
Ingeniería de alimentos

Revista Mexicana de Ingeniería Química
Vol. 14, No. 3 (2015) 567-578

TRACING PHENOLIC COMPOUNDS THROUGH MANUFACTURING OF EDIBLE FILMS FROM ORANGE AND GRAPEFRUIT PEELS

RASTREO DE COMPUESTOS FENÓLICOS EN LA FABRICACIÓN DE PELÍCULAS COMESTIBLES A PARTIR DE CÁSCARAS DE NARANJA Y TORONJA

J.G. Hernández-Carrillo, A. Valdez-Fragoso, J. Welti-Chanes, H. Mújica-Paz*

Escuela de Ciencias e Ingeniería, Tecnológico de Monterrey, Av. Eugenio Garza Sada 2501 Sur, Monterrey, Nuevo León, 64849, México.

Received March 24, 2015; Accepted July 5, 2015

Abstract

Edible films naturally rich in phenolic compounds were prepared from orange and grapefruit peels. Free and total polyphenols were determined by Folin-Ciocalteau method and flavonoids were identified and quantified by HPLC in the manufacturing processes of films. Films from grapefruit and orange peel had 24.95 and 28.18 mg GAE/g (Gallic Acid Equivalents/g), respectively, retaining more than 50% of total phenolics from the raw material. Hesperidin (33.39 mg/g) was the main flavonoid in orange peel based films and naringin (31.42 mg/g) in grapefruit peel films. Recoveries of the identified flavonoids in films were in the range of 31-60%. Residues, mainly those from orange peel process, retained important fraction of phenolics. Edible films with high levels of polyphenols were manufactured from orange and grapefruit peel without the addition of external phenolics extracts.

Keywords: orange peel, grapefruit peel, edible films, phenolic compounds, flavonoids.

Resumen

Se fabricaron películas biodegradables con alto contenido de compuestos fenólicos a partir de cáscaras de naranja y toronja. En el proceso de producción de las películas, se determinaron los polifenoles libres y totales por el método de Folin-Ciocalteau y los flavonoides se identificaron y cuantificaron por HPLC. Las películas de cáscara de naranja y toronja tuvieron 24.95 y 28.18 mg EAG/g (Equivalentes de Acido Gálico/g), respectivamente, con una retención de más del 50% de los fenólicos totales de las materias primas. El principal flavonoide en las películas a base de cáscara de naranja fue la hesperidina (33.39 mg/g) y, la naringina en las de cáscara de toronja. La recuperación de los flavonoides identificados en las películas estuvo entre 31-60%. Los residuos del proceso, principalmente los del proceso de cáscara de naranja, retuvieron una fracción importante de fenólicos. Se fabricaron películas comestibles con altos niveles de polifenoles a partir de cáscaras de naranja y toronja sin la adición de extractos externos de fenólicos.

Palabras clave: cáscara de naranja, cáscara de toronja, películas biodegradables, compuestos fenólicos, flavonoides.

1 Introduction

There is an increasing interest in the development of packaging materials from biopolymers, due to their biodegradability and potential use in the food industry (Woranuch *et al*., 2014). Biodegradable films are made from naturally occurring polymers, as lipids, proteins and polysaccharides, or from a combination of them (Rodríguez-Marín *et al*., 2013; Villagómez-Zavala *et al*., 2008).

A current research trend in food packaging is the incorporation of natural additives in edible films. For instance, films with natural antimicrobials (Hernández-Ochoa *et al*., 2011; Romero-Bastida *et al*., 2011) and antioxidant compounds (Pereira de Abreu *et al*., 2011) have been studied to protect foods from deteriorative and oxidant reactions (Siripatrawan and Harte, 2010; Mehdizadeh *et al*., 2012). Extracts rich in phenolic compounds have been efficiently incorporated as natural antioxidants in films made of starch-chitosan (Mehdizadeh *et al*., 2012), chitosan (Siripatrawan and Harte, 2010), gelatin (Gómez-

* *Corresponding author. E-mail*: h.mujica@itesm.mx
Tel.:+52 (81) 83 58 14 00 ext. 4854.

Estaca *et al*., 2009) and soy protein (Pruneda *et al*., 2008).

Several authors have studied the use of raw materials as a source of biopolymers and phenolic compounds for edible films preparation. Antioxidant edible films from red seaweed (Cian *et al*., 2014), quince seed mucilage (Jouki *et al*., 2013) and defatted mustard meal (Kim *et al*., 2012) have been developed. Extracts from red seaweed and protein isolates have also been used to prepare antioxidant edible films (Blanco-Pascual *et al*., 2014; Salgado *et al*., 2012).

Although antioxidant films have been obtained from raw materials rich in phenolic compounds, information about the phenolics retention in the films was not provided. Besides, a detailed tracing of the bioactive compounds through the film production process has not been carried out.

Purified pectin is one of the biopolymers that has been widely used for making edible films (Alves *et al*., 2010; Bagliotti Meneguin *et al*., 2014; Bierhalz *et al*., 2012; Galus and Lenart, 2013; Otoni *et al*., 2014). Pectin is a polysaccharide of the cell wall of plants and it is commonly extracted from peels of citrus as oranges and grapefruits (Mariniello *et al*., 2010; Yapo, 2009). Apart from being rich in pectin, orange and grapefruit peels have also important amounts of antioxidant compounds as flavonoids and other polyphenols (Wang *et al*., 2008).

Considering that edible films can be obtained from orange and grapefruit peel extracts without pectin purification (Mújica-Paz *et al*., 2011), and that citrus peel contains high levels of active compounds (Wang *et al*., 2008), films from citrus peel could retain these valuable compounds after manufacturing process. The objective of this work was to determine total and free phenolics, and the flavonoids profile through the preparation process of pectin films from orange and grapefruit peel.

2 Materials and methods

2.1 Reagents and standards

Gallic acid, hesperidin, naringin, formic acid solution, dimethyl sulfoxide (DMSO) and Folin-Ciocalteau reagent were purchased from Sigma-Aldrich Corp (St. Louis, Missouri, USA). Methanol HPLC grade were obtained from Honeywell Burdick and Jackson (Muskegon, Michigan, USA), HCl and Na_2CO_3 from CTR Scientific (Monterrey, Nuevo León, México). All other chemicals were analytical grade.

2.2 Raw material

Mature oranges (*Citrus sinensis* var. March) and grapefruits (*Citrus paradisi* var. Doble Rojo) without wax or colorant coating added, were obtained from Distribuidora Mexicana de Cítricos, S R. L. de C. V. (Montemorelos, N. L., México). Citrus fruits were washed, cut in half and squeezed. Pulp was manually removed from peels and peels were frozen and stored in sealed plastic bags at -18°C until further use.

