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DEVELOPMENT AND ANTIOXIDANT STABILITY OF EDIBLE FILMS SUPPLEMENTED WITH A TAMARIND SEED EXTRACT

DESARROLLO Y ESTABILIDAD ANTIOXIDANTE DE PELÍCULAS COMESTIBLES AGREGADAS CON UN EXTRACTO DE SEMILLAS DE TAMARINDO

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Abstract

In this study, the mechanical, antioxidants, and color properties of edible films made with native potato starch (NPS) and whey protein isolate (WPI) were evaluated by incorporating tamarind seed extract (TSE) as an antioxidant. Firstly, the conditions to produce edible films were optimized by a response surface model, varying the amount of plasticizer (2.3-6.3% of glycerol), the pH (5-9), and protein denature temperature (75-95 °C) were varied to achieve the best elongation (% ε), tensile strength (σ), and Young's modulus (E) characteristics. The TSE was a source of natural antioxidants by a polyphenol content (187.0 ± 0.11 mg GAE/g) and antioxidant activity (62.5 ± 0.04% for $DPPH^{\bullet}$ and 45.6 ± 0.14% for $ABTS^{+\bullet}$ radical scavenging methods). The TSE supplementation enhanced the mechanical properties, increasing %ε by 8%, and decreasing σ and E by 50% and 14%, respectively; with a reddish coloration and a 2.7 fold increase in antioxidant activity. These parameters remained stable during storage so that edible films supplemented with TSE could be used as part of a system to prevent the oxidation reactions in foods.

Keywords: edible film, tamarind seeds, antioxidant activity, whey protein isolate-native, potato starch.

Resumen

En este estudio se evaluaron las propiedades mecánicas, antioxidantes y de color de películas elaboradas con almidón nativo de papa (NPS) y aislado de proteína de suero de leche (WPI) por efecto de la incorporación de un extracto de semilla de tamarindo (TSE) como antioxidante. Primeramente, se optimizó la elaboración de las películas empleando un modelo de superficie de respuesta, variando la concentración del plastificante (glicerol 2.3-6.3%), el pH (5-9) y la temperatura de desnaturalización (75-95 °C) para alcanzar las mejores características de elongación (% ε), resistencia a la ruptura (σ) y el módulo de Young (E). El TSE es una fuente de antioxidantes naturales por su contenido en polifenoles (187.0 ± 0.11 mg GAE/g) y actividad antioxidante (62.5 ± 0.04% para $DPPH^{\bullet}$ y 45.6 ± 0.14% para $ABTS^{+\bullet}$). La adición de TSE mejoró las propiedades mecánicas de las películas incrementando en 8% el %ε, además disminuyó en 14% y 50% los parámetros de σ y E, respectivamente; generó una coloración rojiza e intensificó 2.7 veces la actividad antioxidante. Estos parámetros permanecieron sin cambio durante el almacenamiento por lo que las películas adicionadas TSE podrían emplearse como parte de un sistema para prevenir reacciones de oxidación en alimentos.

Palabras clave: películas comestibles, semilla de tamarindo, capacidad antioxidante, aislado de proteína de suero de leche, almidón de papa.

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1 Introduction

Oxidation of lipids and proteins is one of the most common deleterious changes observed in foods due to the formation of undesirable odours and flavors, changes in texture, loss of color and nutrients that decrease shelf life. Lipid oxidation also induces accumulation of toxic metabolites that may threat human health, as they are related to the development of cardiovascular diseases, cancer, metabolic syndrome, and others (Skibsted, 2010). The use of natural antioxidants present in fruits and vegetables could prevent oxidation due to their content in polyphenols, which also decrease the absorption of toxic dietary lipid oxidation end-products (Kanner, 2007). From this point of view, tamarind seeds as other vegetable non-edible residues such as seeds and peels, represent an efficient and economical source of natural antioxidants based on their content in polyphenols such as epicatechin, procyanidins in different forms, and tannins that are capable to act as reducing or scavenging agents against reactive oxygen and nitrogen species (Sudjaroen *et al.*, 2005; Tsuda *et al.*, 1994). According to Soong and Barlow (2004), the antioxidant activity assessed by FRAP (2486 μmol/g) and phenolic content (94.5 mg GAE/g) present in the tamarind seeds is higher compared to other fruit seeds, such as avocado (1484 μmol/g and 88.2 mg GAE/g), jackfruit (3 μmol/g and 27.7 mg GAE/g), and longan (1388 μmol/g and 62.6 mg GAE/g). In addition, the starch present in the tamarind seeds possesses antioxidant and antimicrobial activity (Chandra *et al.*, 2016). The tamarind seed represents 33-40% of the whole fruit and the world production of tamarind fruit is approximately 500,000 ton/year (CropIndex, 2016). Therefore, about 200,000 tons of seeds are produced annually that can be used as a source of natural antioxidants.

