Influence of galactomannans/collagen edible coatings in gas transfer rates in fruits

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Abstract

One of the most important problems in fruit conservation is their short shelf life. Shelf-life can be extended by reducing respiration rates. This is done by controlling factors such temperature, relative humidity, gas composition (ethylene, O_2 and CO_2) and light. An important strategy to control some of these factors is the use of modified atmospheres, obtained using galactomannan coatings, those show low caloric contents. Collagen coatings have already been used on meats and sausages to reduce gas permeability and/or water vapor permeability (WVP). The objective of this work was to produce new edible coatings, based on the mixture of galactomannan, collagen and glycerol, and study their influence in gas transfer rates in mangoes and apples. The coatings presenting the best values of wettability were tested in relation to their gas permeability properties (CO_2, O_2 and H_2O). Mangoes coated with a solution of *Adenanthera pavonina* galactomannan, collagen and glycerol, and the gas transfer rates compared with mangoes without coating. The gas transfer rate was calculated. A 28% less O_2 consumption and 11% less CO_2 production were observed in coated mangoes when compared with mangoes without coating. The same procedure was done in apples (in this case using *Caesalpinia pulcherrima* galactomannan). The CO_2 production and the O_2 consumption is approximately 50% lower in apples with coating than in apples without coating. Results suggest that the coatings can reduce gas transfer rates in these fruits, and can be important tools to extend the shelf-life of fruits.

Resumo

Um grande problema na conservação de frutos é a sua curta vida de prateleira. A vida desses frutos pode ser prorrogada reduzindo as taxas respiratórias. Isso pode ser feito controlando fatores como a temperatura, umidade relativa, composição de gases (etileno, O_2 e CO_2) e luz. Uma estratégia importante para controlar alguns desses fatores é o uso de atmosferas modificadas, obtidas usando revestimentos de galactomanana, que possuem baixo teor calórico. Revestimentos de colágeno têm sido usados em carnes e salsichas para reduzir a permeabilidade a gases e/ou ao vapor de água. O objetivo do trabalho foi produzir filmes comestíveis, baseados na mistura de galactomanana, colágeno e glicerol, e estudar sua influência nas transferências de gases em mangas e maçãs. Os filmes que apresentaram melhores valores de molhabilidade foram testados em relação as suas permeabilidades a gases . Mangas foram revestidas com solução de galactomanana de *Adenanthera pavonina*, colágeno e glicerol, e a taxa de transferência de gases comparada com mangas sem revestimento. A taxa de transferência de gases foi calculada. Um consumo 28% menor de O_2 e produção 11% menor de CO_2 observou-se em mangas revestidas quando comparadas com mangas sem revestimento. O mesmo procedimento foi feito em maçãs (neste caso usando galactomanana de *Caesalpinia pulcherrima).* A produção de CO_2 e consumo de O_2 é aproximadamente 50% menor em maçãs revestidas que em maçãs sem revestimento. Os resultados sugerem que estes revestimentos podem reduzir as taxas de transferência de gases,e podem ser ferramentas importantes para prolongar vida de prateleira de frutos.

Introduction

Great losses in quality and quantity of fresh fruits occur between the harvest and the consumption. Estimates show that 25 to 80 % of losses of fresh fruits are due to of their putrefaction. One of the most important problems, in transport, storage and commercialization of fruits and vegetables, is their senescence. When the fruit is harvested, there is an interruption in the gaseous balance, occurring a high influx of oxygen with the proportional loss of carbon dioxide. In this new condition the cells are not renewed and the respiration rate increases, causing a metabolic loss and taking the fruit to a gradual maturation and eventual senescence. The respiration rate depends both of internal and external factors. The internal factors include the species, the cultivar, and the growth state, while the external factors include the atmospheric composition O_2, CO_2 and ethylene rates, the temperature and stresses [1].