2.3 Film preparation

Films were prepared according to Mújica-Paz *et al*. (2011), a process that has been widely studied in our group and it is patent pending. A flow diagram of the films production process is shown in Fig. 1. Peels (P) of each citrus were thawed, cut in small pieces and microwave blanched (LG, MS2047GR, Englewood Cliffs, NJ, USA) for 1 min. Afterwards, each citrus peel was milled in a blender (Osterizer, 450-10, Miami, FL, USA) with aqueous ethanol solution (15%, v/v) in a 1:2 or 1:3 ratio of peels to solution for grapefruit and orange peels, respectively. Purees (Pu) of each citrus peel were homogenized (IKA, Ultra Turrax T-25, Wilmington, NC, USA) and stored at 4°C overnight. Pectin extraction was done by adding ethanol solution to the puree to adjust the ratio of peels to ethanol solution to 1:10. Glycerol was incorporated as plasticizer (0.625 and 1.25% w/w, for grapefruit and orange peel, respectively) and pH was adjusted to 1.5 with 1 M HCl. Mixtures were heated and stirred at 90°C for 30 minutes, and then filtered through double cotton cloth. The filtrate was maintained at 70°C with continuous stirring to concentrate it up to 12.2 (orange) or 15.6 °Brix (grapefruit).

The concentrates were placed on glass plates and laid down with a film applicator (QPI-MAFA3, Qualtech Products Industry, CO, USA). Film forming solutions were dried at room temperature (23.5 ± 1.5°C) for 36 h and edible films (F) were separated from glass plates. Peels, puree, retentate (R, particles that remained on the cotton cloth after filtration) and edible films of each citrus were placed in an oven and maintained at 35°C for 24 hours, grounded and stored in tubes at -18°C until analysis.

2.4 Solids mass balance

The mass balance was calculated by recording the weight of the streams of each process. The solids content of the streams was determined gravimetrically

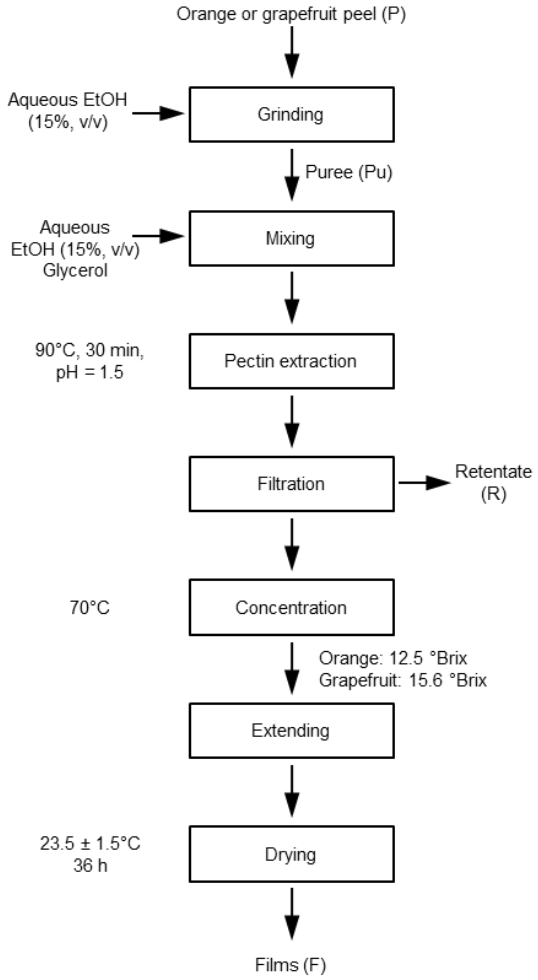

Fig. 1. General process for edible films production based on orange and grapefruit peel.

after drying samples for 24 h at 105°C. The mass balance in the processes was established for batches of 100 g of solids fed (in peels or purees).

2.5 *Free and total phenolic compounds determination*

The extraction process of the free and total phenolics was adapted from Vinson *et al.* (2001). Samples of 50 mg of peel or puree, or 25 mg of retentate or films, were put in glass tubes. For free phenolics (FP) extraction, 2.5 mL of methanol:water (1:1, v/v) solution were added. For total phenolics (TP) extraction, 2.5 mL of HCl (1.2 M) in methanol:water (1:1, v/v) was added. Samples were vortexed (VWR, model 945303, Radnor, PA, USA) at 3000 rpm for 1 min and then placed in a water bath (Fisher Scientific, model 102, Pittsburgh, PA, USA) for 3 hours at 90°C, vortexing every 30 min. Samples were cooled at room temperature. After centrifugation (IEC, Centra MP4R, Thermo Fisher Scientific Inc., Waltham, MA, USA) at 4310 *g* for 10 min at 4°C, supernatants were collected and made up to 5 mL with methanol.

Free and total phenolics were determined using the Folin-Ciocalteau method (Escobedo-Avellaneda *et al.*, 2014). In this assay, 50 μL of blank, standard solutions of gallic acid (50, 100, 150, 200, 250, 300 ppm) or processes samples were mixed with 650 μL of water and 50 μL of Folin-Ciocalteau reagent. After 5 min, 250 μL of 1N Na_2CO_3 were added and samples were incubated at 37°C for 2 h. The samples were transferred to a 96 microwells plate (Corning Costar, Tewksbury, Massachusetts, USA) and absorbance was measured at 765 nm in a microplate reader (Synergy HT, Bio-Tek, Winooski, VT, USA). Concentration was expressed as mg of gallic acid equivalents (GAE)/g, dry basis.

For the mass balance of phenolics, the concentration of these compounds and the solids content in the streams were used, considering 100 g of solids in the inflow streams (P and Pu).

2.6 *Flavonoids analysis by HPLC*

The method described by Escobedo-Avellaneda *et al.* (2014) was slightly modified for flavonoids analysis. Four hundred microliters of methanol:DMSO (1:1, v/v) were added to 25 mg of sample, sonicated for 10 min (VWR, model 50 T, Radnor, PA, USA) and centrifuged (Eppendorf, 5810R, Hamburg, Germany) at 18000 *g* for 10 min. Supernatants were collected and pellets were extracted twice with 2 mL of methanol:DMSO (1:1; v/v) and centrifuged at 18000 *g* for 10 min. Supernatants were mixed and diluted to 1 mL with methanol HPLC grade. Samples were filtered through a 0.45 μm membrane filter and mixed with water (1:1, v/v) before use.