Another strategy to reduce the oxidation in foods is the use packages or natural barriers against oxygen, light, and moisture, as well as the inclusion of bioactive compounds (antioxidants and antimicrobial) within the film matrix in order to extend the shelf life of products to which they are applied (Gómez-Estaca *et al.*, 2014; Piñeros-Hernandez *et al.*, 2017). Polyphenolic extracts can be easily incorporated into edible films and coatings, which are thin layers made of proteins and polysaccharides (Alvarado-González *et al.*, 2012; Falguera *et al.*, 2011; Siracusa *et al.*, 2008; Villagómez-Zavala *et al.*, 2008). However, the incorporation of compounds, such as polyphenols and other bioactive compounds may induce changes in color, mechanical properties, and water/oxygen permeability (Flores-Martínez et al., 2017; Piñeros-Hernandez *et al.*, 2017; Silva-Weiss *et al.*, 2013, Talón *et al.*, 2017). Polyphenols could act as a cross-linkers when interacting with the functional groups of proteins and polysaccharides that conform the film matrix to enhance mechanical strength and flexibility, water resistance and thermal stability accordingly to their nature and concentration (Damodaran *et al.*, 2008; von Staszewski *et al.*, 2011). However, there are few studies in which antioxidants from agro-industrial residues have been added to edible films (Hernández-Carrillo *et al.*, 2015). One of the advantages of using agro-industrial residues is the reduction in the cost of the raw material needed to obtain the antioxidant extracts, while increasing the diversity of the compounds and activities that can be obtained in addition to polyphenols. Even, the same antioxidant may present different activities, according to the polymers or materials that conform the edible film and conditions used for their manufacture, as well as, the concentration at which they are incorporated (Mayachiew and Devahastin, 2010; Martins *et al.*, 2012).

Therefore, the aim of this study was to evaluate the mechanical properties and antioxidant capacity during storage of optimized bi-component WPI-NPS edible films supplemented with an antioxidant tamarind extract.

2 Materials and methods

2.1 Chemicals

Whey protein isolate (WPI) from Davisco Foods International, Inc. (Le Sueur, MN, USA) and native potato starch (NPS) from FABPSA® (Mexico) were used for the elaboration of films. Tamarind seeds were collected in the state of Guerrero (Mexico), dried at 40 °C for 24 h in a natural convection oven (FE291-AD, Felisa®, Mexico), and ground in a gristmill (Thomas Wiley Mill Model 4, Arthur H. Thomas Company, Philadelphia, PA, USA). The ground seeds were sieved (U.S. Standard Sieve Series, No. 100, Dual Manufacturing Co. Chicago, IL, USA), and kept at a final moisture content of 9.5%.

Table 1. Mechanical properties for the experimental combinations in the production of protein-starch films for the response surface model.[a]

Combination			Tensile strength (σ, MPa)	Elongation ($\%\varepsilon$)	Young's modulus (E, MPa)
Glycerol	pH	Temperature			
3.3	6	80	12.15 ± 1.236	58.51 ± 3.505	0.31 ± 0.010
3.3	6	90	14.01 ± 0.903	46.91 ± 6.624	0.42 ± 0.035
3.3	8	80	11.60 ± 0.659	70.48 ± 1.931	0.36 ± 0.013
3.3	8	90	13.58 ± 1.419	60.27 ± 3.550	0.47 ± 0.026
5.3	6	80	8.09 ± 0.132	53.08 ± 4.357	0.37 ± 0.027
5.3	6	90	8.69 ± 0.469	49.66 ± 5.155	0.36 ± 0.017
5.3	8	80	4.74 ± 0.160	70.65 ± 8.090	0.21 ± 0.022
5.3	8	90	5.81 ± 0.385	75.40 ± 5.820	0.23 ± 0.028
2.3	7	85	2.47 ± 0.514	82.01 ± 4.058	0.04 ± 0.020
4.3	5	85	3.94 ± 0.796	6.80 ± 1.024	0.48 ± 0.027
4.3	7	95	7.82 ± 0.093	68.02 ± 5.118	0.26 ± 0.018
4.3	7	75	8.83 ± 0.488	111.68 ± 4.154	0.08 ± 0.011
4.3	9	85	7.02 ± 0.663	72.05 ± 4.284	0.13 ± 0.041
6.3	7	85	9.51 ± 0.173	73.42 ± 3.183	0.14 ± 0.028
4.3	7	85[b]	12.54 ± 1.246	89.17 ± 6.043	0.13 ± 0.018
RMSE			0.943	5.237	0.022

[a]Data are expressed as mean ± standard deviation (n=3). [b]Mean ± standard deviation (n=6). RMSE = Root mean square error.

Folin-Ciocalteu's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$), and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic) (ABTS$^{+\bullet}$) were analytical grade and purchased from Sigma-Aldrich (Steinheim, Germany).

2.2 *Polyphenolic tamarind seed extract characterization*

2.2.1 Extraction

The extraction was performed according to Siddhuraju (2007). Tamarind seed extract (TSE) was obtained through ethanol extraction from the milled seeds (1:10) at 25 °C and stirring (Laboratory Stirrer/hot plate, Corning PC-420D, Mexico) at 400 rpm for t24 h and filtered (Whatman paper No. 4). The solvent was evaporated using a rotary vacuum-evaporator (RV0551, Janke & Kunkel, Staufen, Germany) at 40 ± 0.8 °C. The final dry extract (tamarin seed extract = TSE) was stored in amber vials at -20 °C, until analysis.

