

Elaboration of edible starch films using CaCO_3 nanoparticles and plasticizers by optimized extrusion-casting process

Elaboración de películas de almidón comestibles, a base de nanopartículas de Carbonato de Calcio y plastificantes mediante la optimización del proceso extrusión-casting

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ABSTRACT

Edible films (EF) can be produced and developed for preservation of fruits and vegetables. Starch has been considered one of the biopolymers with the greatest potential to produce edible films through different processing techniques, such as extrusion-casting. Our objective was to study the effect of calcium carbonate nanoparticles (NPsCC) and plasticizers (sorbitol- glycerol (80-20%; w/w)) produced by extrusion (EXT)-casting (CT) combination technologies, to obtain EF with improved mechanical and barrier properties. Corn starch-plasticizers were processed in a twin-screw extruder to produce modified thermoplastic starch, followed by the addition of calcium carbonate nanoparticles (CCNP) in casting to EF formation. A hybrid design (four factors; twenty-one treatments) of surface response methodology was used to optimization process. The optimal EF mechanical and barrier properties were characterized according to microstructural properties (X-ray diffraction, Scanning Electron Microscopy) to determine the damage caused in the starch during EXT and CT. In conclusion, it is possible to obtain EF with greater both Breaking Strength and Deformation, as well as, lower Water Vapor Permeability and Solubility (mechanical and barrier properties), through the use of CCNP and plasticizers, using EXT-CT combination processes.

Keywords: Starch edible films, CaCO_3 nanoparticles, extrusion, microstructural properties.

RESUMEN

Para la conservación de frutas y hortalizas, se pueden producir y desarrollar películas comestibles (PC). Para producir PC, el almidón es considerado uno de los biopolímeros con mayor potencial mediante diferentes técnicas de procesamiento como la extrusión-casting. El objetivo fue estudiar el efecto de las nanopartículas de carbonato de calcio (NPsCC) y plastificantes [sorbitol-glicerol (80-20%; p/p)] producidas mediante tecnologías combinadas de extrusión (EXT)-casting (CT) para obtener PC con propiedades mecánicas y de barrera mejoradas. La mezcla almidón de maíz-plastificantes fueron procesados en un extrusor de doble tornillo para obtener almidón termoplástico modificado seguido de la adición de NPsCC en el casting para la formación

final de la PC. Se utilizó un diseño híbrido (cuatro factores; veintiún tratamientos) de la metodología de superficie de respuesta para el proceso de optimización. Se caracterizó PC óptima acorde a propiedades mecánicas y de barrera, además, de propiedades microestructurales (difracción de rayos X, Microscopía Electrónica de Barrido) para determinar el daño causado en el almidón durante EXT y CT. En conclusión, es posible obtener PC con mayor resistencia a la rotura y deformación, así como, menor permeabilidad al vapor de agua y solubilidad, mediante el uso de NPsCC y plastificantes, utilizando la combinación EXT-CT.

Palabras clave: películas de almidón, nanopartículas de CaCO_3 , extrusión, casting, propiedades microestructurales.

INTRODUCTION

Starch has been considered one of the biopolymers with the greatest potential to produce biodegradable and edible films by different processing techniques such as casting, injection or blow molding and as well as in combination with others techniques (Teixeira *et al.*, 2012, Chiumarelli y Hubinger, 2014, Li *et al.*, 2015, Calderón-Castro *et al.*, 2018). In this sense, corn starch has many advantages over other starches, and is considered the best of them due to its versatility, high availability, low cost and also, its biodegradability (Aguirre-Joya *et al.*, 2019, Galindez *et al.*, 2019, Kim *et al.*, 2019, Yildirim-Yalcin *et al.*, 2019). Although, it has acceptable physicochemical and good mechanical properties, these could be improved. Calcium carbonate nanoparticles (CCNP) are commonly used since to they add characteristics of greater elongation and resistance in the paper breakage, paint and plastics industry (Hu *et al.*, 2009, Sahebian *et al.*, 2009, Duncan, 2011, Ghadam *et al.*, 2012,), as well as, improved opacity, whiteness and brightness, and printing properties in quality paper (Buri *et al.*, 2012). Sun *et al.* (2014) used calcium carbonate nanoparticles with the purpose to improve the mechanical properties, such as flexibility and strength, of films based on corn starch and glycerol by casting. Latest, the use of nanoparticles could be considered for the production of edible starch-based films with characteristics that may improve the post-harvest preservation of fruits (Maftoonazad y Ramaswamy, 2005). Calderón-Castro *et al.* (2018) evaluated

a combined method using extrusion and casting to modify the starch macromolecular matrix (which is exposed to a variety of changes at both levels, intra and intermolecular) and for Edible Films (EF) formation. Thin layers of edible materials can be applied to the preservation, distribution and marketing of post-harvest products (McHugh, 2000, Falguera *et al.*, 2011, Fakhouri *et al.*, 2015, Buso-Ríos *et al.*, 2020). These materials act as barriers, producing modified atmospheres, reducing moisture exchange, controlling microbial growth, and carrying functional ingredients such as antioxidants and antimicrobials (Treviño-Garza *et al.*, 2015). For this reason, the aim of this work was to use calcium carbonate nanoparticles and plasticizers during extrusion (EXT)-casting (CT) combination technologies to obtain EF with improved mechanical and barrier properties.