Reversed-phase HPLC was used to determine flavonoids profile in the processes streams, with an Agilent equipment (1200 series, Santa Clara, CA, USA) with PDA detector and a Zorbax SB-C18 column (100 mm large, 3 mm internal diameter, 3.5 μm). The mobile phase consisted of (A) 0.1% formic acid (aqueous) and (B) methanol. The gradient elution was performed as follows: 0-3 min: 20% B; 3-10 min, linear to 55% B; 10-15 min: 55% B; 15-20 min, linear to 90% B; 20-36 min, 90% B; and 9 min of post-time. Two microliters of sample were analyzed at 35°C with a mobile phase

flow of 0.4 mL/min. Compounds detection was carried out at 280 nm, and the identification was based on retention times and absorption spectrums of hesperidin and naringin standards, and of stored standards in the HPLC computer. Quantification of flavonoids in orange and grapefruit samples was based on calibration curves of hesperidin (10 points of 2-600 ppm, R^2 >0.999) and naringin (9 points from 5 to 300 ppm, R^2 >0.999), respectively. Results were expressed as mg of hesperidin or naringin equivalents per gram of dry sample. For the mass balance of flavonoids, their concentration and the solids content in the stream were used, considering 100 g of solids in the inflow streams (P and Pu).

2.7 Statistical analysis

Unless otherwise indicated, results were expressed as mean ± standard deviation (S. D.) of three determinations. Data were analyzed using Minitab 14 by the Tukey´s test and p values < 0.05 were considered significant.

3 Results and discussions

3.1 Mass balance of solids

The dried solids balance of each film production process is shown in Table 1. Almost half (49.6 ± 0.1%) of the solids in orange peel occurred in the films, whereas 44.6 ± 0.2% occurred in the retentate. About 6% of the solids in orange peel were lost during films manufacturing.

A total of 87.3% of the solids from grapefruit peel were obtained in the outflow streams, with 58.5 ± 0.1% in the films and 28.8 ± 0.2% in the retentate. Therefore almost 13% of the solids were lost. The solids losses in both processes might be due to leftovers of the streams on surfaces of the labware used during films manufacturing.

Orange retentate had more solids than grapefruit retentate, indicating that grapefruit peel puree might have smaller particles. This could be caused by differences in the firmness of the citrus peels (Aluja *et al*., 2011) and in the used ratios during peel grinding. Particle size is a factor that has been reported as significant in phenolics extraction (Luthria, 2008). Moreover, films from grapefruit peel had higher mass of solids than orange peel films, which could lead to a higher phenolics retention.

3.2 Phenolic compounds

3.2.1 Orange peel process

The free and total phenolics concentration in peels, puree, retentate and films from orange are reported in Table 2. FP concentration ranged from 20.98 ± 0.67 to 23.56 ± 0.63 mg GAE/g and TP concentration was between 22.92 ± 0.64 and 28.18 ± 0.30 mg GAE/g in the different orange streams, where films exhibited the highest FP and TP concentration. Significant differences (p <0.05) were not observed in concentration or mass of FP or TP between orange peel and puree (Table 2).

The TP concentration of orange peel (Table 2) was slightly lower than that reported by Lagha-Benamrouche and Madani (2013) in orange peel (Thompson Navel) (25.6 mg/g, db). TP concentrations of about 6.2-6.5 mg/g in orange flavedo (Washington Navel) (Ramful *et al*., 2010) and of 5.53-7.30 mg/g for Valencia orange albedo and flavedo (Escobedo-Avellaneda *et al*., 2014) have been reported (both values on wet basis, wb). The TP concentration in orange peel was 6.97 mg/g of fresh peel, which was similar than those mentioned above.

The phenolic compounds were present mainly in free form (Table 2), with about 80% of the TP in orange peel. Escobedo-Avellaneda *et al*. (2014) also obtained higher fraction of FP than bound phenolics, in orange flavedo (65-77%) and albedo (71-79%). The obtained levels of FP in orange peel were higher than the previously reported (Escobedo-Avellaneda *et al*., 2014) probably because slow freezing of the peels could form large ice crystals that damage cellular structures (Sun and Li, 2003). These damages might have allowed the release of some of the bound phenolics, increasing the FP concentration.

The mass balances of phenolics in fabrication of films from orange peels (Table 2) indicated that the fraction of FP and TP was slightly higher in films (>50%) than in retentate (<50%). However, the retentate had an important mass of phenolics, so this stream could be used as source of phenolics that inclusive could be added to the films to enhace their antioxidant content.

3.2.2 Grapefruit peel process

In the process of film manufacture from grapefruit peel, the FP concentration ranged from 10.77 ± 0.24 to 17.46 ± 0.62 mg GAE/g, and of TP from 11.98 ± 0.33 to 24.95 ± 0.33 mg GAE/g (Table 2). Retentate had the lowest concentration and mass of both types of

phenolics, while films exhibited the highest ones. The fraction of free phenolics in grapefruit peel was about 90% of the TF.

Significant differences (p <0.05) were not detected in concentration or mass of total phenolics after grapefruit peel grinding. However, FP concentration, and therefore the mass, presented a slight reduction in puree possibly by oxidation reactions during storage of puree overnight at 4°C, as the polyphenols are substrates for many oxidoreductases (Robards *et al*., 1999). This FP reduction was also probably due to the low ascorbic acid content of grapefruit peel that would be unsufficient to inhibit polyphenoloxidase and consequently, phenolics degradation (Manthey and Grohmann, 1996; Nagy, 1980).

In this process, 253.81 ± 88.09 and 153.73 ± 129.43 mg GAE of FP and TP, respectively, were lost. The retention of polyphenols in films was about 65% of FP and 75% of TP, with respect to the mass of these compounds in grapefruit peel.

3.2.3 Orange and grapefruit processes

Comparison of both processes showed that orange peel had higher mass of FP and TP than grapefruit peel. Previous studies have also showed higher levels of phenolic compounds in orange peel than in grapefruit peel (Gorinstein *et al*., 2001; Goulas and Manganaris, 2012; Guimarães *et al*., 2010). The mass of both types of phenolic compounds (FP and TP) in the retentate of grapefruit peel was significantly (p <0.05) lower than in the orange peel retentate. Grapefruit peel films had less FP mass than orange peel films, but there was not significant difference in TP concentration in both films. This might be due to both, a higher fraction of bound phenolics (Table 2) and a higher mass of solids in grapefruit films than in orange peel films (Table 1).

The extraction conditions used for fabrication of films from citrus peels and some conditions for phenolics extraction are similar (Li *et al*., 2006; Vinson *et al*., 2001). This enabled an important recovery of citrus peel polyphenols in film forming solutions during films manufacturing. For example, phenolics have been extracted from citrus peels for 3 h with 20% aqueous ethanol at 80°C (Li *et al*., 2006) and from the edible portion of citrus fruits with acidified methanol:water (1:1) at 90°C (Vinson *et al*., 2001).