The films act as semipermeable barriers which are able to assure the quality of the food. Some of them, besides being biodegradable, offer alternative packaging systems which cause reduced environmental damages. The modified atmosphere created by the cover, generates a physical capture of CO_2 inside the fruit and a partial occupation of the pores, reducing in such a way the gaseous exchange and reducing the respiration rate. If the permeation of oxygen (O_2) to the fruit´s interior is reduced, a prolongation of the maturation time occurs [2]. Film formation and properties of several polysaccharide materials such as starch and starch derivatives, alginates, cellulose derivatives, carrageenan, various plant and microbial gums, chitosan and pectinates have been studied in the last years. In general, due to their hydrophilic nature, polysaccharide films generally exhibit limited water vapor barrier ability. However, certain polysaccharides, applied in the form of high moisture gelatinous coatings, can retard moisture loss from coated foods by functioning as sacrificing agents rather than moisture barriers [3]. In accordance with Banker [4] an inverse relation between the permeability to water vapor and the permeability to oxygen, is sometimes observed. Galactomannans as reserve carbohydrates are found in cell wall storage polysaccharides of various albuminous or endospermic seeds. The physicochemical and conformational properties of the galactomannans are related with the ratio mannose/galactose (M/G) and the distribution of galactose residues throughout the main chain. The polar nature of proteins confers to protein films the property of being excellent barriers to oxygen (apolar), possibly due to their impermeability to apolar substances and the high value of cohesive energy that they contain. Collagen is an abundant protein constituent of connective tissue in vertebrate (about 50 % of total human protein) and invertebrate animals [5].

Blending has acquired importance in improving the performance of the polymeric materials. It has become an economical and versatile way to obtain materials with a wide range of desirable properties [6]. Biodegradable protein and polysaccharide films with satisfactory mechanical properties and good appearance are potential and ecological alternatives for substituting synthetic packaging in pharmaceutical and food applications. The formation of protein–polysaccharide complexes has been related to enhancements of the functionality of proteins adsorbed at the fluid interfaces [7]. Protein–polysaccharide interactions are sensitive to details of protein and polysaccharide structures as well as to pH [7, 8].

Having for base this type of knowledge, a good strategy to be evaluated is the development of films produced from blends of galactomannans and collagen, with the purpose of improving coating properties through the possible synergism between them. The objective of this study was to produce new edible coatings, based in the mixture of novel galactomannans (*Adenanthera pavonina and Caesalpinia pulcherri-*

ma), collagen and glycerol, to characterize the coatings with the best wettability values in terms of their physical-chemical properties and to evaluate the use of this coatings in some fruits (apple and mango).

Materials and Methods

The **seeds** of *A. pavonina* and *C. pulcherima* were collected in Fortaleza, Ceará (Brazil) during June 2006 and kept in a dry place until further use. The soluble anionic **collagen** was prepared by alkaline treatment of bovine intestinal submucosal tissue, at 20 °C for a period of 72 h, followed by homogenization in 0.5 mol L^{-1} acetic acid solution and brought to a final collagen concentration of 10 g L^{-1} [9]. The **galactomannan extraction** was performed with ethanol and distilled water. In this process the seeds are removed from the pods, cleaned and put in a blender. The endosperm is separated from the cotyledon, peeled and suspended in previously warmed ethanol (70 °C) during 15 minutes (to inactivate enzymes that could degrade the polysaccharide). The ethanol is decanted and distilled water is then added in the proportion of 1:100. This mixture is left during approximately 1 hour and then mixed in a blender during 5 min. The purification of the galactomannan is achieved by filtering it through nylon, followed by centrifugation at 3200 g during 30 minutes. The galactomannan is precipitated in ethanol with the proportion 2:1 (ethanol – galactomannan solution). In the end of this process the precipitated galactomannan is lyophilized, and kept in the freezer until further use. The **coating solutions** (blends) were prepared dissolving the lyophilized galactomannans in distilled water followed by the addition of the collagen solution and the plasticizer (glycerol). Each blend was homogenized during 5 minutes at room temperature (21 °C) and left to stabilize during 10 more minutes at the same temperature. This study was preceded by a "screening" with 12 different ratios of collagen, galactomannan and glycerol. The **critical surface tension** of the fruits was determined according to Zisman [10]. In systems having a surface tension lower than 100 mN/m (low-energy surfaces), the contact angle formed by a drop of liquid on a solid surface will be a linear function of the surface tension of the liquid. The Zisman method is applicable only for low energy surfaces; therefore it is necessary to determine the surface energy of the fruits. The estimation of the critical surface tension was performed by extrapolation from Zisman plots [10]. The liquids used to determine the surfaces properties from the fruits have: the surface tension, the dispersive and the polar component were, respectively, 72.10, 19.90 and 52.20 mN/m for water, 44.40, 44.40 and 0.00 mN/m for bromonaphtalene and 56.90, 23.50 and 33.40 mN/m for formamide [11].