2.2.2 TSE characterization

2.2.2.1 Polyphenols and flavonoids content

Total polyphenol content was determined according to Singleton and Rossi (1965). An aliquot of 100 μL (1 g/100 mL ethanol) of TSE was mixed with 600 μL of Folin reagent (2N) and 2 mL of Na_2CO_3 (20% w/v). After 40 min of incubation at room temperature, the absorbance was measured at 725 nm in a spectrophotometer (Varian Cary 50 Bio UV/Vis, Varian Inc. Agilent, Palo Alto, CA, USA). The content of polyphenols was reported as milligrams of gallic acid equivalent (GAE)/g of dry TSE. Flavonoids content was determined following the method reported by Miliauskas *et al.* (2004). The results were expressed as milligrams of catechin equivalent/g of dry TSE.

2.2.2.2 Antioxidant activity

Antioxidant activity (AnA) of TSE was measured by the DPPH$^{\bullet}$ radical scavenging method (Brand-Williams *et al.*, 1995) at 515 nm, and the ABTS$^{+\bullet}$

method (Re *et al.*, 1999) at 734 nm. The DPPH$^{\bullet}$ reagent was prepared in ethanol (0.025 g/L), and ABTS$^{+\bullet}$ was formed by mixing ABTS (7 mM) with potassium persulfate (2.45 mM). After 16 h, the absorbance was adjusted to 0.70. The reaction was carried out with 10 μL of TSE and 990 μL of ABTS$^{+\bullet}$ or DPPH$^{\bullet}$ solution, incubated at room temperature. DPPH$^{\bullet}$ and ABTS$^{+\bullet}$ were reported as percentage of inhibition after 7 and 30 min of incubation, respectively.

2.3 Bi-component film formation

Films were elaborated as previously described by McHugh and Krochta (1994). Whey protein isolate (6.96 g) and glycerol were dissolved in 80 mL of distilled water and homogenized at 12,000 rpm for 1 min (Ultra Turrax IKA-T18, Staufen, Germany). Then, the pH was adjusted using a pH-meter (Hanna HI 3222 pH/ORP/ISE meter and pH glass probe HI 1131, Hanna Ins. Romania) and HCl (0.1M) or NaOH (0.1M) according to Table 1; the solution was placed in a water-bath for 25 min with stirring (400 rpm) at different studied protein denaturation temperatures (PDT). Native potato starch (10.44 g) solutions were dissolved in 20 mL of distilled water at 65 °C and agitated for 3 min (Romero-Bastida *et al.*, 2005), and adjusted at different studied pH values. Both solutions were mixed, and the mixture was called "film-forming solution" (FFS). Thirty milliliters of each FFS were poured on glass surfaces of 225 cm^2 and placed in a cabinet at controlled temperature and moisture (25 ± 0.8 °C and 32 ± 1.0% relative humidity) for 48 h.

2.4 Mechanical properties of edible films

The mechanical properties (percentage of elongation = $\%\varepsilon$, tensile strength before breaking = σ, and Young's modulus = E) of films were evaluated after being conditioned to 50 ± 3% of relative humidity with a $Mg(NO_3)_2$ saturated solution at 20 °C for 24 h. A texture analyzer was used (CT3 Texture Analyzer, and TexturePro v1.2 Build-9-CT software, Brookfield Eng. Lab, Inc., Middleboro, MA, USA) with stress clamps separated at 50 mm, a stretching velocity of 10 mm/min, load capacity of 1 N, and sensitivity of 0.5 N (Perez-Gago & Krochta, 2001). The mechanical properties were obtained directly by the software.

2.5 Films optimization by surface response methodology

Film optimization was based on the variation of the amount of the plasticizer, the pH of the WPI-NPS solution and the temperature for protein denaturation (PDT), factors that can regulate the film network. The glycerol content (Gly) varied from 2.3 to 6.3 g/100 mL solution, PDT from 75 to 95 °C, and pH of the WPI solution from 5 to 9. The methodology included a central rotary design with three factors to generate a surface response in order to obtain a film with a high $\%\varepsilon$, and low values in σ and E, according to McHugh & Krochta (1994), which are properties that characterize synthetic films.

The experimental array included eight combinations from the basic design 2^3, six axial points and the central point (replicated 6 times), obtaining 15 experimental runs (Table 1). The model evaluated was $Y = A + Bx + Cy + Dz + Ex^2 + Fy^2 + Gz^2 + Hxy + Ixz + Jyz$, where capital letters are lineal, quadratic and interactive regression coefficients, and "A" the constant term. Small letters are optimized factors (x = glycerol, y = pH and z = protein denaturalization temperature). Later, films adding with the tamarind seed extract, used the final combination selected from these factors.

2.6 Bi-component films with tamarind seed extract and their characterization

2.6.1 Edible film elaboration

The incorporation of TSE to edible films was studied at two concentrations: 1.26 and 12.6 g/100 mL and a control film (without incorporation of TSE). Tamarind seed extract was added to the FFS of the optimized film (obtained by surface response methodology) after it was cooled at 20 ± 0.8 °C following the procedure described in section "bi-component film formation".