Thereby, manufacture of films from citrus wastes could be easier and cheaper than the actual methods that use purified biopolymers and phenolic rich extracts to develop antioxidant films.

Table 1. Mass balance (g) of dried solids in the manufacturing of edible films from citrus peel.

Stream	Orange	Grapefruit
Peel	100.0 ± 0.3	100.0 ± 0.0
Puree	100.0 ± 1.2	100.0 ± 0.6
Retentate	44.6 ± 0.2	28.8 ± 0.2
Films	49.6 ± 0.1	58.5 ± 0.1

3.2.4 Phenolic compounds in edible films

The performed analysis through the process indicated that films from orange and grapefruit peel had high levels of total phenolic compounds (24.95-28.18 mg/g) coming from the fruit peels. These levels are comparable to those of films containing extracts rich in phenolic compounds (Mehdizadeh *et al*., 2012; Siripatrawan and Harte, 2010). Starch-chitosan films incorporated with 2% of *Thymus kotschyanus* essential oil (Mehdizadeh *et al*., 2012) presented a total phenolic content of approximately 13 mg GAE/g. Siripatrawan and Harte (2010) made films from chitosan with 20% of green tea extract, achieving a total phenolics content of 33 mg/g of film.

Total phenolic concentration of films from citrus peels was also in the range of films made with different extracts from red seaweed (Blanco-Pascual *et al*., 2014), whose concentrations were from 6.08 to 41.32 mg GAE/g. However, the films with the highest concentration of polyphenols were prepared by adding an aqueous extract with high content of TP (28.53 mg GAE/g), which was obtained from the same raw material.

The obtained films from orange and grapefruit peels could have elevated scavenging activity due to their phenolic compounds content. The TP content of films enriched with phenolic extracts was strongly related to the antioxidant activity of the films, measured by DPPH (1,1-diphenyl 1-2-picrylhydrazyl) scavenging activity (Mehdizadeh *et al*., 2012; Siripatrawan and Harte, 2010). The TP concentrations in orange and grapefruit peel films (Table 2) were similar to the TP concentrations of citrus peels (25.6-31.62 mg GAE/g) with high antioxidant activity (81.7-88.0%) reported by Lagha-Benamrouche and Madani (2013), where a strong correlation was observed.

Orange and grapefruit peel films have potential to be used as antioxidant packaging. For example to reduce the lipid oxidation and to avoid the oxygen negative effect on the color of meat and fish products

Table 2. Concentration and mass[A] of free (FP) and total phenolics (TP) in the manufacturing process of edible films from citrus peel.

Stream	Concentration (mg GAE[B]/g, db[C])		Mass (mg GAE)	
	FP	TP	FP	TP
Orange				
Peel	21.18 ± 0.27[a]	22.92 ± 0.64[a]	2117.63 ± 32.86[a]	2292.24 ± 70.77[a]
Puree	20.98 ± 0.67[a]	23.33 ± 0.53[a]	2098.24 ± 91.51[a]	2332.96 ± 81.18[a]
Retained	22.69 ± 0.39[b]	25.51 ± 0.63[b]	1011.55 ± 21.94[b]	1136.94 ± 74.80[b]
Films	23.56 ± 0.63[c]	28.18 ± 0.30[c]	1169.49 ± 32.80[c]	1397.47 ± 34.15[c]
Grapefruit				
Peel	15.67 ± 0.41[d]	19.58 ± 0.51[d]	1567.37 ± 41.24[d]	1958.36 ± 51.68[d]
Puree	14.29 ± 0.47[e]	19.01 ± 0.29[d]	1429.29 ± 56.25[e]	1900.74 ± 41.23[d]
Retained	10.77 ± 0.24[f]	11.98 ± 0.33[e]	310.66 ± 9.36[f]	345.41 ± 42.59[e]
Films	17.46 ± 0.62[g]	24.95 ± 0.33[a]	1021.00 ± 37.49[b]	1459.22 ± 35.16[c]

[A]Mass balance is based on 100 g of dried solids in inflow streams (peel and puree). [B]GAE, gallic acid equivalents.[C]Dry basis. [a-g]Means with different superscripts in the same column are significantly different at $p < 0.05$).

(Kim *et al*., 2012; Pereira de Abreu *et al*., 2010; Qin *et al*., 2013), and to prevent the enzymatic browning and the vitamin C loss in vegetables and fruits (Bonilla *et al*., 2012; Tapia *et al*., 2008; Pérez-Gago *et al*., 2006).

3.3 Flavonoids

3.3.1 Orange peel process

Representative HPLC chromatograms of the flavonoid profiles in samples of the process based on orange peel are given in Fig. 2. The identified flavonoids in peel, puree, retentate and films from orange (Fig. 2) were two flavanone glycosides (hesperidin and didymin) and three polymethoxylated flavones (sinensetin, auranetin and nobiletin). These flavonoids have been found before in orange peel (Escobedo-Avellaneda *et al*., 2014; Li *et al*., 2006; Nogata *et al*., 2006; Ramful *et al*., 2010; Wang *et al*., 2008).

The concentration and mass of all identified flavonoids in samples from orange peel did not present significant differences (p <0.05) between peel and puree (Table 3). Hesperidin was the main flavonoid in orange peel, which is in accordance with Ramful *et al*. (2010) and Wang *et al*. (2008), and also in the others streams (Pu, R and F).

Polymethoxylated flavones were more concentrated in films than in retentate, while flavanones glycosides were more concentrated in R than in F. Hesperidin was the only flavonoid whose concentration significantly decreased in the films, with respect to the peel.

The concentration of hesperidin in orange peel (53.77 ± 2.26 mg/g) was similar to that of orange albedo reported by Escobedo-Avellaneda *et al*. (2014) (about 50 mg/g), where a direct correlation with the antioxidant activity was observed. The antioxidant activity of those samples was between 85.67 and 154.84 mmol TE/g (Trolox Equivalents), for concentrations of hesperidin between 17 and 50 mg/g, approximately (Escobedo-Avellaneda *et al*. 2014). Hence, the developed films could have antioxidant activity.