When a solid is contacted by a liquid in the presence of vapour, the liquid will adhere well on the solid surface if the total free energy required for the creation of the new interface decreases. The physical significance of this energy change is the work needed to separate the solid and liquid from the solid/liquid interface, being the equilibrium the **spreading coefficient** (*Ws*). Contact angle and liquid-vapor surface tension were measured in a face contact angle meter (OCA 20, Dataphysics, Germany). The **surface tension** of the coating solution was measured by the pendant drop method using the Laplace-Young approximation [12]. The samples of the coatings were taken with a 500 µL syringe (Hamilton, Switzerland), with a needle of 0.75 mm of diameter. The contact angle at the fruit surfaces was measured by the sessile drop method [13], in which a droplet of the tested liquid was placed on a horizontal surface and observed with a face contact angle meter.

Oxygen permeability (O_2 *P*) was determined based on the ASTM (2002) method. A film was sealed between two chambers; having each one two channels. In the lower chamber O_2 is supplied at a controlled flow rate to keep its pressure constant in that compartment. The other chamber was purged by a stream of nitrogen, also at a controlled flow. This nitrogen acted as a carrier for the O_2 and the flow leaving this chamber was connected to an O_2 sensor. The flows of the two chambers were connected to a manometer to ensure the equality of pressures between both compartments. As the O_2 was carried continuously by nitrogen flow, it was considered that O_2 partial pressure in the upper compartments is null, therefore ΔP is equal to 1 atm. **Carbon dioxide permeability** (CO_2P) was determined based on the ASTM (2002) method. The films were sealed between two chambers, having each one two channels. In the lower chamber CO_2 is supplied at a controlled flow rate to keep its pressure constant in that compartment. The other chamber was purged by a stream of nitrogen, also at a controlled flow. This nitrogen acted as a carrier for the CO_2 and the flow leaving this chamber was collected for CO_2 quantification. The flows of the two chambers were connected to a manometer to ensure the equality of pressures between both compartments. As the CO_2 was carried continuously by nitrogen flow, it was considered that CO_2 partial pressure in the upper compartment is null, therefore ΔP is equal to 1 atm. To determine CO_2 concentration 1 mL of sample was injected in a gas chromatograph (Chrompack 9001, Middelburg, Netherlands) at 110 ºC with a column Porapak Q 80/ 100 mesh 2 m x 1/8" x 2 mm SS, using a flame ionization detector (FID) at 110 ºC. Helium at 23 mL/min was used as carrier gas. A standard mixture containing 10 % CO_2, 20 % O_2 and 70 % N_2 was used for calibration. The measurements were repeated three times for each film. The **water vapor permeability** (*WVP*) of the films was determined gravimetrically based on ASTM E96-92 method [14,15]. The test film was sealed on the top of a permeation cell containing distilled water (100 % RH; 2.337×10^3 Pa vapor pressure at 20 °C), placed in a desiccator which was maintained at 20 °C and 0 % RH (0 Pa water vapor pressure) with silica gel. The water transferred through the film and adsorbed by the desiccant was determined from weight loss of the permeation cell. The cups were weighed at intervals of 2 hours during 10 hours. Steady-state and uniform water pressure conditions were assumed by keeping the air circulation constant outside the test cup by using a fan inside the desiccator [14]. The slope of weight loss versus time was obtained by linear regression. The measured (*WVP*) of the films was determined as follows:

$$WVP = (WVTR.\ L) / \Delta P$$

where *WVTR* is the measured water vapor transmission rate ($g/m^{-2}.s^{-1}$) through a film, *L* is the mean film thickness (m), and ΔP is the partial water vapor pressure difference (Pa) across the two sides of the film. For each type of film, *WVP* measurements were replicated three times.