2.6.2 Characterization and stability of film with TSE during storage

The color, mechanical and antioxidant properties of films were measured after 72 h (48 h of film formation plus 24 h of conditioning). Twelve films of each TSE level were used. Samples were rolled to an internal diameter of 1 cm, placed in a sealable bag of 18 × 20 cm, and kept inside a cabinet with controlled temperature and humidity (25 °C and 32% relative humidity [RH]) during 1, 15, 25, and 35 days. At each time, the following determinations were performed:

mechanical properties were measured as described in "Mechanical properties of edible film" section. The color (L*, a* and b*) of films was measured according to Siripatrawan & Harte (2010) with a Minolta colourimeter (Minolta CM-3600d; Tokyo, Japan) at 1.5 cm opening and D-65/2° illumination. The thickness of the films was measured with a digital micrometer (293-811/MDC-1, Mitutoyo, Japan), and the percentage of soluble material (%SM, after solubilizing them in distilled water at 25 °C) was determined by weight differences (Romero-Bastida *et al.*, 2005). The water soluble film fraction (WSF) was separated from the non-solubilized film through filtration; the WSF was stored at -20 °C for AnA determination. The AnA of films was measured in both WSF and FFS by $DPPH^{\bullet}$ and $ABTS^{+\bullet}$ methods. Both assays were adapted as methods to evaluate indirectly AnA of films (Arcan and Yemenicioğlu, 2014).

2.7 Statistical analysis

For optimization, response surface models were analyzed using the software STATGRAPHICS Centurion XV, V.15.2.05 (StatPoint Technologies, Inc., Warrenton, VA, USA). Physical and mechanical properties, color, and AnA data were expressed as the mean ± standard deviation or root of error mean square. Two-way ANOVA and Duncan's multiple comparison were analyzed using the SPSS 10.0 software (SPSS Inc., Chicago, IL, USA). Stability during storage of films was analyzed by a principal component analysis (XLSTAT 2015.4 software, Addinsoft, NY, USA).

3 Results and discussion

3.1 Optimization of WPI-NPS edible films

Figure 1 shows the response surface of each mechanical property (A to F) analyzed on films and the final optimization model (G and H). Few researches perform optimization processes in the films, although it is an effective tool to evaluate their properties (Perez-Gago & Krochta, 2001). Response surface plots are the result of the data presented in Table 1, where the contours represent the area of the response projected on two axes and the squared surface correspond to the three-dimensional response. Table 2 shows the coefficients for each variable's response ($p < 0.05$).

The tensile strength refers to the maximum resistance to stress that a film can withstand while being stretched before its rupture, and depends on the elaboration conditions of the film. Tables 1 and 2 show that σ values ranged from 2.5 to 14.0 MPa and only the quadratic terms of all factors affected the response ($p < 0.001$).

Table 2. Regression coefficients of the response surface model in the elaboration of optimized protein-starch films.

Coefficient	Tensile strength (σ, MPa)			Elongation (ε, %)			Young's modulus (E, MPa)		
	RC	SE	P	RC	SE	P	RC	SE	P
A	-351.99			-616.27			8.61		
Bx	12.09	0.81	N.S.	-32.90	2.98	N.S.	0.32	0.03	N.S.
Cy	22.42	0.81	N.S.	200.48	2.98	0.001	-0.66	0.03	0.001
Dz	6.12	0.81	N.S.	-1.35	2.98	0.001	-0.16	0.03	0.010
Ex^2	-1.48	0.65	0.001	-3.86	2.38	0.001	-	-	-
Fy^2	-1.61	0.65	0.001	-13.43	2.38	0.001	0.06	0.02	0.001
Gz^2	-0.04	0.65	0.010	-	-	-	0.001	0.02	0.030
Hxy	-	-	-	-	-	-	-0.05	0.04	0.020
R^2		0.55			0.81			0.62	

Linear, quadratic and interaction terms are shown for each mechanical property. Capital letters correspond to coefficient regression; and x, y, z, correspond to glycerol, pH and denaturalization temperature effects, respectively. RC = coefficient regression value. SE = standard error. P = probability value. R^2 = Regression coefficient. N.S. = not significant. Absent values (-), the values were not considered by the software.