Films had lower mass of the flavanone hesperidin than the retentate, but higher mass of the polymethoxylated flavones. The mass of sinensetin, auranetin and nobiletin in films was about 58, 54 and 54% of the original mass of each flavonoid in orange peel, respectively. The recovered mass of hesperidin and dydimin in films was approximately 31 and 43%, respectively. The retentate had about 50% of the mass of the identified flavonoids, while the mass that remained in the films was about 33%. Therefore, the mass loss of the identified flavonoids in the process based on orange peel was approximately 17%. The fraction of flavonoids retained in films was mainly due to hesperidin, since its concentration was much higher than the concentration of the other identified flavonoids. The retentate, which is the residue of the process, could still be used as source of natural antioxidants because of its high fraction of flavonoids.

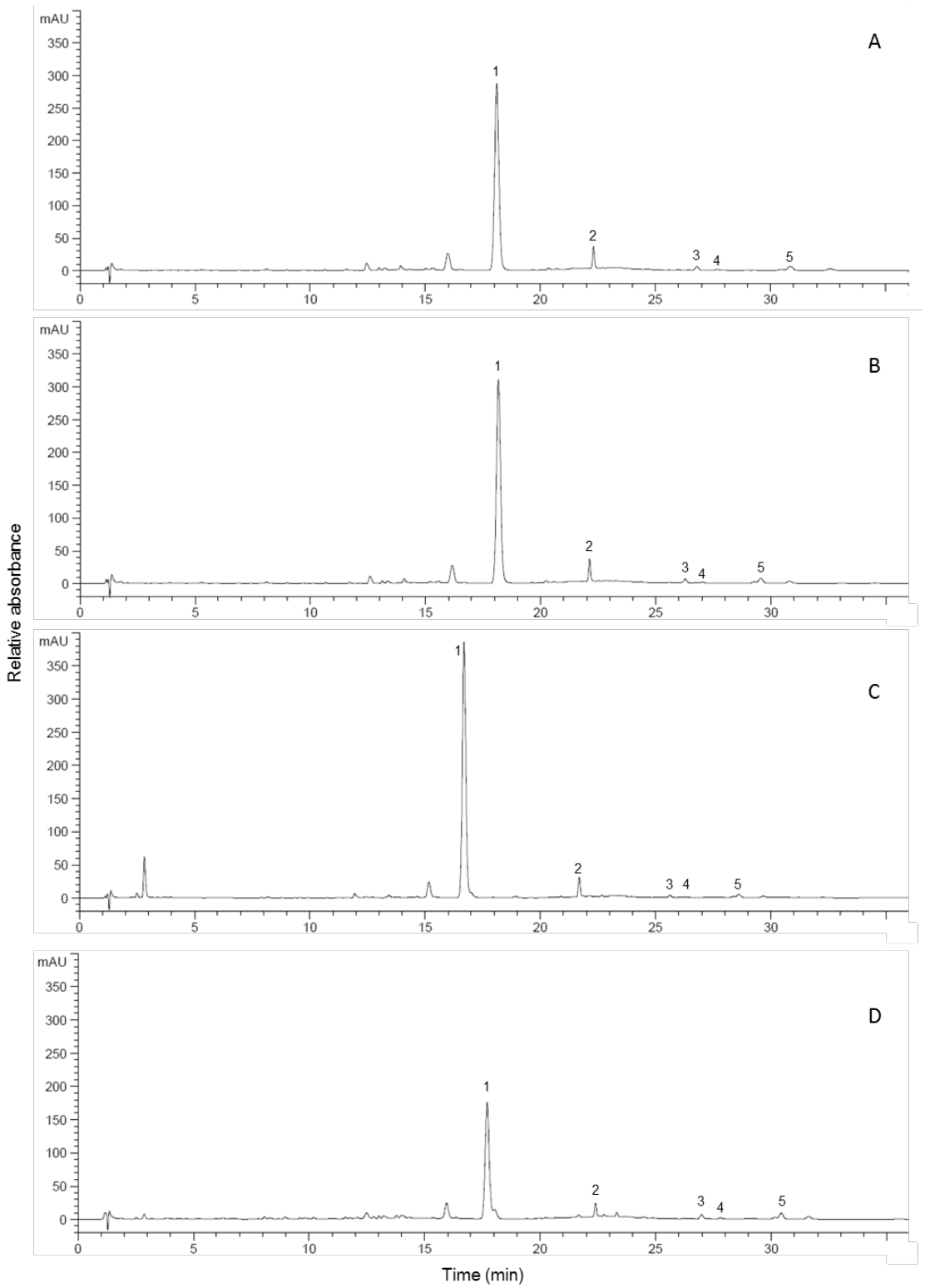

Fig. 2. HPLC chromatograms at 280 nm of orange peel (A), puree (B), retentate (C) and films (D). Hesperidin (1), didymin (2), sinensetin (3), auranetin (4) and nobiletin (5).

Table 3. Concentration and mass[A] of flavonoids in the manufacturing of edible films from orange peel.

Stream	Hesperidin	Didymin	Sinensetin	Auranetin	Nobiletin
Concentration[B]					
Peel	53.77 ± 2.26[a]	3.08 ± 0.28[a]	1.06 ± 0.05[a]	0.49 ± 0.03[a]	1.76 ± 0.08[a]
Puree	55.17 ± 1.84[a]	3.25 ± 0.13[a]	1.03 ± 0.02[a]	0.47 ± 0.04[a]	1.72 ± 0.06[a]
Retentate	61.68 ± 0.07[b]	3.22 ± 0.05[a]	0.72 ± 0.05[b]	0.39 ± 0.03[b]	1.27 ± 0.08[b]
Films	33.39 ± 1.31[c]	2.62 ± 0.29[a]	1.23 ± 0.03[c]	0.53 ± 0.01[a]	1.84 ± 0.06[a]
Mass[C]					
Peel	5376.20 ± 211.26[a]	308.03 ± 27.38[a]	106.38 ± 5.09[a]	49.24 ± 2.88[a]	175.56 ± 7.32[a]
Puree	5516.93 ± 199.45[a]	325.34 ± 13.63[a]	103.27 ± 2.57[a]	47.00 ± 3.65[a]	171.66 ± 3.65[a]
Retentate	2749.29 ± 15.87[b]	143.71 ± 2.96[b]	32.17 ± 2.22[b]	17.49 ± 1.45[b]	57.07 ± 3.66[b]
Films	1657.54 ± 72.65[c]	130.07 ± 14.90[b]	61.28 ± 1.88[c]	26.16 ± 0.60[c]	94.31 ± 3.43[c]

[A]Mass balance is based on 100 g of dried solids in inflow streams (peel and puree). [B]mg hesperidin equivalents/g of sample, dry basis. [C]mg hesperidin equivalents. [a–d]Means with different superscripts in the same column for concentration or mass are significantly different at $p < 0.05$.

Table 4. Concentration and mass[A] of flavonoids in the manufacturing of edible films from grapefruit peel.

