvation of phenolic molecules. This, along with the strong decrease of solvent−solvent polar interactions leads to a high concentration of o-quinones compounds towards the fruit cut surface. Such concentration induces coupling and polymerisation transforming the quinones into dark pigments (Escríbano-Bailón & Santos-Buelga, 2003). In reality, the phenolic oxidation followed by surface quinone polymerisation is a natural healing process which has the objective to seal the injured tissue (Friedman, 1997).

Additionally, the zein proteins have a light yellow colour attributed to the presence of carotenoids β-carotene, zeaxanthin and lutein (Kurilich & Juvick, 1999, Sessa et al., 2003) and the resultant cured films retains some residual pale yellow in colour (Trezza & Krochta, 2000). Such effect is confirmed by the chromatic ordinate b* (Fig. 3(b)). As b* values increases, yellow colour intensity increases. These data confirm that the application of both films and, mainly the zein formulation, accentuate the brown appearance instead of inhibiting it.

The L* component decrease for all samples over time (Fig. 3(C)), due to surface dehydration which reduces gloss and causes wilting appearance (Garcia & Barret, 2005). From a physical point of view, brightness is related to amount of light scattered and usually is associated with surface irregularities. For zein-coated samples the inferior level of gloss can also be attributed to the natural opaqueness of the maize protein films (Bai et al., 2003; Trezza & Krochta, 2000). Anyway, the differences between the control and coated samples were also somewhat small and less obvious to the naked eye.

The total colour difference Δ*E* and the browning index BI (calculated by equations /1/ and /2/), which are a combination of L*, a* and b* medium values, have been extensively used to characterize variations in colour perception. For these, a same tendency is followed by all samples (Figure 4). Δ*E* summarizes the progressive deviation between the original colour, as found at first measure (just after the cutting), and each subsequent measurement, that is, identify numerically the variability in colour over periods of time. All cut surfaces suffer colour alteration and it is quite apparent that the application of both coating formulations here used, was not effective in preserving, or even in decreasing the enzymatic browning. Such inactivity is better visualized by the browning index BI (Figure 4(b)) whose values are proportionally higher for the coated slices.

Taken together, these results demonstrate that the coatings themselves, in spite of having positive effects on mass and firmness conservation, bring no benefits in

controlling apple mesocarp enzymatic browning during non-refrigerated storage.

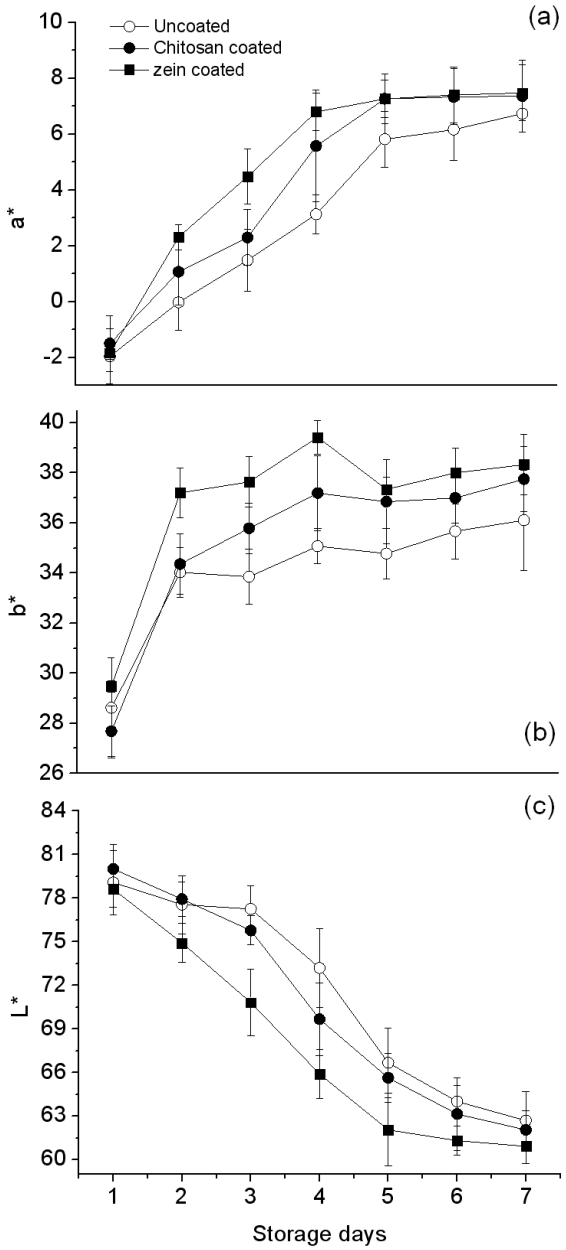

Figure 3 - Effect of coating treatments on the a*, b* and L* values of the fresh-cut apples surfaces.