The **O_2 and CO_2 consumption/production in apple and mango** were measured by placing fruits inside a hermetic jar and closing it. The air circulation was promoted inside the jar by using a miniature fan. The atmosphere inside the jar was measured by drawing the gas samples with a 1 mL syringe through a septum fitted in the jar lid. The O_2 and CO_2 content in the jar was determined using a gas chromatograph (Chrompack 9001, Middelburg, Netherlands) at 110 ºC with a column mol.sieve 5A 80/ 100 mesh 1 m x 1/8" x 2 mm to separate the O_2 and a column Porapak Q 80/ 100 mesh 2 m x 1/8 "x 2 mm SS to separate the CO_2 using a flame ionization detector (FID) at 110 ºC. Helium at 23 mL/min was used as carrier gas. A standard mixture containing 10 % CO_2, 20 % O_2 and 70 % N_2 was used as standard for calibration.

Results and Discussion

Critical Surface Tension

Table 1 displays the values of the surface tension of the fruits, and their two components. The estimated values of the polar and dispersive components of the surface tension, are 1.71 and 24.77 mN/m, respectively for the mango and 0.68 and 27.13 mN/m, respectively for the apple, being the surface tensions of the mango and apple the sum of the two components (26.48 and 27.81 mN/m). Both vegetables are therefore, low energy surfaces. This type of surface interacts with liquids primarily through dispersion forces [16].

Table 1 - Surface tension from mango and apple, at the temperature of 20 °C

Fruit	Surface tension / (mN/m)	Polar component / (mN/m)	Dispersive component / (mN/m)
Apple	27.81 ± 0.03	0.68 ± 0.01	27,13 ± 0.02
Mango	26.48 ± 0.02	1.71 ± 0.01	24,77± 0.01

Once both values of the surface tension are lower than 100 mN/m the Zisman method can be applied to estimate the critical surface tension by extrapolation from the corresponding Zisman plot.

Wettability

Wettability determinations were performed with different galactomannan, collagen and glycerol concentrations. The wettability was studied by determining the values of the spreading coefficient (*Ws*). The values of the spreading coefficient from the galactomannans on each fruit were analysed and are presented below. The best values are filled in red. The best wettability values obtained for apple are found for the coating with no glycerol. This fact is probably associated to the particularity that apple surface presents a high dispersive component, denoting a predominance of apolar forces, once glycerol is a polar substance, probably this can explain the observed results.The optimum values of the spreading coefficients, in mango, were obtained with blends of 0.5 % galactomannan of *A. pavonina*, 1.5 % collagen and 1.5 % glycerol. The optimum values of the spreading coefficients, in apple, were obtained with blends of 0.5 % galactomannan of *C. pulcherrima*, 1.5% collagen and no glycerol.

Table 2 - Values from the spreading coefficient (Ws) for different collagen, galactomannan and glycerol blends (95% of confidence level – Tukey Test).