Stream	Naringin	Didymin	Nobiletin
Concentration[B]			
Peel	47.58 ± 1.31[a]	4.90 ± 0.22[a]	0.47 ± 0.02[a]
Puree	42.23 ± 0.28[b]	4.10 ± 0.06[b]	0.44 ± 0.01[a]
Retained	5.08 ± 0.07[c]	1.21[c]**	ND
Films	31.42 ± 0.55[d]	4.50 ± 0.05[d]	0.49 ± 0.05[a]
Mass[C]			
Peel	4758.46 ± 132.74[a]	490.00 ± 22.49[a]	46.89 ± 2.07[a]
Puree	4222.69 ± 29.35[b]	409.73 ± 6.39[b]	43.72 ± 1.36[a]
Retained	146.56 ± 2.85[c]	34.96 ± 1.73[c]	-
Films	1837.48 ± 46.78[d]	262.99 ± 5.24[d]	28.57 ± 3.19[b]

[A]Mass balance is based on 100 g of dried solids in inflow streams (peel and puree). [B]mg naringin equivalents/g of sample, dry basis. [C]mg naringin equivalents. [a–d]Means with different superscripts in the same column for concentration or mass are significantly different at $p < 0.05$.

3.3.2 Grapefruit peel process

The flavanone glycosides naringin and dydimin, as well as the polymethoxylated flavone nobiletin, were the identified flavonoids in peel, puree and films from grapefruit (Fig. 3A, 3B, 3D). Nogata *et al*. (2006) and Zhang *et al*. (2011) also identified these flavonoids in grapefruit peel. Didymin was identified in two of three retentate samples, while nobiletin was not found in any of the analyzed samples of the retentate (Fig. 3C).

Table 4 shows the concentration and mass balance of the identified flavonoids in peel, puree, retentate and films from grapefruit. Puree presented a concentration and mass reduction of naringin and didymin with respect to peel, possibly because of oxidation reactions (Robards *et al*., 1999), which was reflected in concentration and mass reduction of FP (Table 2). The concentration of nobiletin was the same in grapefruit peel, puree and films. All the identified flavonoids presented the lowest concentration in R.

The concentration of naringin in grapefruit peel was at least two fold higher than the values reported (10.26-15.7 mg/g) by Goulas and Manganaris (2012) for other grapefruit varieties, being the most abundant flavonoid in this citrus peel. A direct correlation of grapefruit flavonoids concentration and antioxidant activity was also reported (Goulas and Manganaris, 2012).

The mass balance in the development of edible films from grapefruit peel (Table 4) indicated a significant (p <0.05) loss in the flavonoids mass at the end of the process, probably due to their degradation and to the solids lost during manufacturing. Films retained about 39% of the naringin from grapefruit peel, 54% of didymin and 60% of nobiletin. In general, grapefruit films retained approximately 40% of the identified flavonoids, while the retentate conserved 3.4% of them.

3.3.3 Orange and grapefruit processes

Although losses of flavonoids in the process based on grapefruit peel were high (57%, approximately), the films obtained by this process retained a higher percentage of the flavonoids (≈40%) from the raw material than the films from orange peel (≈33%).

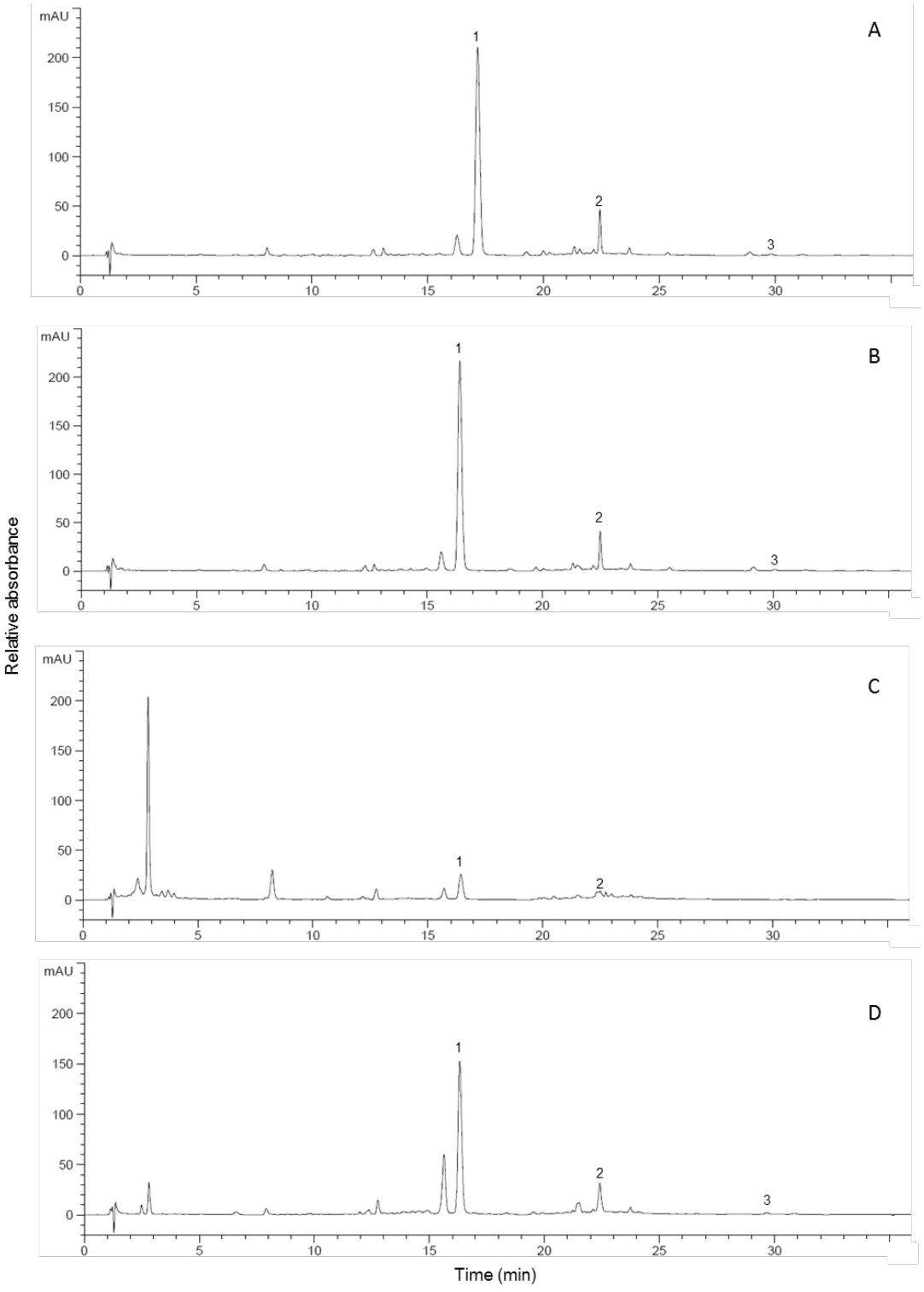

Fig. 3. HPLC chromatograms at 280 nm of grapefruit peel (A), puree (B), retentate (C) and films (D). Naringin (1), didymin (2) and nobiletin (3).

