Universitat de Lleida

Escola Tècnica Superior d'Enginyeria Agrària

Departament de Tecnologia d'Aliments

Recubrimientos comestibles y sustancias de origen natural en manzana fresca cortada: Una nueva estrategia de conservación

María Alejandra Rojas Graü

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CONCLUSIONES

De acuerdo con los resultados obtenidos y de su interpretación se deducen las siguientes conclusiones:

- El uso individual de agentes antioxidantes tales como, 4-hexylresorcinol (<0,5% p/v) y N-acetilcisteína (>0,75% p/v), así como la combinación de glutatión y N-acetilcisteína (>0,60% p/v) fueron efectivos en el mantenimiento del color de trozos de manzana cortada.

- Las manzanas de la variedad Fuji en un estado de madurez intermedio mostraron la mejor aptitud para ser procesadas mínimamente. Además, el uso de una atmósfera modificada baja en O_2 combinado con una inmersión previa en una solución de N-acetilcisteína (1% p/v), permitió mantener el color y la textura de los trozos de manzanas durante por lo menos 1 mes de almacenamiento refrigerado.

- La resistencia al vapor de agua del recubrimiento aplicado en trozos de manzana mejoró notablemente debido a la incorporación de aceite de girasol en una formulación de alginato (2% p/v) y gelano (0,5% p/v), conteniendo glicerol como agente plastificante en concentraciones de 1,5% y 0,6% p/v respectivamente. Además, se demostró la capacidad para el transporte de agentes antioxidantes de ambos recubrimientos, evidenciado por la ausencia de pardeamiento superficial en trozos de manzana expuestos a condiciones extremas de almacenamiento.

- La vida útil de manzanas frescas cortadas se vio incrementada por el uso de recubrimientos de alginato y gelano, los cuales ayudaron a mantener sus características de frescura durante dos semanas de almacenamiento. Dichas coberturas redujeron la producción de etileno por debajo de 50 $\mu l\ l^{-1}$ y mantuvieron la textura de los trozos de manzana con una firmeza de aprox. 10 N, así como también su estabilidad microbiológica. Se confirmó que los recubrimientos de alginato y gelano además de transportar agentes antioxidantes son capaces de retenerlos en la superficie durante un período prolongado de almacenamiento.

- La incorporación de aceites esenciales (orégano, hierba de limón y canela) como agentes antimicrobianos dentro de una película de puré de manzana, mejoró ligeramente la permeabilidad al vapor de agua y no afectó las propiedades mecánicas de las mismas, aunque produjo un aumento de la permeabilidad al O_2 de 22,64±1,28 a 38,12±0,80 cm^3-$\mu m/m^2$-d-kPa cuando se incorporó 0.1% de aceite de orégano en la formulación. Sin embargo, la incorporación de alginato en este tipo de películas originó una mejora en la permeabilidad al O_2, causando una disminución de casi el 50% de este parámetro. Las propiedades mecánicas de la película no se vieron apenas afectadas.

- Dentro de los antimicrobianos evaluados, el aceite de orégano (0,1% p/v) y su compuesto activo carvacrol, mostraron el mayor efecto antimicrobiano frente al microorganismo patógeno *E. coli* O157:H7, en películas de puré de manzana y puré de manzana-alginato, respectivamente, siendo necesarias concentraciones cinco veces mayores (0,5% p/v) de aceite de hierba de limón y canela o de sus compuestos activos citral y cinamaldehído, para obtener resultados similares a los observados con aceite de orégano o carvacrol.

- Los recubrimientos de alginato y puré de manzana conteniendo agentes antimicrobianos, redujeron la producción de etileno de los trozos de manzana a niveles por debajo de 50 $\mu l\ l^{-1}$ durante el período de almacenamiento. Adicionalmente, la incorporación de altas concentraciones de aceite de hierba de limón (1 y 1,5% p/v) y de orégano (0,5% p/v), permitieron la difusión del oxigeno a través del recubrimiento, evitando así condiciones de anaerobiosis interna en los trozos de fruta.

- La adición de N-acetilcisteína al 1% en una solución de cloruro de calcio empleada para lograr el entrecruzamiento con las moléculas de alginato, ayudaron a mantener el color y la firmeza de los trozos de fruta recubiertos. Sin embargo, la presencia de aceite de hierba de limón en la formulación, causó una apreciable degradación de la textura alcanzando valores de 1.9 N después de 21 días de almacenamiento, así como la aparición de tonalidades oscuras en los trozos de manzana.

- La efectividad antimicrobiana del aceite de orégano, aceite de hierba de limón y vainillina incorporados ahora en un recubrimiento de alginato-puré de manzana se demostró gracias a la completa inhibición de *Listeria inocua* inoculada en trozos de manzana, así como también por la inhibición del crecimiento de bacterias aerobias psicrófilas, mohos y levaduras durante el almacenamiento.

- Desde el punto de vista sensorial, la aplicación de un recubrimiento comestible elaborado a base de puré de manzana y alginato no causó ningún efecto perjudicial en las características sensoriales originales de los trozos de manzana. Sin embargo, la incorporación de aceite de hierba de limón causó un efecto perjudicial en el color y la textura de los trozos de manzana, limitando su vida útil. Aunque el uso de aceite de orégano no afectó las características de firmeza y de color de los trozos de manzana, su olor y sabor si se vieron modificados, no siendo totalmente compatible con el producto donde fue aplicado. Por el contrario, la incorporación de vainillina dentro del recubrimiento comestible fue sensorialmente aceptable, obteniendo la mayor puntuación en cuanto a preferencias por parte del consumidor.

CONSIDERACIONES FUTURAS

La hierba de limón demostró ser un buen agente antimicrobiano, sin embargo ocasionó efectos adversos tanto en la textura como en el color de los trozos de manzanas recubiertos, por lo que se debería estudiar su uso en combinación con otros métodos de conservación, que permitan evitar consecuencias perjudiciales en la fruta además de obtener un producto microbiológicamente estable. Por otra parte, sería necesario ampliar los estudios a compuestos antimicrobianos que no modifiquen el sabor original de la fruta o que en su defecto resulten más compatibles con el sabor de la misma.