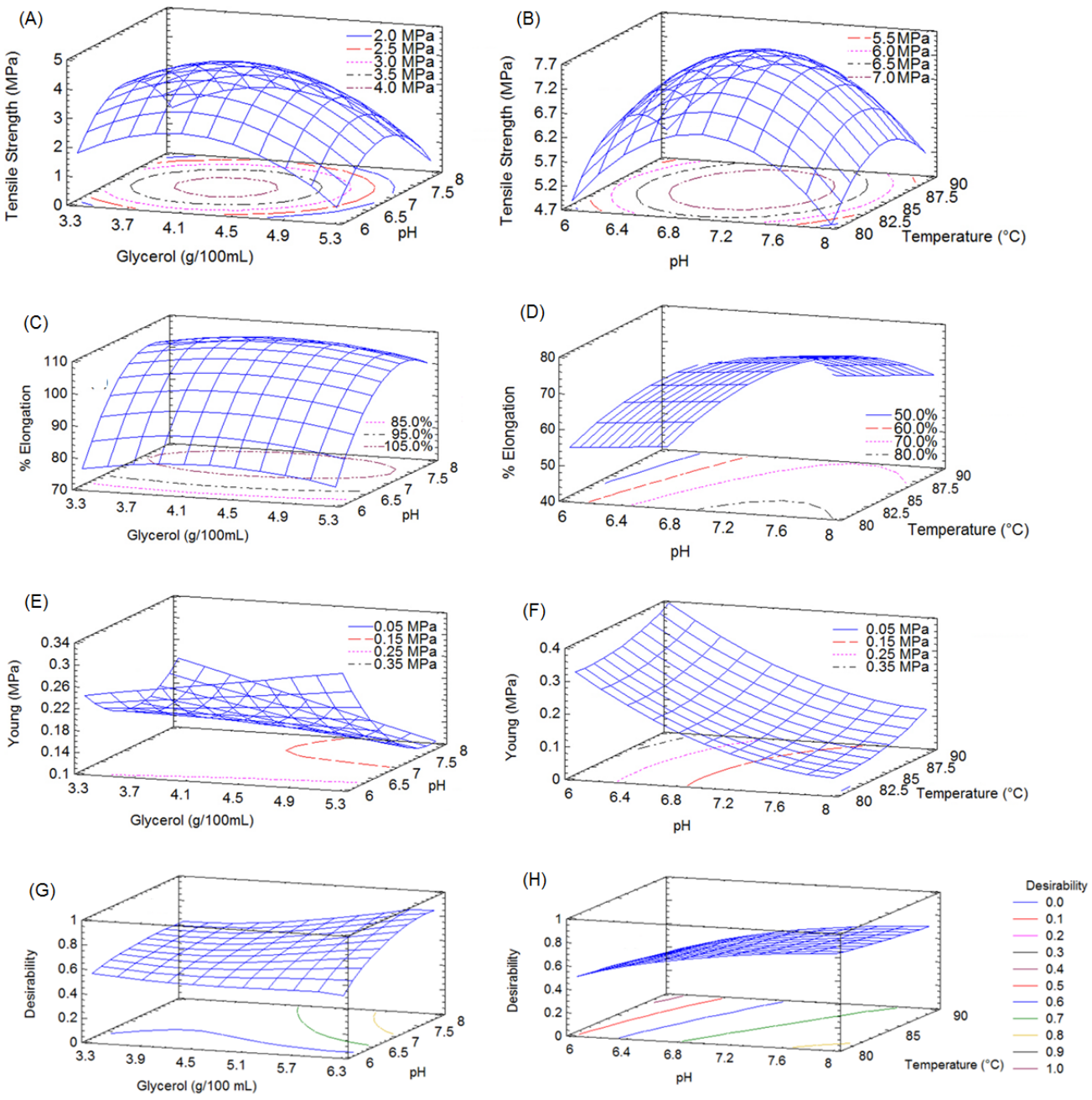

Fig. 1. Response surfaces for: (A) tensile strength at PDT 70 °C; (B) tensile strength at Gly 6.0 g/100 mL of FFS; (C) elongation at PDT 70 °C; (D) elongation at Gly 6.0 g/100 mL of FFS; (E) Young's module at PDT 70 °C; (F) Young's module at Gly 6.0 g/100 mL of FFS; (G) Response surface for optimized conditions in WPI-NPS films at PDT 70 °C and (H) Response surface for optimized conditions in WPI-NPS films at Gly 6.0 g/mL of FFS.

Figure 1A shows the response surface of σ at 70 °C, where the lowest values (< 2.5 MPa) were obtained with Gly values > 4.9 g/100 mL of FFS. But, if Gly is increased at 6.0 g, regardless of pH (6-8) and PDT (80-90 °C) conditions, σ was > 5.0 MPa (Figure 1B). This response was affected by low Gly concentrations that promote protein-protein interactions, thereby reducing the flexibility. The best WPI properties for films were found at pH > 7.0, and similar results have been reported (Anker *et al.*, 2000; McHugh and Krochta, 1994).

Figure 1C and 1D show the response for $\%\varepsilon$, in which the highest value (≈ 105%, as predicted by the model) was observed at 70 °C of PDT in the pH range from 7.0 to 8.0 with different concentrations of Gly (3.3 to 5.3 g/100 mL of FFS). Several reports indicate that when the temperature of the process is > 75 °C, the formation of polymers and copolymers of globular proteins is induced, forming a less resistant complex network (Floris *et al.*, 2008; von Staszewski *et al.*, 2011). This phenomenon was observed for films formed at PDT > 80.0 °C with lower pH values (6.0 to 7.0), which presented elongation values < 80%. Table 2 shows the regression coefficients, where the pH factor affected positively the $\%\varepsilon$, and negatively the PDT in linear terms, whereas quadratic terms were significant for Gly and pH ($p < 0.001$). High pH value favors S-S bonds, but at low values, -SH groups are protonated and lose reactivity, decreasing the elastic properties of films (Shimada and Cheftel, 1988).