Galactomannan/ Collagen/Glycerol	**Apple** *C. pulcherrima*	 *A. pavonina*	**Mango** *C. pulcherrima*	 *A. pavonina*
0.5% - 1.5% - 0%	-42.79^{h}	-50.01^{g}	-49.85^{c}	-35.87^{g}
0.5% - 1.5% - 0.5%	-49.36^{ef}	-56.22^{de}	-49.31^{c}	-36.73^{fg}
0.5% - 1.5% - 1%	-45.46^{g}	-53.21^{ef}	-36.60^{d}	-38.61^{f}
0.5% - 1.5% - 1.5%	-47.49^{fg}	-55.21^{de}	-38.38^{d}	-29.07^{h}
1% - 1% - 0%	-50.12^{def}	-64.80^{a}	-52.45^{b}	-53.09^{a}
1% - 1% - 0.5%	-57.78^{b}	-60.26^{b}	-56.73^{a}	-41.63^{e}
1% - 1% - 1%	-49.32^{ef}	-56.55^{d}	-48.20^{c}	-48.42^{c}
1% - 1% - 1.5%	-51.48^{de}	-61.76^{b}	-52.10^{b}	-53.38^{a}
1.5% - 0.5% - 0%	-55.18^{c}	-52.11^{fg}	-47.85^{c}	-45.20^{d}
1.5% - 0.5% - 0.5%	-63.51^{a}	-57.50^{cd}	-58.59^{a}	-50.01^{bc}
1.5% - 0.5% - 1%	-52.26^{cd}	-59.53^{bc}	-59.63^{a}	-49.33^{bc}
1.5% - 0.5% - 1.5%	-54.19^{c}	-62.00^{ab}	-57.15^{a}	-51.72^{ab}

Water Vapor, Oxygen and Carbon dioxide permeabilities

Figure 1 shows the differences of oxygen permeability (*O_2P*), carbon dioxide permeability (*CO_2P*) and water vapor permeability (*WVP*) between the samples with best wettability values. The sample with 0.5 % of *A. pavonina* galactomannan; 1.5 % of collagen and 1.5 % glycerol is less permeable to oxygen (*O_2P*) than the sample with 0.5 % *C .pulcherrima* galactomannan; 1.5% of collagen and no glycerol. The addition of plasticizer decreases the presence of cracks and pores, improving the dispersion and decreasing the gas permeability [17]. Similar results were obtained to carbon dioxide permeability (*CO_2P*). The film with 0.5 % of *A. pavonina* galactomannan; 1.5 % of collagen and 1.5 % glycerol is approximately 18 times less permeable to CO_2 than the one with 0.5 % of *C.pulcherrima* galactomannan; 1.5% of collagen and no glycerol. In the water vapor permeability (*WVP*) the opposite occurs as observed for CO_2 and O_2 permeability. The coating with 0.5 *% C.pulcherrima* galactomannan; 1.5 % collagen and no glycerol is approximately 60 % less permeable to water vapor than the coating with 0.5 % *A. pavonina* galactomannan; 1.5 % collagen and 1.5 % glycerol. The plasticizer decreases the intermolecular attractions between polymeric chains, facilitating the penetration of water vapor molecules [3]. Glycerol is a hydrophilic molecule (polar) and its increase causes an amplification on water vapor mass transfer.

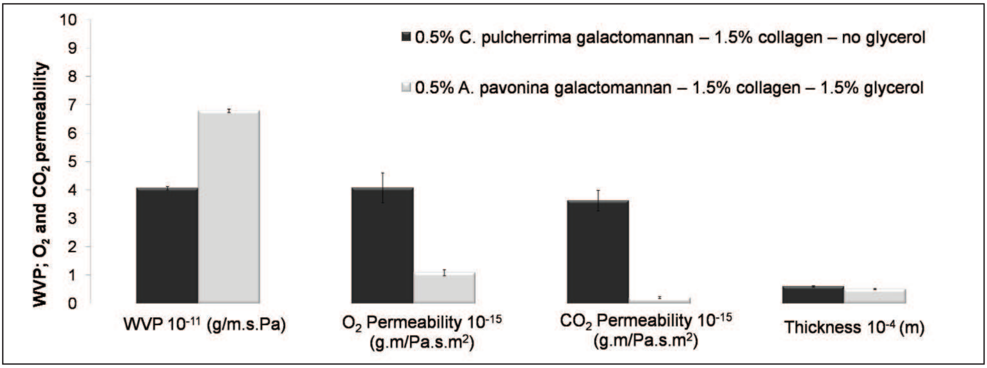
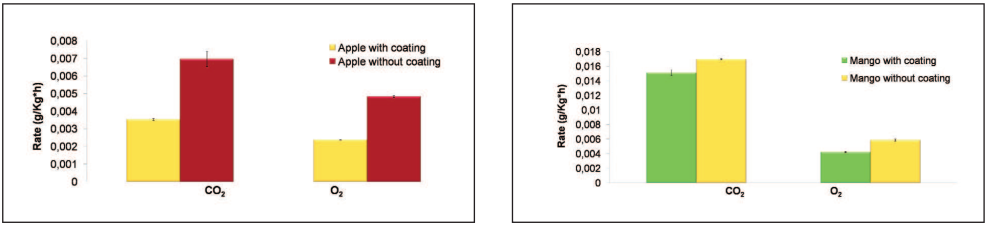