Los recubrimientos comestibles empleados en esta investigación resultaron adecuados para la conservación de manzana fresca cortada. En el futuro sería conveniente investigar otros tipos de recubrimientos comestibles, que además de servir de barrera a los gases y a la pérdida de agua, también puedan ser útiles para transportar sustancias con finalidades tecnológicas, nutricionales u organolépticas, permitiendo alargar la vida útil de frutas frescas cortadas, así como también el desarrollo de nuevos productos.

ALGINATE AND GELLAN BASED EDIBLE FILMS FOR PROBIOTIC COATINGS ON FRESH-CUT FRUITS

M.S. Tapia, M.A. Rojas-Graü, F.J. Rodríguez, J. Ramírez, A.Carmona, O. Martín-Belloso

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ABSTRACT

Alginate (2% w/v) and gellan (0.5% w/v) edible films with glycerol (0.6 - 2.0% w/v) and 1% w/v N-acetylcysteine, 1% w/v ascorbic and 1% w/v citric acid, were formulated for later coating of fresh-cut apple and papaya. Water vapor permeability (WVP) in alginate films was significantly ($p<0.05$) higher (0.30 and 0.31 x 10^{-9} g / Pa s m) than in gellan films (0.26-0.27 x 10^{-9} g / Pa s m). Addition of 0.025 % (w/v) commercial sunflower oil improved WVP in the gellan films to 0.20 - 0.22 x 10^{-9} g/ Pa s m. Water solubility (0.47-0.59 and 0.74-0.79 for gellan and alginate films respectively) at 25ºC and swelling ratio (1.6-2.0 and 1.6-2.0 for gellan and alginate films respectively) seem to indicate an adequate potential of the films for coating high moisture fresh-cut fruits. It was possible to coat fresh-cut apple and papaya with alginate and gellan film-forming solutions to which 2 % (w/v) viable bifidobacteria were successfully added. WVP in alginate-papaya and apple coatings (6.31 and 5.52 x 10^{-9} g / Pa s m in) and in gellan-papaya and apple coatings (3.65 and 4.89 x 10^{-9} g / Pa s m) were higher than in the corresponding cast films. The gellan coatings/films exhibited better water vapor properties than the alginate ones. Values >10^6 cfu/g *B. lactis Bb*-12 were maintained throughout 10 days of refrigerated storage, demonstrating the feasibility of the alginate and gellan-based edible coatings to carry and support probiotic organisms on fresh-cut fruits.

Key words: Edible films, edible coatings, alginate, gellan, probiotic, fresh-cut fruits

INTRODUCTION

Edible films are known to improve shelf life and food quality by serving as selective barriers to moisture transfer, oxygen uptake, lipid oxidation and loss of volatile aromas and flavors (Kester and Fennema 1986). When used as coatings on fresh-cut fruits and vegetables they reduce the deleterious effects imposed by minimal processing. The moisture-barrier properties of edible films and coatings have been extensively studied by measuring water vapor properties of films due to the key role played by water in deteriorative reactions and to the rather simple methods required. Edible films and coatings made from naturally occurring polymers such as polysaccharides as well as from proteins are expected to be very good oxygen barriers due to their hydrogen-bonded network structure which is very tightly packed and ordered (McHugh and others 1994). Their major drawback is their relatively low water resistance and poor vapor barrier properties resulting from their hydrophilic nature (Yang and Paulson 2000).

Alginate and gellan are biopolymers very well suited for edible films and food coatings because of their colloidal properties and their ability to form strong gels or insoluble polymers upon reaction with multivalent metal cations (King 1983; Rhim 2004). This interaction is produced by mixing the

components and casting them as films, and also by pouring the cation solution onto a previously cast and dried film (Pavlath and others 1999; Rhim 2004). Plasticizers are required for polysaccharide and protein-based edible films to increase film flexibility and processability by increasing the free volume and intermolecular spacing. Plasticizers though, affect the ability of the system to attract water and also generally increase film permeability to oxygen (McHugh and Krochta 1994; Sothornvit and Krochta, 2000). Lipids and antioxidants (AO) are included in the films formulations to improve their barriers properties to moisture vapor and oxygen, and to help prevent oxidative degradation and respiration reactions when coating vegetable tissues (García and others 1998, 2000; Yang and Paulson 2000). Edible films/coatings may serve as carriers of food additives such as antibrowning agents, antimicrobials, colorants, flavors, nutrients and spices (Pena and Torres, 1991; Wong and others 1996; Li and Barth, 1998; Pranoto and others 2005. Different authors have studied the incorporation of antioxidant agents into edible films for coating minimally processed fruits (Wong and others 1994a; Baldwin and others 1996; Lee and others 2003; Perez-Gago and others 2004). Rojas-Graü and others (2007) and Tapia and others (2005) applied alginate and gellan based coatings to fresh-cut apple and papaya that proved to be good carriers for antioxidant agents like cysteine, glutathione, ascorbic and citric acid.

The addition of probiotic organisms to confer functional properties to films and coatings has not been reported. Reported potential health benefits and biological functions of bifidobacteria include production of lactic acid and acetic acid, inhibition of pathogens, reduction of colon cancer risks and of cholesterol in serum, calcium absorption, activation of the immune system, etc. (Mitsuoka 1991; Gibson and Roberfroid 1995; Kim and others 2002). For bifidobacteria to provide health benefits, a viable population of 5 log cycle cfu/g of the final product has been suggested as the therapeutic minimum (Naidu and others 1999).

The objective of this work was to formulate alginate and gellan-based edible films intended for coating fresh-cut fruits. The water vapor permeability (WVP) of the cast films formulated with glycerol and antioxidants used in fresh-cut fruits, with addition of sunflower oil, was examined. The formulated film forming solutions were used to coat apple and papaya pieces and the feasibility of the alginate and gellan-based edible coatings as carriers of organisms like bifidobacteria -in order to get probiotic functional coated fruits- was investigated as well as the WVP of the bifidus-containing coatings.