Young's modulus (E) is a calculation of the films rigidity, in which high values suggest a better stability of the network (Perez-Gago & Krochta, 2001). In this study, the pH and PDT (Table 2) exerted a linear ($p < 0.01$) and quadratic ($p < 0.03$) effect, aside from the pH × Gly interaction ($p < 0.02$). At 70 °C of PDT, the use of Gly (4.5 to 5.3 g/100 mL of FFS) and pH (7.0 to 8.0) generated low E values (< 0.15 MPa) in the films (Figure 1E). Figure 1F shows the response surface for E at 6.0 g of Gly/100 mL of FFS; where the modulus decreased from 0.35 to 0.05 MPa in function of PDT < 80 °C and high pH values (> 6.8). Similar results were reported by other authors that have developed films with WPI in similar conditions (Anker *et al.*, 2000; Gounga *et al.*, 2007). Films with an acidic pH (5-6) showed deficiencies in their mechanical properties, as suggested by the generation of insoluble complexes that precipitated during the drying process, which may compromise the physical integrity of the films (Anker *et al.*, 2000).

Figures 1G and 1H show the desirability for Gly and pH at a constant PDT of 70 °C; and pH and PDT at a constant Gly value of 6.2 g/100 mL, respectively. The optimal elaboration conditions were 6.2 g/100 mL of Gly in FFS at pH 7.9, and 76 °C of PDT, which predicted the following mechanical values: E = 0.07 MPa, σ = 1.94 MPa and $\%\varepsilon$ = 83.6, with a desirability maximum of 0.86. These conditions were used in the next stage for the incorporation of TSE.

Table 3. Thickness, mechanical properties, soluble matter, and antioxidant activity of WPI-NPS optimized films supplemented with different amounts of TSE.

	Tamarind seed extract (g/100mL of FFS)			
	Control	1.26	12.6	RMSE
Thickness (mm)	0.20 ± 0.012^{a}	0.17 ± 0.012^{b}	0.17 ± 0.012^{b}	0.012
Mechanical properties				
Tensile strength (σ; MPa)	4.69 ± 0.177^{a}	4.41 ± 0.213^{a}	4.04 ± 0.357^{b}	0.261
Elongation (ε; %)	83.04 ± 2.096^{c}	92.56 ± 1.262^{a}	90.31 ± 1.275^{b}	1.593
Young's modulus (E; MPa)	0.04 ± 0.003^{a}	0.03 ± 0.005^{b}	0.02 ± 0.006^{b}	0.005
Soluble material (%SM)	13.48 ± 3.677^{b}	20.72 ± 4.758^{a}	22.90 ± 3.937^{a}	4.150
Antioxidant activity on film forming solution (FFS)				
$DPPH^{\bullet}$, % Inhibition	19.11 ± 1.838^{c}	35.95 ± 2.065^{b}	55.19 ± 2.530^{a}	2.163
$ABTS^{+\bullet}$, % Inhibition	12.81 ± 0.875^{c}	19.30 ± 1.856^{b}	32.67 ± 1.402^{a}	1.435
Antioxidant activity in solution after solubilization of films (WSF)				
$DPPH^{\bullet}$, % inhibition	10.48 ± 1.754^{c}	14.56 ± 1.136^{b}	21.80 ± 2.110^{a}	1.714
$ABTS^{+\bullet}$, % inhibition	9.26 ± 0.575^{c}	11.46 ± 1.163^{b}	15.97 ± 1.375^{a}	1.091

Data are expressed as the mean ± standard deviation (n=6); RMSE = Root mean square error; FFS = film forming solution; WSF = water-soluble film fraction. ^{a,b,c} Different letters within a row indicate significant differences ($p < 0.05$).

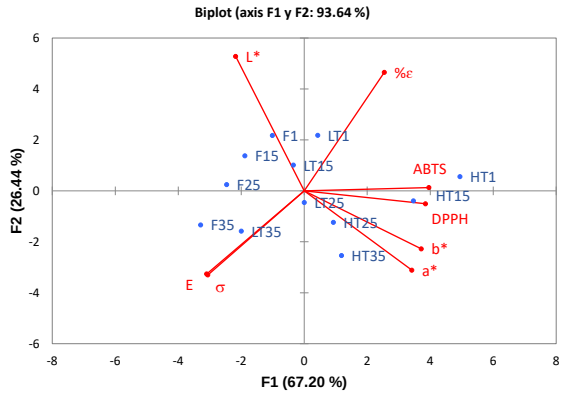

Fig. 2. Principal component analysis of storage data of optimized films with tamarind seed extract (TSE). In red vectors are shown the variable responses: L* = luminosity, a* = blue-red scale, b* = yellow-green scale, $ABTS^{+\bullet}$ and $DPPH^{\bullet}$ activities, E = Young module, σ = tensile strength, and $\%\varepsilon$ = elongation. In blue are the samples during shelf life; the first letter means "F" = optimized film, "LT" = optimized film with 1.26 g/100mL or "HT" = optimized film with 12.6 g/100mL; and the number corresponds to the storage day.