Figure 1 - Water Vapor Permeability (*WVP*), Oxygen Permeability (*O_2P*) and Carbon Dioxide Permeability (*CO_2P*) properties of coatings based on galactomannan-collagen blends, and the respective standard deviations.

O_2 and CO_2 consumption/production in apple and mango

Apples were coated using a solution with 0.5 % of *C. pulcherrima* galactomannan, 1.5 % of collagen and no glycerol and its O_2 and CO_2 transfer rates were compared with some apples without coating. The gases were measured during 60 hours and the gas transfer rate was calculated and the results are presented in Figure 2a. The coated apple permits a lowest gas exchange. The CO_2 production and the O_2 consumpttion is approximately 50 % lower in apples with coating than in apples without coating. The rate of CO_2 production is higher than that of O_2 consumption. Mangoes were coated using a solution with 0.5 % of *A. pavonina* galactomannan, 1.5 % of collagen and 1.5 % of glycerol and its O_2 and CO_2 transfer rates were compared with mangoes without coating.The gases were measured during 120 hours and the gas transfer rate was calculated and the results are presented in Figure 2b. The coated mango permits lowest gas exchange. A 28 % less O_2 consumption and 11 % less CO_2 production is observed in coated mangoes when compared with mangoes without coating.



[A]

[B]

Figure 2 – O_2 and CO_2 consumption/production in apple (A) and mango (B)

Conclusions

Mango and apple have low energy surfaces with a surface tension of 26.48 and 27.81 mN/m, respectively and a polar and dispersive component of 1.71 and 24.77mN/m for the mango and 0.68 and 27.13 mN/m for the apple, respectively. The critical surface tensions are 19.5 and 25.4 mN/m, respectively. The optimum values of the spreading coefficients, in mango, were obtained with blends of 0.5 % of galactomannan of *C. pulcherrima*, 1.5 % of collagen and 1 % of glycerol. When the galactomannan used was the *A. pavonina* the best values were obtained with blends of 0.5 % of galactomannan of *A. pavonina*, 1.5 % of collagen and 1.5 % of glycerol. The optimum values of the spreading coefficients, in apple, were obtained with blends of 0.5 % of galactomannan of *C. pulcherrima*, 1.5 % of collagen and no glycerol. When the galactomannan used was the *A.. pavonina* the best values were obtained with the same ratios of 0.5 % of galactomannan of *A. pavonina*, 1.5 % of collagen and no glycerol.

The coatings presenting the best values of *Ws* were tested in relation to their gas permeability properties. The coating with 0.5 % *C. pulcherrima* galactomannan, 1.5 % collagen and no glycerol shown that it is less pemeable to water vapor. The coating with 0.5 % *A. Pavonina* galactomannan, 1.5 % collagen and 1.5 % glycerol shown that it is less permeable to O_2 and CO_2.
A 28% less O_2 consumption and 11% less CO_2 production were observed in coated mangoes when compared with mangoes without coating. The same procedure was done in apples (in this case using *C. pulcherrima* galactomannan). The CO_2 production and the O_2 consumption is approximately 50% lower in apples with coating than in apples without coating. Results suggest that these coatings can reduce gas transfer rates in these fruits, and can be important tools to extend shelf-life of fruits.

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