MATERIALS AND METHODS

Food grade sodium alginate (Keltone® LV, ISP, San Diego, CA., USA) and gellan gum (Kelcogel®, CPKelco, Chicago, IL., USA) were used as the carbohydrate biopolymers for films and coating formulations. Glycerol (Merck, Whitehouse Station, N.J., USA) was added as plasticizer. N-acetylcysteine (Sigma-Aldrich Chemic, Steinhein, Germany), and ascorbic acid and citric acid (Sigma-Aldrich Co. St. Louis, MO.) were the added antioxidant compounds. Sunflower oil (SO) (La Española, Spain, with the following composition: 11g monosaturated, 30g monounsaturated and polyunsaturated

57.4g; 3.5g ω-3 and 55-60g ω-6) was used as the lipid source. Calcium chloride ($CaCl_2$) (Sigma-Aldrich Chemic, Steinhein, Germany) was used to induce crosslinking reaction. Magnesium chloride ($MgCl_2.6H_2O$) and sodium chloride (NaCl) (Panreac Quimica SA, Barcelona, Spain) were employed in experiments of WVP determination of films and coatings. Freeze-dried pure cultures of *Bifidobacterium lactis* Bb-12 (Christian-Hansen, Denmark) were used as the probiotic to be incorporated into the film-forming solutions for coating the fruit pieces. For cultivation of the microorganism, Man-Rogosa-Sharpe (MRS) broth and agar (Oxoid, Unipath Ltd, Basingstoke, UK), cysteine-HCl and a solution of antibiotics (polymixin B sulphate and kanamycin sulphate) and sodium propionate, lithium chloride, nalidixic acid, iodoacetate and 2,3,5 triphenyltetrazolium chloride (Sigma-Aldrich, Missouri, USA) were used. Maradol papaya (*Carica papaya* L.) and Fuji apple (*Malus domestica* Borkh) used as model fruits for coating were obtained in local supermarkets.

Preparation of the film forming solutions and dipping solutions

Film forming solutions were prepared by dissolving alginate (2% w/v) and gellan (0.5% w/v) powders in distilled water and heated on hot plate with stirring until the mixtures become clear. Glycerol was added as plasticizer in various concentrations to overcome film brittleness and to help obtain freestanding films (Table 1). For improving water vapor barrier properties, solutions were then emulsified with three different concentrations of sunflower oil (0.025, 0.05, and 0.125 % w/w) which were added using an Ultra Turrax T25 (IKA® WERKE) and a dispersing device S25N-G25G, for 5 min at 24,500 rpm and degassed under vacuum. Dried films obtained by casting were examined regarding visible -non desirable- presence of oil, appearance and easiness of peeling off the plates without tearing, and integrity under the microscope. These criteria were used for selection of film formulations that were used for film casting on plates to determine some physical properties of cast films: WVP, solubility and swelling ratio. The film formulations that rendered the best WVP values of the cast films were selected for use in further experiments for coating apple and papaya cylinders previous incorporation of viable bifidobacteria into the film forming solutions in order to determine if the alginate and gellan-based edible coatings can carry probiotic organisms. WVP of the bifidus-fruit coatings was determined as well as the viable bifidus population in the film forming solution and coated fruits.

The gellan and alginate-based films used for coating apple and papaya respectively, were crosslinked with a 2% (w/v) calcium chloride solution containing 1 % (w/v) N-acetylcysteine (apples) and 1% (w/v) ascorbic acid / 1% citric acid (papaya) (Table 1). These concentrations were selected based on results of water vapor resistance (WVR) and inhibition of browning and other oxidative reactions in coated fresh-cut Fuji apples and papayas reported by Rojas-Graü and others (2007) and Tapia and others (2005).

Table 1. Formulations investigated in alginate and gellan-based film forming solutions intended for coating fresh-cut apple and papaya

Polymeric estructural matrix	Fruit	% (w/v) glycerol	% (w/v) sunflower oil	Antioxidants in $CaCl_2$ crosslinking solution (w/v)
Gellan 0.5% (w/v)	Papaya	1.0	0.000	Ascorbic.acid 1% Citric acid 1%
			0.025	
			0.050	
			0.125	
	Apple	0.6	0.000	N-acetylcysteine 1%
			0.025	
			0.050	
			0.125	
Alginate 2% (w/v)	Papaya	2.0	0.000	Ascorbic acid 1% Citric acid1%
			0.025	
			0.050	
			0.125	
	Apple	1.5	0.000	N-acetylcysteine 1%
			0.025	
			0.050	
			0.125	

Films formation and conditioning

To obtain films, 12 g suspensions were cast on polyethylene petri dishes of 5.5 cm diameter and placed in a controlled temperature oven. Samples were dried at 60ºC as described by Garcia and others 2000). Crosslinking with calcium ions was brought about by the immersion technique described by Rhim (2004) of soaking the dried films for 2 min in a 2% (w/v) $CaCl_2$ solution. The treated films were dried - after discarding the calcium solution- at the laboratory ambient conditions (62 ± 5 % RH) and 25 ± 2ºC for 3 h. Films were peeled off from the plates and stored at 25 ± 2 ºC and conditioned at 33.3% RH in a closed chamber with a saturated solution of magnesium chloride until used for the WVP determination and other tests.

Incorporation of viable *Bifidobacterium lactis Bb12* into the gellan and alginate film forming solutions

The lyophilized pure culture *Bifidobacterium lactis* Bb-12 was activated (48 h) and propagated next (24 h) in MRS broth with 0.05% (w/v) cysteine-HCl, at 37°C under anaerobic incubation using Oxoid gas jars (HP11) and anaerobic gas packs (Oxoid, Unipath Ltd, UK). Bacterial cells were harvested by centrifugation at 6000 g for 15 min at 5°C. Selected formulation of the 0.5% (w/v) gellan and of the 2% (w/v) alginate film forming solutions were used for coating the apple and papaya pieces. The biomass sediment of *Bifidobacterium lactis* Bb-12 was aseptically added at a 2% (w/v) concentration to the film forming solutions and mixed with very slow agitation to obtain suspensions for coating the fruits as described by Rodríguez and others (2005).