It is important to mention that the thickness of coating can interfere on the degree of colour stability. The effectiveness of barrier in edible coatings, such as to control the moisture migration, resistance to CO_2 and O_2 transmission are quite dependent on the film homogeneity and thickness (Park & Chinnan, 1994). A low oxygen environment in combination with moderate levels of CO_2 has been successfully used to maintain the visual appearance of several fresh-cut fruits (Rojas-Graü et al., 2009). Actually, oxygen is needed for the enzymatic reactions and a restriction on O_2 permeation can reduce the level of browning. An internal increasing in CO_2 level and a decreasing in O_2 level can be attained by increasing coating thickness. Thicker coatings can be produced either by increasing polymer concentration or by using layer-by-layer assembly (Skurtys et al., 2011).

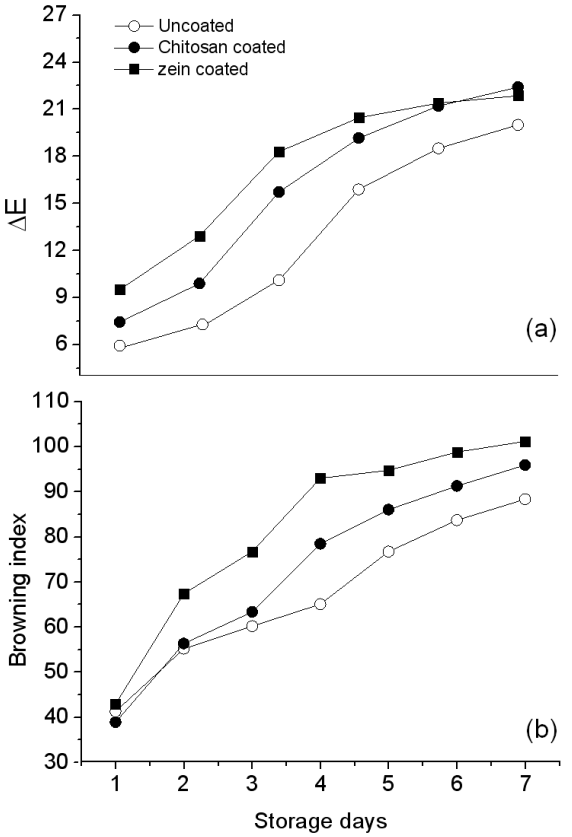

Figure 4 - Effect of coating treatment on total color difference (a) and on browning index (b).

Additionally, the use of common antioxidants agents such as ascorbic, citric and oxalic acids; L-cysteine, 4-hexylresorcinol and

N-acetylcysteine, should be considered. These compounds have been reported as efficient in preventing browning on cut surfaces by competitive reaction with polyphenol oxidase forming stable colourless compounds (Perez-Gago et al., 2006; Raybaudi-Massilia et al., 2007).

CONCLUSION

Both chitosan and zein are edible, safe for humans and could be used as a protective coating applied near harvest time or on fresh-cut fruits surfaces. Our present study showed however that the both basic formulations which have been reported as efficient on protecting intact apples, do not evidence good results when applied on cut samples. The coatings have some positive effect on reducing mass losses and preserving firmness, most significantly for the zein formulation with a better performance on reducing loss of mass via transpiration. The latter may be attributed to the hydrophobic feature of this protein. Concerning colorimetric measurements, the coatings, by themselves, were not efficient in inhibiting enzymatic browning. On the contrary, changes were introduced in the colours when compared with the untreated–control samples. It is worth noting that these changes are only perceptible via instrumental colorimetric measurements. The incorporation of proper antioxidants in the film formulations should be considered for attaining a reduction on browning effect.

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