In both processes, although polymethoxylated flavones (auranetin, sinensetin and nobiletin) showed higher degree of retention in films than flavanone glycosides (naringin, hesperidin and didymin), the mass of the latter was higher.

Conclusions

Films from orange and grapefruit peel retained more total phenolic compounds (>50% and >70%, respectively) than retentates (<50% and <20%, respectively). However, rententates still had an important fraction of phenolics, especially orange peel retentate, which could also be recuperated and used as natural antioxidant in different applications, including their addition to films. The high content of flavonoids (mainly hesperidin and naringin) and other polyphenols in the films could provide them with important antioxidant properties which help in preservation of foods susceptible to oxidation.

Acknowledgements

The authors thank the financial support from CONACYT-México (Grant 223775) and Tecnológico de Monterrey. J.G. Hernández-Carrillo is grateful to CONACYT for the doctoral scholarship. Special thanks to Z. J. Escobedo-Avellaneda for her advices in laboratory techniques.

References

Aluja, M., Bigurra, E., Birke, A., Greany, P., and McDonald, R. (2011). Delaying senescence of 'Ruby Red' grapefruit and 'Valencia' oranges by gibberellic acid applications. *Revista Mexicana de Ciencias Agrícolas 2*, 41-55.

Alves, V.D., Costa, N., and Coelhoso, I.M. (2010). Barrier properties of biodegradable composite films based on kappa-carrageenan/pectin blends and mica flakes. *Carbohydrate Polymers 79*, 269-276.

Bagliotti Meneguin, A., Stringhetti Ferreira Cury, B., and Evangelista, R.C. (2014). Films from resistant starch-pectin dispersions intended for colonic drug delivery. *Carbohydrate Polymers 99*, 140-149.

Bierhalz, A.C.K., da Silva, M.A., and Kieckbusch, T.G. (2012). Natamycin release from alginate/pectin films for food packaging applications. *Journal of Food Engineering 110*, 18-25.

Blanco-Pascual, N., Gómez-Guillén, M.C., and Montero, M.P. (2014). Integral *Mastocarpus stellatus* use for antioxidant edible film development. *Food Hydrocolloids 40*, 128-137.

Bonilla, J., Atarés, L., Vargas, M., and Chiralt, A. (2012). Edible films and coatings to prevent the detrimental effect of oxygen on food quality: Possibilities and limitations. *Journal of Food Engineering 110*, 208-213.

Cian, R.E., Salgado, P.R., Drago, S.R., González, R.J., and Mauri, A.N. (2014). Development of naturally activated edible films with antioxidant properties prepared from red seaweed *Porphyra columbina* biopolymers. *Food Chemistry 146*, 6-14.

Escobedo-Avellaneda, Z., Gutiérrez-Uribe, J., Valdez-Fragoso, A., Torres, J.A., and Welti-Chanes, J., (2014). Phytochemicals and antioxidant activity of juice, flavedo, albedo and comminuted orange. *Journal of Functional Foods 6*, 470-481.

Galus, S., and Lenart, A. (2013). Development and characterization of composite edible films based on sodium alginate and pectin. *Journal of Food Engineering 115*, 459-465.

Gorinstein, S., Martín-Belloso, O., Park, Y.-S., Haruenkit, R., Lojek, A., Ĉíž, M., Caspi, A., Libman, I., and Trakhtenberg, S. (2001). Comparison of some biochemical characteristics of different citrus fruits. *Food Chemistry 74*, 309-315.

Goulas, V., and Manganaris, G.A. (2012). Exploring the phytochemical content and the antioxidant potential of *Citrus* fruits grown in Cyprus. *Food Chemistry 131*, 39-47.

Guimarães, R., Barros, L., Barreira, J.C.M., Sousa, M.J., Carvalho, A.M., and Ferreira, I.C.F.R. (2010). Targeting excessive free radicals with peels and juices of citrus fruits: Grapefruit, lemon, lime and orange. *Food and Chemical Toxicology 48*, 99-106.

Gómez-Estaca, J., Giménez, B., Montero, P., and Gómez-Guillén, M.C. (2009). Incorporation

of antioxidant borage extract into edible films based on sole skin gelatin or a commercial fish gelatin. *Journal of Food Engineering 92*, 78-85.

Hernández-Ochoa, L., Gonzales-Gonzales, A., Gutiérrez-Mendez, N., Muñoz-Castellanos, L. N., and Quintero-Ramos, A. (2011). Estudio de la actividad antibacteriana de películas elaboradas con quitosano a diferentes pesos moleculares incorporando aceites esenciales y extractos de especias como agentes antimicrobianos. *Revista Mexicana de Ingeniería Química 10*, 455-463.

Jouki, M., Tabatabaei Yazdi, F., Mortazavi, S.A., and Koocheki, A. (2013). Physical, barrier and antioxidant properties of a novel plasticized edible film from quince seed mucilage. *International Journal of Biological Macromolecules 62*, 500-507.

Kim, I.-H., Yang, H.-J., Noh, B.-S., Chung, S.-J., and Min, S.C. (2012). Development of a defatted mustard meal-based composite film and its application to smoked salmon to retard lipid oxidation. *Food Chemistry 133*, 1501-1509.

Lagha-Benamrouche, S., and Madani, K. (2013). Phenolic contents and antioxidant activity of orange varieties (*Citrus sinensis* L. and *Citrus aurantium* L.) cultivated in Algeria: Peels and leaves. *Industrial Crops and Products 50*, 723-730.

Li, B.B., Smith, B., and Hossain, M.M. (2006). Extraction of phenolics from citrus peels: I. Solvent extraction method. *Separation and Purification Technology 48*, 182-188.

Luthria, D.L. (2008). Influence of experimental conditions on the extraction of phenolic compounds from parsley (*Petroselinum crispum*) flakes using a pressurized liquid extractor. *Food Chemistry 107*, 745-752.

Manthey, J.A., and Grohmann, K. (1996). Concentrations of hesperidin and other orange peel flavonoids in citrus processing byproducts. *Journal of Agricultural and Food Chemistry 44*, 811-814.