Fruit coating

Apple and papaya fruits were washed and cut in cylinders of 1.42 cm diameter x 2.06 cm of height. Fruit pieces were immersed for 2 minutes into the 2% alginate (w/v) or 0.5% gellan (w/v) film forming solutions containing *Bifidobacterium lactis* Bb-12. Residual solution was allowed to drip off for 1 min before immersing the fruits for 2 min in a sterile $CaCl_2$ (2% w/v) solution required for crosslinking, containing the respective antioxidant agents at the same concentrations used for films (Table 1). Coated fruits were used for WVP determination and for determination of the population of viable bifidobacteria for which approximately 80g of coated fruit cylinders were packed into commercial plastic click bags of 16.5 x 14.9 cm. All samples were stored for 10 days at 2ºC and microbiological examination of bifidobacteria was made at 0, 3, 5, 7 and 10 days.

Water vapor permeability of films and coatings

WVP of films were determined gravimetrically at 25ºC using modifications of the standard procedure of the ASTM standard method E96-93 (ASTM E96-95, 1995) described by Rhim (2004) for alginate films. WVP of the coatings were determined as described by García and others (1998, 2000).

For films, two specimens were cut circularly from each film after conditioning, mounted and sealed over methylmethacrylate test cups (with an internal diameter of 3 cm, an outer diameter of 4.5 cm and a depth of 2.0 cm) by a cap with a rubber O-ring. Diameter of the specimens was slightly larger than the diameter of the cup. The cups were filled with 5 mL of distilled water leaving an air gap of 1 cm between the film underside and the water. The cups were placed in air-tight desiccators containing in the bottom saturated solutions of $MgCl_2.6H_2O$ that render 33.3% RH at 25ºC. Weights of the cups with their contents were recorded at 30 min intervals for six hours.

The water vapor transfer through the film (m_1, slope of the curve of weight loss vs time in g/s) was estimated by regression analysis. Water vapor transmission rate (WVTR) through the films under investigation, and WVP were obtained as described by Kaya and Kaya (2000) and Chinnan and Park (1995).

$$WVTR = m_1/A = g/m^2\ s \quad (1)$$

$$WVP = L \times WVTR/(p_i - p_a) \quad (2)$$

Where:

A= exposed film area (p_i - p_a) is the difference in water vapor pressure inside and outside the cup p_i and p_a

are the vapor pressure of a saturated air and air with 33% of R.H. respectively to 25°C. L is the average film thickness (m).

For WVP of coatings, coated apple and papaya cylinders were equilibrated for 24 h in desiccators maintained at 98.9% RH with a 0.6 molal solution of NaCl at room temperature. Fruit cylinders were placed in small test cups and weighed in an analytical scale prior to be placed in sealed chambers equilibrated at 33.3% RH with saturated $MgCl_2.6H_2O$ at 25 ºC. Samples were weighed at regular time intervals. Weight was taken during 24 h periods and the water vapor transfer through the coating (m_2, slope of the curve of weight loss vs. time in g/s) was estimated by linear regression analysis. Additionally, a control assay was performed with uncoated apple and papaya, to determine the mass transfer coefficient of water vapor in air (k_{air} = 7.06 10^{-6} g m^{-2} s^{-1} Pa^{-1}, 7.28 10^{-6} g m^{-2} s^{-1} Pa^{-1} for apple and papaya respectively) (Tapia and others 2005). Water activity of fresh fruit was measured with an Aqualab CX-3 (Pullman, WA).

Water vapor flux, Fl (g/m^2 s) was calculated from the slope of the weight loss vs time curves (m_2) and the mass transfer areas (A), this last was considered as the upper surface plus the lateral area of the apple and papaya cylinders (9.25 10^{-4} m^2).

As stated by Garcia and others (1988, 2000), coating permeance, P (g/m^2 s Pa), was determined considering that water vapor is transferred in series through the coatings and the surrounding air, using the following equation:

$$Fl = (p_{if}-p_a)/ [(1/P) + (1/ k_{air})] \quad (3)$$

In eq 3 p_{if} is the partial water vapor pressure at the fruit-coating interface (3139.88 Pa and 3122.45 Pa for papaya and apple respectively), which was calculated considering that a_w of papaya was 0.991 and a_w of apple was 0.985, and the total water vapor pressure (P_{tot}) at 25ºC is 3170 Pa; p_a is the partial water vapor pressure in the environment with 33.3% RH at 25ºC expressed in Pa, and K_{air} is the water vapor mass transfer coefficient in air [$g/(m^2$ s Pa)] of the uncoated fruits.

WVP was calculated according to the following equation that includes coating thickness (e) in m.

$$WVP = Pe \quad (4)$$

Films and coatings thickness

Film thickness was measured with a micrometer (Dial thickness gauge 7301, Mitutoyo Co., Japan) at 0.001 mm accuracy. Thickness measurements were taken in each specimen at five random positions in the film following WVP tests as described by Kaya and Kaya, (2000). Mean values were used for

calculations. Coatings thickness was measured in a stereomicroscope Leica (model MZ8, Leica AG, Heerbrugg, Switzerland).

Water solubility and swelling ratio of alginate and gellan-based films

The water solubility (WS) and swelling ratio (SR) of films containing SO were determined according to the method described by Rhim (2004) and Gontard and others (1992). Three samples selected at random from each type of film, were dried at 105ºC for 24 h to obtain the initial dry matter. Other three samples of each film were placed in a 50-ml beaker containing 30 ml distilled water, which were sealed with parafilm and placed in a drying cabinet at 25 ºC for 24 h stirring occasionally. The films were removed from the beakers, rinsed gently with distilled water, and the dry matter was determined by placing them in an oven at 105 ºC for 24 h so as to calculate the unsolubilized dry matter. The water solubility of the films was calculated by subtracting the weight of unsolubilized dry matter from the weight of initial dry matter and expressed as a fraction of the initial dry matter content using following relationship:

$$WS = (So - S) / So = g \text{ soluble solids} / g \text{ total solids} \quad (5)$$

where, So is the initial dry matter and S the unsolubilized dry matter.