3.2 *Variation of the optimized WPI-NPS films by incorporation of the TSE*

In this section, the predicted mechanical properties of the film (control film) were experimentally verified, and the changes induced on films by the inclusion of the TSE (1.26 and 12.6 g/100 mL FFS) were analyzed and results are shown in Table 3.

3.2.1 *Physical and mechanical properties*

The films without TSE present behaviour according to the variation of the properties predicted by the optimized response surface model. Incorporation of TSE diminished the thickness of films ($p < 0.004$). This difference could be attributed to the interaction between polyphenols and polymers of the film matrix, as there could be a stronger polyphenol-amino acid interaction than that of glycerol-amino acid (Sothornvit and Krochta, 2005). Additionally, when increasing the proportion of WPI, thickness and permeability to oxygen increase (Gounga *et al.*, 2007). It has been demonstrated that thickness depends on the proportion of Gly content because it can compete with water for sites inside WPI polymers (Sothornvit and Krochta, 2005; Gounga *et al.*, 2007). It is possible that polyphenols diminished the water presence in the polymer and increased the interactions among polymers, reflecting a low thickness. In agar films with catechin, thickness decreases with higher amounts of the antioxidant, whereas $\%\varepsilon$ and σ do not differ (Ku *et al.*, 2008). The addition of compounds with -OH groups can act as plasticizers, which is the case for TSE polyphenols. The protein-polyphenol interactions include hydrophobic bonds between aromatic amino acids and polyphenols, as well as hydrogen bridges with protein carbonyl groups (Damodaran *et al.*, 2008; von Staszewski *et al.*, 2011). In this study, σ and E values decreased with the addition of TSE polyphenols ($p < 0.001$) only at high extract concentration. These results had a similar behaviour to that of chitosan films with high levels of green tea extract (5-20%), where σ and $\%\varepsilon$ decrease (Siripatrawan and Harte, 2010). In another study, the incorporation of polyphenols on zein films reduced E values from 4.7 to 0.1 MPa (Arcan and Yemenicioğlu, 2011).

Solubility can be increased by the intensity of the thermal treatment (high temperatures for short times) and a pH close to neutral (Perez-Gago & Krochta, 2001). The time and PDT for this study were considered to be of mild intensity. The addition of TSE increased in 7 to 9 units of %SM ($p < 0.004$), suggesting that more hydrophilic compounds could be released to the medium (Perez-Gago & Krochta, 2001).

3.2.2 *Polyphenols, flavonoids, and antioxidant activity of TSE*

The polyphenol content in TSE was 187.0 ± 0.11 mg equivalents of gallic acid/g of dry extract, and flavonoids content was 30.3 ± 1.01 mg catechin equivalent/g of dry extract. The antioxidant activities of TSE were 62.5 ± 0.04% for $DPPH^{\bullet}$ inhibition and 45.6 ± 0.14% for $ABTS^{+\bullet}$. Several studies have reported polyphenol content of 26.6 ± 13.6 mg GAE/g, $ABTS^{+\bullet}$ inhibition of 0.77 ± 0.01 and $DPPH^{\bullet}$ of 0.98 mmol Trolox/g dw (Razali *et al.*, 2012). Additionally, Siddhuraju (2007) reported the presence of tannins in tamarind seeds.

3.2.3 *Antioxidant activity of films with TSE*

The TSE added to FFS increased the AnA, depending on the concentration ($p < 0.05$): approximately 2.8 × for $DPPH^{\bullet}$ inhibition and 2.5× for $ABTS^{+\bullet}$ inhibition with the highest amount of TSE (Table 3). The control film (without TSE) had AnA because it possesses free

aromatic amino acids from WPI and hydroxyl groups from glycerol. When zein film was incorporated with red ginseng extract, the AnA increased up to 33.6% for DPPH$^{\bullet}$ inhibition (Norajit *et al.*, 2010). Additionally, polyphenols of thyme extract increased the AnA of chitosan and starch films (Talón *et al.*, 2017). The AnA, after solubilizing the film, also increased with the concentration of TSE; this suggests that the polyphenols dispersed in the WSF during the drying process could become oriented due to the polarity towards the surface of the film. After solubilization, a certain number of polyphenols was released from the film. Therefore, the AnA of films applied to food could be sufficient to reduce part of the oxidation reactions at the surface level.

3.3 Films' stability during storage

Integrity of an edible film during storage is essential to maintain its functions. The film materials and the bioactive compounds are involved in the stability during storage (Rodríguez-Marín *et al.*, 2016). Table 4 shows the results of the mechanical properties, color, and AnA of films with or without TSE during storage. These data were also organized on a PCA graph (Figure 2), which correlated the samples according to the time and responses variables at 93.64%.