Mariniello, L., Giosafatto, C.V., Di Pierro, P., Sorrentino, A., and Porta, R. (2010). Swelling, mechanical, and barrier properties of albedo-based films prepared in the presence of phaseolin cross-linked or not by transglutaminase. *Biomacromolecules 11*, 2394-2398.

Mehdizadeh, T., Tajik, H., Razavi Rohani, S.M., and Oromiehie, A.R. (2012). Antibacterial, antioxidant and optical properties of edible starch-chitosan composite film containing *Thymus kotschyanus* essential oil. *Veterinary Research Forum 3*, 167-173.

Mújica-Paz, H., Valdez-Fragoso, A., Hernández-Carrillo, J., and Welti-Chanes, J., 2011. Proceso para la elaboración de películas a partir de cítricos, las películas y su uso como empaque biodegradable y vehículo de compuestos activos. *Solicitud de patente MX-a-2011-008209*, 4 de Agosto del 2011, México.

Nagy, S. (1980). Vitamin C contents of citrus fruit and their products: a review. *Journal of Agricultural and Food Chemistry 28*, 8-18.

Nogata, Y., Sakamoto, K., Shiratsuchi, H., Ishii, T., Yano, M., and Ohta, H. (2006). Flavonoid composition of fruit tissues of citrus species. *Bioscience, Biotechnology and Biochemistry 70*, 178-192.

Otoni, C.G., Moura, M.R.D., Aouada, F.A., Camilloto, G.P., Cruz, R.S., Lorevice, M.V., Soares, N.D.F.F., and Mattoso, L.H.C. (2014). Antimicrobial and physical-mechanical properties of pectin/papaya puree/cinnamaldehyde nanoemulsion edible composite films. *Food Hydrocolloids 41*, 188-194.

Pereira de Abreu, D.A., Losada, P.P., Maroto, J., and Cruz, J.M. (2010). Evaluation of the effectiveness of a new active packaging film containing natural antioxidants (from barley husks) that retard lipid damage in frozen Atlantic salmon (*Salmo salar* L.). *Food Research International 43*, 1277-1282.

Perez-Gago, M.B., Serra, M., and del Río, M.A. (2006). Color change of fresh-cut apples coated with whey protein concentrate-based edible coatings. *Postharvest Biology and Technology 39*, 84-92.

Pruneda, E., Peralta-Hernández, J.M., Esquivel, K., Lee, S.Y., Godínez, L.A., and Mendoza, S. (2008). Water vapor permeability, mechanical

properties and antioxidant effect of Mexican oregano-soy based edible films. *Journal of Food Science 73*, C488-C493.

Qin, Y.Y., Yang, J.Y., Lu, H.B., Wang, S.S., Yang, J., Yang, X.C., Chai, M., Li, L., and Cao, J.X. (2013). Effect of chitosan film incorporated with tea polyphenol on quality and shelf life of pork meat patties. *International Journal of Biological Macromolecules 61*, 312-316.

Ramful, D., Bahorun, T., Bourdon, E., Tarnus, E., and Aruoma, O.I. (2010). Bioactive phenolics and antioxidant propensity of flavedo extracts of Mauritian citrus fruits: Potential prophylactic ingredients for functional foods application. *Toxicology 278*, 75-87.

Robards, K., Prenzler, P.D., Tucker, G., Swatsitang, P., and Glover, W. (1999). Phenolic compounds and their role in oxidative processes in fruits. *Food Chemistry 66*, 401-436.

Rodríguez-Marín, M. L., Bello-Perez, L. A.,Yee-Madeira, H., and Gonzalez-Soto, R. A. (2013). Propiedades mecánicas y de barrera de películas elaboradas con harina de arroz y plátano reforzadas con nanopartículas: estudio con superficie de respuesta. *Revista Mexicana de Ingeniería Química 12*, 165-176.

Romero-Bastida, C. A., Zamudio-Flores, P. B., and Bello-Pérez, L. A. (2011). Antimicrobianos en películas de almidón oxidado de plátano: Efecto sobre la actividad antibacteriana, microestructura, propiedades mecánicas y de barrera. *Revista Mexicana de Ingeniería Química 10*, 445-453.

Salgado, P.R., López-Caballero, M.E., Gómez-Guillén, M.C., Mauri, A.N., and Montero, M.P. (2012). Exploration of the antioxidant and antimicrobial capacity of two sunflower protein concentrate films with naturally present phenolic compounds. *Food Hydrocolloids 29*, 374-381.

Siripatrawan, U., and Harte, B.R. (2010). Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract. *Food Hydrocolloids 24*, 770-775.

Sun, D.-W., and Li, B. (2003). Microstructural change of potato tissues frozen by ultrasound-assisted immersion freezing. *Journal of Food Engineering 57*, 337-345.

Tapia, M.S., Rojas-Graü, M.A., Carmona, A., Rodríguez, F.J., Soliva-Fortuny, R., and Martin-Belloso, O. (2008). Use of alginate- and gellan-based coatings for improving barrier, texture and nutritional properties of fresh-cut papaya. *Food Hydrocolloids 22*, 1493-1503.

Villagómez-Zavala, D. L., Gómez-Corona, C., San Martín Martínez, E., Pérez-Orozco, J.P., Vernon-Carter, E.J., and Pedroza-Islas., R. (2008). Comparative study of the mechanical properties of edible films made from single and blended hydrophilic biopolymer matrices. *Revista Mexicana de Ingeniería Química 7*, 263-273.

Vinson, J.A., Su, X., Zubik, L., and Bose, P. (2001). Phenol antioxidant quantity and quality in foods: fruits. *Journal of Agricultural and Food Chemistry 49*, 5315-5321.

Wang, Y.C., Chuang, Y.C., and Hsu, H.W. (2008). The flavonoid, carotenoid and pectin content in peels of citrus cultivated in Taiwan. *Food Chemistry 106*, 277-284.

Woranuch, S., Yoksan, R., and Akashi, M. (2014). Ferulic acid-coupled chitosan: Thermal stability and utilization as an antioxidant for biodegradable active packaging film. *Carbohydrate Polymers 115*, 744-751.

Yapo, B.M. (2009). Pectin quantity, composition and physicochemical behaviour as influenced by the purification process. *Food Research International 42*, 1197-1202.

Zhang, M., Duan, C., Zang, Y., Huang, Z., and Liu, G. (2011). The flavonoid composition of flavedo and juice from the pummelo cultivar (*Citrus grandis* (L.) Osbeck) and the grapefruit cultivar (*Citrus paradisi*) from China. *Food Chemistry 129*, 1530-1536.