The swelling ratio of films was also determined as described by Lee and others (2004) and Rhim (2004). Triplicate pre-weighed film samples were immersed in beakers with water at 25ºC; films were taken out of the water after 10 min, the surface water was gently removed with blotting paper for 1 min and the final weight of the swollen samples was measured. SR was expressed as a fraction of g of water gained against g of total solids of the film.

Determination of viable bifidobacteria in coated fruits

Viable population in the coated fruits, as well as in the film forming solution for coating, was determined by making appropriate dilutions and culturing in Miller-Pricket tubes with MRS agar, 0.05% cysteine-HCl and solution of antibiotics (polymixin B sulphate and kanamycin sulphate) and sodium propionate, lithium chloride, nalidixic acid, iodoacetate and 2,3,5 triphenyltetrazolium chloride, as described by Payne and others (1998) and Arroyo and others (1995) with and overlay of the same medium.

Statistical analysis

Measurements of WVP, WS, and SR of the films prepared of each of the selected formulation were performed in triplicate. Statgraphics Plus 4.0 was used to run an ANOVA analysis and the significance ($p<0.05$) of the mean values was determined with Duncan's multiple range tests.

RESULTS AND DISCUSSION

Characterization of the gellan and alginate films

Six films formulations with SO were selected according to the criteria described of integrity under the microscope, absence of brittleness and of oil exudation. Thickness of each type of film was measured. Thickness values varied from 0.047 to 0.048 for gellan films, and from 0.050 to 0.052 mm for alginate. WVP of the films was investigated including the respective controls without addition of SO. These formulations along with the respective controls without SO are presented in Table 2.

Figure 1 presents the curves of weight loss vs. time -under an atmosphere of 33.3% RH- of the test cups filled with distilled water, on which each one of films prepared from the six selected formulations with SO, as well as the control films without oil, had been mounted and sealed. Results of the latter are not presented here. The graph shows that the water vapor transfer through the films varies with the type of film. m_1 values (slopes of the curves) ranged from 0.022 to 0.025 x 10^{-5} g / h in gellan films, and from 0.032 - 0.035 x 10^{-5} g/h in the alginate films. It is clear that the films formulated with alginate are the ones that offer less resistance to water transfer when comparing them with the gellan-formulated films. The nature of each hydrophilic polymer and the higher concentration used of alginate may be the cause of the higher WVP of its films.

In Table 2, it can be seen that values of the gellan and alginate control films (without oil) did not show significant differences ($p<0.05$) among them, and are comparable to values of WVP of the alginate-based films even with addition of SO. As stated by Kester and Fennema (1986), due to the hydrophilic nature of alginate and gellan, minimal water vapor barrier properties are expected in films and coatings based on these polymers. Additionally, if ascorbic and citric acid are incorporated -which are hydrophilic compounds that may increase water vapor permeability and water loss when incorporated into films and coatings (Ayrancy and Tunc 2004)- as well as the hydrophilic N-acetylcysteine and glycerol, the addition of lipids is expected to improve the water vapor barrier properties of the films.

Table 2. Water vapor permeability (WVP) of alginate (Al) and gellan (Ge) -based films with/without sunflower addition (SO), with glycerol (Gly), and/or N-acetylcysteine (Cys), ascorbic acid (AA) and citric acid (CA) in the calcium crosslinking solutions.

Film	Polymeric matrix		% (w/v) Gly	% (w/v) SO	WVP	
	Alginate (% w/v)	Gellan (% w/v)			(x 10^9 g m / Pa s m^2)	(g-mm / kPa-h-m^2)
F_1	-	0.5	1.0	0.025	$0.21^{a} \pm 0.02$	$0.75^{a} \pm 0.02$
F_2	-	0.5	1.0	0.050	$0.20^{a} \pm 0.02$	$0.73^{a} \pm 0.02$
F_3	-	0.5	0.6	0.025	$0.22^{a} \pm 0.02$	$0.80^{a} \pm 0.02$
F_4	-	0.5	0.6	0.050	$0.21^{a} \pm 0.02$	$0.77^{a} \pm 0.02$
F_5	2.0	-	2.0	0.025	$0.32^{c} \pm 0.02$	$1.16^{c} \pm 0.02$
F_6	2.0	-	1.5	0.025	$0.29^{bc} \pm 0.02$	$1.04^{bc} \pm 0.02$
F_7	-	0.5	1	0	$0.26^{b} \pm 0.01$	$0.95^{b} \pm 0.01$
F_8	-	0.5	0.6	0	$0.27^{b} \pm 0.01$	$0.96^{b} \pm 0.01$
F_9	2.0	-	2.0	0	$0.30^{bc} \pm 0.01$	$1.09^{bc} \pm 0.01$
F_{10}	2.0	.	1.5	0	$0.30^{bc} \pm 0.01$	$1.08^{bc} \pm 0.01$

Each value is the mean of three experiments with three replicate each. Means followed by different letters are significantly different ($p < 0.05$).
F_1**:** Ge 0.5%, Gly 1%, SO 0.025%, AA 1.0%, CA 1.0%; F_2**:** Ge 0.5%, Gly 1%, SO 0.050%, AA 1.0%, CA 1.0%; F_3**:** Ge 0.5%, Gly 0.6%, SO 0.025%, 1.0% Cys; F_4**:** Ge 0.5%, Gly 0.6%, SO 0.050%, Cys 1.0%; F_5**:** Al 2%, Gly 2%, SO 0.025%, AA 1.0%, CA 1.0%; F_6**:** Al 2 %, Gly 1.5%, SO 0.025%, Cys 1.0%; F_7**:** Ge 0.5%, Gly 1%, AA 1.0%, CA 1.0%; F_8**:** Ge 0.5%, Gly 0.6%, Cys 1.0%; F_9**:** Al 2 %, Gly 2%, AA 1.0%, CA 1.0%; F_{10}**:** Al 2 %, Gly 1.5%, Cys 1.0%.