The films without TSE were mainly characterized based on the luminosity, which was lost during storage. These films lost elongation ($p < 0.05$) and became more brittle. High TSE films were characterized during the first days of storage by their antioxidant response ($p < 0.05$) and on the last days by their red color ($p < 0.05$) and opacity (low luminosity). These two responses (opacity and red color) are very important in food systems to decrease the velocity of oxidation reactions. The magnitude of color change is determined by the origin of the extract and density; changes in transparency (greater opacity) could improve the light barrier (Siripatrawan & Harte, 2010). Polyphenols of TSE could interact with a greater amount of water molecules, fostering a less moist and brilliant surface. The stability of red pigments on films was low at high TSE concentrations, possibly due to darkening reactions between these compounds and the light. Values of b* (yellow shades) also increased with the incorporation of TSE (Table 4).

The films with low levels of TSE presented a similar behavior to that of films without TSE but had higher elongation and AnA responses (Figure 2). The AnA is one of the most important activities to be conserved over time and was representative of films elaborated with TSE. The addition of high concentrations of TSE may help to maintain stability of mechanical properties of films during storage, as they did not present significant changes in $\%\varepsilon$, σ, and E.

Table 4. Storage stability on color, mechanical and antioxidant properties from WPI-PS optimized films supplemented with different amounts of TSE.*

TSE (g/100mL FFS)	Time (days) 1	15	25	35	RMSE	Time (days) 1	15	25	35	RMSE
	Lightness (L)					Red-green scale (a)				
0	96.44±1.32	94.91±0.22	90.71±1.53	85.91±1.64	0.706	-0.79±0.02	-0.72±0.02	-0.66±0.02	-0.62±0.03	0.018
1.26	91.86±1.15	88.57±1.36	85.86±6.48	80.91±1.41		2.40±0.03	2.48±0.03	2.54±1.28	2.60±0.03	
12.26	80.18±2.37	78.36±1.89	76.54±3.59	75.16±2.31		11.43±0.05	12.50±0.04	12.83±1.32	13.22±0.10	
	Blue-yellow scale (b)					Tensile strength (MPa)				
0	3.29±0.06	3.04±0.04	2.82±0.03	2.71±0.05	0.029	4.53±0.16	4.67±0.15	4.82±0.17	4.99±0.16	0.057
1.26	7.43±0.05	7.18±0.06	6.60±1.67	6.49±0.05		4.19±0.19	4.33±0.15	4.54±0.12	4.68±0.20	
12.26	17.43±0.06	17.06±0.06	15.66±1.69	14.88±0.05		4.04±0.05	4.28±0.04	4.47±0.11	4.82±0.09	
	Elongation (%)					Young's modulus†				
0	86.07±1.33	81.16±3.83	72.54±2.46	59.94±1.11	1.780	0.04±0.005	0.06±0.008	0.08±0.015	0.10±0.010	0.006
1.26	97.55±1.51	88.41±4.00	80.62±4.10	66.56±4.82		0.06±0.024	0.08±0.015	0.09±0.012	0.11±0.017	
12.26	93.49±3.81	88.32±2.52	75.47±5.77	72.37±2.31		0.02±0.011	0.04±0.008	0.06±0.031	0.07±0.015	
	DPPH$^{\bullet}$ (%inhibition)					ABTS$^{+\bullet}$ (%inhibition)				
0	11.46±0.46	9.79±0.11	9.35±0.17	9.01±0.13	0.473	8.77±1.23	5.42±0.47	3.90±0.26	2.55±0.42	0.223
1.26	12.17±0.46	11.56±0.53	10.63±2.27	9.25±0.24		10.96±0.49	8.73±0.54	6.48±2.10	5.09±0.19	
12.26	28.72±1.36	22.68±2.57	18.48±2.41	16.45±1.92		24.47±0.60	17.37±0.88	14.79±2.42	13.51±0.38	

Data are expressed as mean ± standard deviation (n=6); and RMSE = Root mean square error. TSE = tamarind seed extract. DPPH$^{\bullet}$ and ABTS$^{+\bullet}$ were determined in WSF (water-soluble film fraction). *Significance per treatment ($p < 0.0027$), time ($p < 0.0001$), and interaction ($p < 0.0007$). † Not differences per interaction ($p > 0.5$)

Conclusions

Polyphenols of the TSE apparently acted as plasticizer within the bi-component WPI-NPS edible films by weakening the protein-polysaccharide interactions with an increase in elongation and reduction of the tensile strength. Addition of this extract not only imparts an antioxidant feature; but enhances the film mechanical properties that remained stable during storage. Therefore, the edible films supplemented with TSE could be used as part of a system to prevent the oxidation reactions in foods due to antioxidant properties, but also as protectors for light incidence due to their opacity. Moreover, it is worth mention that TSE constitutes a low-cost alternative for natural antioxidants derived form an agro-industrial waste.

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Nomenclature

ABTS	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic)
AnA	Antioxidant activity
DPPH	2,2-diphenyl-1-picrylhydrazyl
FFS	Film forming solution
WSF	Water-soluble film fraction
GAE/g	Gallic acid equivalents per gram
Gly	Glycerol
NPS	Native potato starch
PDT	Protein denaturation temperature
TSE	Tamarind seed extract
WPI	Whey protein isolate

Greek symbols

$\%\varepsilon$	Elongation
σ	Tensile strength
E	Young's modulus

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