Table 2 also shows that alginate films with 1.5 or 2% glycerol and presence or absence of SO, do not exhibit significant differences among their WVP values, which ranged for films with oil, between 0.29 and 0.32 x 10^{-9} g / Pa s m (1.04 and 1.16 g-mm / kPa-h-m^2) and for films without oil between 0.30 - 0.31 g m / Pa s m^2 (1.08 and 1.09 g-mm / kPa-h-m^2) respectively. In this case, oil addition did not improve the WVP values of the alginate films, even though permeabilities values are lower than the ones reported by Parris and others (1995) and Rhim (2004) which are 2.35 x 10^{-9}g / Pa s m, and between 0.93 and 1.08 x 10^{-9} g m / Pa s m^2 for (70% /30%) alginate/glycerol films and 2% alginate respectively, both without oil addition.

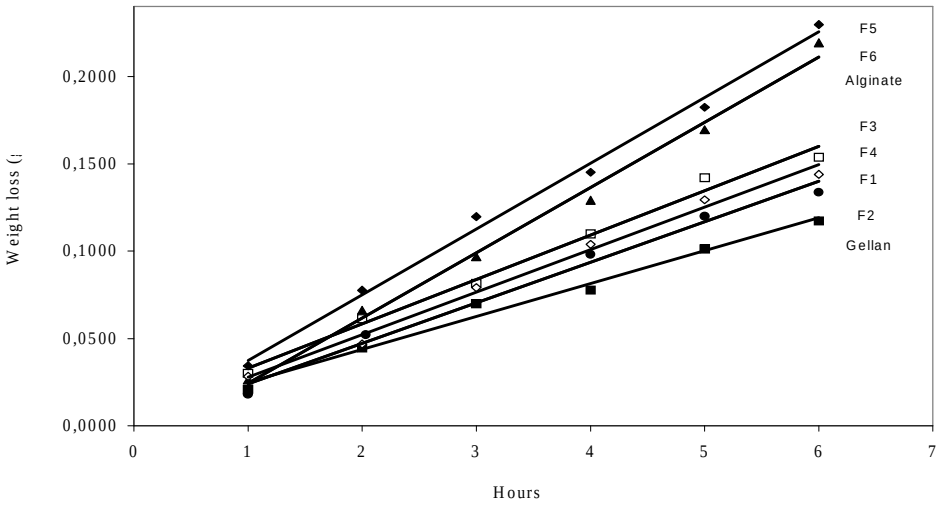

Figure 1: Weight loss over time (in a 33% RH atmosphere) of alginate (Al) and gellan (Ge) films, with sunflower addition (SO), glycerol (Gly), and/or N-acetylcysteine (Cys), ascorbic acid (AA) and citric acid (CA) in the calcium crosslinking solutions. Gellan films: F_1**:** Ge 0.5%, Gly 1%, SO 0.025%, AA 1.0%, CA 1.0%; F_2**:** Ge 0.5%, Gly 1%, SO 0.050%, AA 1.0%, CA 1.0%; F_3**:** Ge 0.5%, Gly 0.6%, SO 0.025%, 1.0% Cys and F_4**:** Ge 0.5%, Gly 0.6%, SO 0.050%. Alginate films: F_5**:** Al 2%, Gly 2%, SO 0.025%, AA 1.0%, CA 1.0% and F_6**:** Al 2 %, Gly 1.5%, SO 0.025%, Cys 1.0%.

Pranoto and others (2005) studied the addition of garlic oil (0.1 to 0.4 % v/v) as antibacterial agent to 1% (w/v) alginate films with 0.4 % (v/v) glycerol, and the addition of oil did not either affect significantly the WVP of the films, which varied from 18.73 with 0.1 % oil to 23.42 g mm/ day m^2 kPa with 0.3% oil. When 0.4 % garlic oil was incorporated, the WVP increased to 30.89 g mm/ day m^2 kPa. The authors attribute this to the hydrophobic properties of garlic oil that can contribute to extend intermolecular interactions of the structural matrix allowing moisture transfer since for alginate films without oil they found values of 20.32 g mm/ day m^2 kPa. Zactiti and Kieckbusch (2005) found for 1.5% (w/v) alginate films and 0.6% glycerol (w/w), WVP values of 11.93 g mm/ day m^2 kPa when crosslinking was complemented with 2% $CaCl_2$. The authors did not use oil but water barrier properties improved greatly with crosslinking degree.

On the other hand, as seen in Table 2, the 0.5% (w/v) gellan-based films with 1.0 and 0.6% glycerol (w/v) and 0.025 and 0.05% SO (w/v) did not exhibit significant differences in WVP values. Yang and Paulson (2000a) investigated the WVP of 2% (w/v) gellan films with addition of different concentrations of glycerol, and encountered that WVP varied from approximately 0.7 to 2.8 g mm / kPa h m^2 as the glycerol concentration increased. In this study, the gellan-based formulations exhibit WVP mean

values in the range of 0.20 and 0.22 x 10^{-9} g / Pa s m (0.73 and 0.78 g-mm / kPa-h-m^2), and contrary to the alginate films, when values of WVP of gellan control films without oil are examined: 0.26 and 0.27 g/Pa s m (0.95 and 0.96 g-mm / kPa-h-m^2), it is evident that addition of oil improves water barrier properties of gellan films in approximately 20%. Yang and Paulson (2000b) investigated the incorporation of different lipids to gellan films finding that oil addition in values as high as 20%, significantly decreased WVP from values of 1.5 to approx. 1.2 g mm / kPa h m^2 with stearic and palmitic acid, and to approx. 0.7 g mm / kPa h m^2 with beewax, but the high levels of lipids were difficult to handle in the films. In our case, the addition of 0.025 and 0.05 % (w/v) SO reduced the WVP of the gellan films from 0.95-0.96 g mm / kPa h m^2 to 0.73-0.78 g mm / kPa h m^2 regardless the concentration of glycerol used.

Swelling ratio and solubility.

Table 3 presents the swelling ratio (SR) (g of water gained /g of total solids of the film) and water solubility (WS) (g soluble solids/ g total solids) of the gellan and alginate films obtained with the six selected formulations. In this study, the solubility values found for found for gellan films at 25ºC, are significantly lower (0.47-0.59 g soluble solids/ g total solids, than the ones found for alginate films (0.74-079). No significant differences among treatments with SO and Gly were encountered. Lee and others (2004), reported for films made out with gellan (2% w/v) and glycerol 1% (w/v) a solubility value of 0.52 g soluble solids/ g total solids (52 %), similar to the ones found in this work; while Rhim (2004) found for alginate films, values around 0.164 g soluble solids/ g total solids at 25ºC exhibiting much more resistance that the alginate films of this work, probably due to the type of alginate used. Solubility values of other biopolymer –based films such as cellulose or carrageenan have been reported as 0.55-0.84 and 0.41 g soluble solids/ g total solids respectively by Lee and Lee (2004). WS is one of the most important properties in food or pharmaceutical applications.

Table 3. Swelling ratio (SR) (g of water gained /g of dry solids of the film) and water solubility (WS) (g soluble solids/ g total solids) at 25°C of gellan (Ge) and alginate (Al) films, with glycerol (Gly) and sunflower oil (SO) and/or 1% N-acetylcysteine (Cys), ascorbic acid (AA), citric acid (CA) in the calcium crosslinking solutions.

Film	Polymeric structural matrix				SR	WS
	Alginate (% w/v)	Gellan (% w/v)	Gly (% w/v)	SO (% w/v)		
F_1		0.5	1.0	0.025	2.6 [a]	0.55 [a]
F_2		0.5	1.0	0.050	2.6 [a]	0.57 [a]
F_3		0.5	0.6	0.025	2.6 [a]	0.47 [a]
F_4		0.5	0.6	0.050	2.3 [a]	0.59 [a]
F_5	2.0		2.0	0.025	1.6 [a]	0.79 [b]
F_6	2.0		1.5	0.025	2.0 [a]	0.74 [b]

Each value is the mean of three experiments with three replicate each. Means followed by different letters are significantly different ($p< 0.05$). F_1**:** Ge 0.5%, Gly 1%, SO 0.025%, AA 1.0%, CA 1.0%; F_2**:** Ge 0.5%, Gly 1%, SO 0.050%, AA 1.0%, CA 1.0%; F_3**:** Ge 0.5%, Gly 0.6%, SO 0.025%, 1.0% Cys and F_4**:** Ge 0.5%, Gly 0.6%, SO 0.050%. Alginate films: F_5**:** Al 2%, Gly 2%, SO 0.025%, AA 1.0%, CA 1.0% and F_6**:** Al 2 %, Gly 1.5%, SO 0.025%, Cys 1.0%.

The water solubility, unlike the water permeability, is determined by chemical structure and defines the resistance or tolerance to water, indicating stability in water. As stated by Lee and Lee (2004) films can be used as coating materials to inhibit for instance, exudation from frozen foods, and the higher the water solubility the poorer the stability of films, but also with the advantage that food coated with edible films temporarily, can be easily eaten after washing. In the case of the high moisture fresh-cut fruits, resistance of the coatings to be dissolved by water is relevant. SR defines the amount of water absorbed by films and is an important property of carbohydrate and protein films since these biopolymers initially swell when they absorb water with resulting changes of their structure. SR has been used as a measure of the extent of cross- linking, similar to its use for protein films like collagen (Lee and Lee 2004). Thus, examination of SR is necessary for the efficient application of biopolymer films. Low values of SR indicate a high tolerance for water. No significant ($p< 0.05$) differences in SR were observed among the treatments applied and the type of films. The values of SR (1.6-2.0 g of water gained /g of dry solids of the film) are inferior to those reported by Lee and others (2004), who found in films made out with 2% (w/v) gellan and 1% (w/v) a SR around 6. On the other hand Rhim (2004) found for alginate films (2% w/v and glycerol 1% w/v) a SR of 0.8 g of water gained /g of total solids of the film, which is lower than the one found in this work (1.6-2.0). These results lead to infer an adequate tolerance of the gellan/alginate based coatings containing SO to water and an appropriate potential for use to coat fresh-cut fruits.

Water vapor permeability of the bifidus-coatings

Each fruit (apple and papaya) was coated with one formulation of the 0.5% (w/v) gellan and one of the 2% (w/v) alginate film forming solution with SO and antioxidants, to which 2% (w/v) viable biomass of *Bifidobacterium lactis* Bb12 had been added (Table 4). For the gellan coatings, a concentration of 0.025 % (w/v) of SO was selected over the 0.050 % since no significant differences in WVP ($p < 0.05$) were encountered between them (Table 2), the lower oil concentration being then chosen.

Table 4 shows the results of the WVP determination of these alginate and gellan coatings containing viable bifidobacteria. Thickness of each type of coating was measured, varying from 0.150-0.155 for alginate and 0.170-0.178 mm for gellan. It can be seen that the gellan coatings exhibited better water barrier properties ($p<0.05$) than the alginate coatings for both papaya and apple, even if there seemed to be some differences linked to the type of vegetable matrix, since WVP values of the papaya coatings were significantly lower ($p<0.05$) than the same coating on apple.

Table 4. Water vapor permeabilities (x 10^9 g m / Pa s m^2) of selected alginate (Al) and gellan (Ge) coatings on fresh-cut papaya and apple, with 2% (w/v) viable bifidobacteria plus sunflower oil (SO) and glycerol (Gly), and/or N-acetylcysteine (Cys), ascorbic acid (AA), citric acid (CA) in the calcium crosslinking solutions.

Model fruits coated			
Papaya		Apple	
Alginate coatings (F_5)	Gellan coatings (F_1)	Alginate coatings (F_6)	Gellan coatings (F_3)
6.31[a]	3.65[d]	5.52[b]	4.89[c]

Each value is the mean of three experiments with three replicate each.
Means followed by different letters are significantly different ($p< 0.05$).
F_1**:** Ge 0.5%, Gly 1%, SO 0.025%, AA 1.0%, CA 1.0%; F_3**:** Ge 0.5%, Gly 0.6%, SO 0.025%, 1.0% Cys; F_5**:** Al 2%, Gly 2%, SO 0.025%, AA 1.0%, CA 1.0%; F_6**:** Al 2 %, Gly 1.5%, SO 0.025%, Cys 1.0%.

These results are in agreement with the ones obtained in the previous work by Rojas-Graü and others (2007), in which the 0.5% (w/v) gellan and SO coatings (without bifidus) were more effective in increasing the resistance to water vapor than the 2.0% (w/v) alginate/SO oil coatings (also without bifidus). According to results of these authors, water vapor resistance (WVR) values were in all cases higher (19.2 and 27.6 s/cm for alginate and gellan coatings) than the resistance values of the corresponding coatings with bifidus formulated in this work, whose WVR values were also calculated here, rendering resistance values approximately 40 to 50% lower (11.87 – 12.72 s/cm) for alginate and gellan respectively. In terms of WVP, when values found for the alginate (0.32- 0.29 x 10^{-9} g / Pa s m) and gellan (0.22- 0.21 x 10^{-9} g / Pa s m) cast films are compared to the WVP values of the alginate coatings with bifidus (6.31 - 5.52 x 10^{-9} g / Pa s m), and to those of the gellan coatings with bifidus (3.65- 4.89 x 10^{-9} g / Pa s m), values of WVP in coatings are approximately 20 times higher. It may be inferred than the probiotic biomass addition (2% w/v) to the alginate and gellan/SO composite coatings may probably cause an increase in the spacing between the chains due to the inclusion of bacterial cells among the polymer chains and thus promote the diffusivity through the coatings accelerating the water transmission. Also, in this case, for WVP determination, the very humid fruit surface is in contact with a high moisture coating contrary to what happens in the dry films that for WVP determination were conditioned to 33% RH and were in contact with a side of the cup containing distilled water. Finally coating on the fruit surface might have set in a non-uniform way. All this may contribute to less effective barrier properties of the alginate and gellan bifidus-containing coatings.

Viable bifidobacteria in the coated fruits

The gellan and alginate film forming solutions to which bifidobacteria were incorporated, rendered upon culturing, a viable population of 9.93 and 9.67 Log_{10} cfu/g *B. lactis Bb-12* respectively. Figure 2 shows that immediately after coating (day 0), viable counts of *B. lactis Bb-12* in the coated papaya pieces were 6.89 and 7.52 Log_{10} cfu/g for alginate and gellan respectively, while in apples values were 7.91 and 7.78 Log_{10} cfu/g for alginate and gellan respectively. This represents approximately a two log cycles decrease as compared to the concentration of the original film forming solution, which is explained by dilution effects. Figure 2 shows that the bifidus population stays viable and constant during the10 day storage period at 2 °C.

The survival and maintenance of *B. lactis Bb-12* into the alginate and gellan based edible coatings, both on fresh-cut papaya and apple is considered satisfactory since values remained between 6 and 7 log_{10} cfu/g. It may be probable that the AO present might help to maintain the ox-red conditions that are favourable to bifidobacteria. To confer health benefits, the viable count of bifidobacteria at the time of consumption should be 10^6 cfu/g (Samona & Robinson, 1991). Ingestion in numbers $\geq 10^6$ cells per gram are recommended for a classic probiotic food such as yoghurt (Kurman & Rasic, 1991). It important for manufacturers and retailers to be able to confirm the viable count of these organisms in the bifidus-containing products.

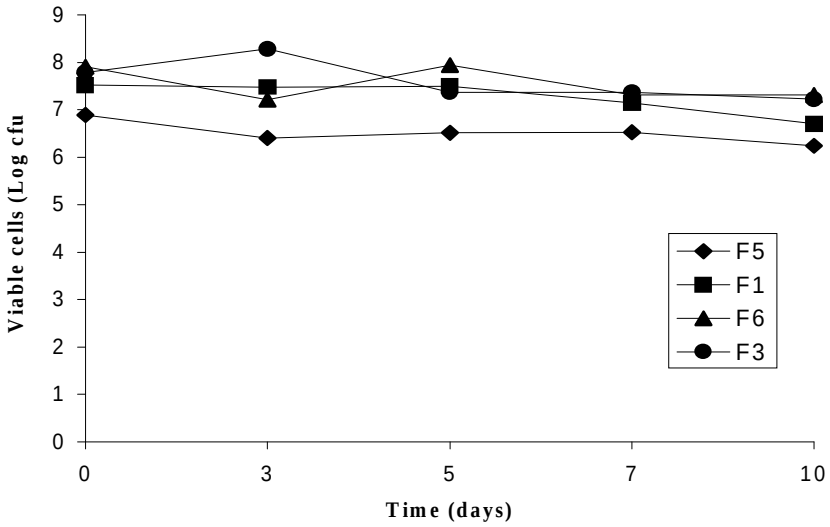

Fig.2- Viable cells (Log cfu/g) of *Bifidobacterium lactis Bb-12* incorporated into alginate (Al) and gellan (Ge) coatings with sunflower addition (SO) and glycerol (Gly); and/or N-acetylcysteine (Cys), ascorbic acid (AA) and citric acid (CA) in the calcium crosslinking solutions, applied on fresh cut papayas and apples along refrigerated (2ºC) storage (days). F_1**:** Ge 0.5%, Gly 1%, SO 0.025%, AA 1.0%, CA 1.0%; F_3**:** Ge 0.5%, Gly 0.6%, SO 0.025%, 1.0% Cys; F_5**:** Al 2%, Gly 2%, SO 0.025%, AA 1.0%, CA 1.0%; F_6**:** Al 2 %, Gly 1.5%, SO 0.025%, Cys 1.0%.

CONCLUSIONS

Gellan and alginate-based films were formulated satisfactorily with glycerol incorporation, SO and antioxidants. WS and SR values seem to indicate an adequate potential of the films for coating high moisture products like fresh-cut fruits. The gellan films exhibited better vapor barrier properties than the alginate films, and the WVP values of the former were improved by incorporation of SO, what was not the case for the alginate films. The bifidus-containing coatings were more vapor permeable than the corresponding cast films, and again, gellan coatings were more resistant to water transfer that the alginate ones. The alginate and gellan-based edible coatings seem to be efficient to support *Bifidobacterium lactis Bb-12* on fresh-cut apple and papaya. Edible films or coatings can carry diverse food additives, and films that sustain viable bifidus may extend the type of biologically active compounds to be supported by edible films, opening the possibilities of developing probiotic fresh-cut fruit products.

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