MATERIALS AND METHODS

Film preparation

Corn starch (Ingredion, Jalisco, Mexico), sorbitol (Cedrosa, Edo. Mexico, Mexico) and glycerol (JT Baker®, Pa., U.S.A.) as well as CCNP (SkySpring Nanomaterials, Inc., TX, USA) were used for EF preparation. The corn starch composition was: 25.58 % amylose and 74.72 % amylopectin. The film preparation was carried out according to Yan *et al.* (2012) and Fitch-Vargas *et al.* (2016) with some modifications. At the first

stage, with the use of the extrusion technology, thermoplastic starch was obtained. The mixture was prepared with corn starch and plasticizers, according to the experimental design (Table 1) [sorbitol (80 %) and glycerol (20 %)]. A co-rotating twin screw extruder (Shandong Light M&E, Model LT32L, China) with a L/D of 18.5, screw compression ratio of 2:1, and a circular die with 19 mm of length, 42 mm of diameter and an output of 5 mm was used. The feed rate kept constant at 120 g/min, while moisture content ranged at different ratios according to the experimental design. Barrel temperatures in the feeding (60 °C) and transition (75 °C) zones were constant with respect to the output die temperature (E.g. ET: 90 °C); they varied according to the experimental design by 15 °C between them. The 3 heating zones were independently and electrically heated and water-cooled to maintain the temperature. The thermoplastic starch was collected in water at a 1:4 ratio and stored until use. The product obtained was named extruded formulation. In a second stage, EFs elaborated with the casting technique were obtained. First, CCNP were dissolved in 50 mL of distilled water by ultrasonic mixing (Branson Ultrasonic Cleaner, Danbury, CT, EUA). Briefly, 200 mL of extruded formulation were collected and heated for 10 minutes on a plate (Fisher Scientific, Mass., U.S.A.) at 80 °C; when the temperature reached 30 °C, calcium carbonate nanoparticles were added according to the experimental

Table 1. Experimental design of the extrusion process for four factors.

Tabla 1. Diseño experimental del proceso de extrusión para cuatro factores.

Trat*	X_1	X_2	X_3	X_4	CaCO_3	ET (°C)	PC (%)	MC (%)	BS	D	WVP	WS
1	0	0	0	1.7317	1.52	90	20	25.2	13.37	11.42	1.826E-11	29.56
2	0	0	0	-0.2692	1.52	90	20	19.2	22.39	05.26	1.044E-11	25.63
3	-1	-1	-1	0.6045	0.52	80	15	21.81	18.03	07.00	1.465E-11	29.16
4	1	-1	-1	0.6045	2.52	80	15	21.81	12.28	13.40	3.092E-11	43.48
5	-1	1	-1	0.6045	0.52	100	15	21.81	25.15	06.75	1.248E-11	25.99
6	1	1	-1	0.6045	2.52	100	15	21.81	15.44	14.83	2.122E-11	36.13
7	-1	-1	1	0.6045	0.52	80	25	21.81	12.82	5.47	9.529E-12	25.49
8	1	-1	1	0.6045	2.52	80	25	21.81	26.00	4.86	8.710E-12	24.66
9	-1	1	1	0.6045	0.52	100	25	21.81	7.69	12.61	3.390E-11	41.66
10	1	1	1	0.6045	2.52	100	25	21.81	13.23	13.48	1.635E-11	31.94
11	1.5177	0	0	-1.0498	3.03	90	20	16.85	18.60	14.22	2.075E-11	32.48
12	-1.5177	0	0	-1.0498	0	90	20	16.85	24.62	11.29	1.685E-11	29.25
13	0	1.5177	0	-1.0498	1.52	105.177	20	16.85	20.32	5.66	1.238E-11	25.10
14	0	-1.5177	0	-1.0498	1.52	74.823	20	16.85	12.33	10.77	1.570E-11	28.22
15	0	0	1.5177	-1.0498	1.52	90	27.59	16.85	15.65	10.41	2.654E-11	34.85
16	0	0	-1.5177	-1.0498	1.52	90	12.41	16.85	18.08	11.00	2.725E-11	32.54
17	0	0	0	0	1.52	90	20	20	20.99	4.91	1.129E-11	23.97
18	0	0	0	0	1.52	90	20	20	17.37	9.87	1.534E-11	31.57
19	0	0	0	0	1.52	90	20	20	21.50	5.36	1.031E-11	25.26
20	0	0	0	0	1.52	90	20	20	11.13	12.66	2.572E-11	38.73
21	0	0	0	0	1.52	90	20	20	21.30	5.30	1.111E-11	25.51

*The order not corresponding to experimental run

X_1 = CaCO_3 = Calcium Carbonate Nanoparticles X_2 = ET = Extrusion Temperature, X_3 = PC = Plasticizers Content, X_4 = MC = Moisture Content.

design. Subsequently, 25 mL of the gelled formulation were poured spilled out into acrylic molds, and then placed into an oven at 60 °C for 2 h ± 0.25 h. The films thickness were measured using a digital micrometer (Digital Insize, Model 3109-25A, Spain), obtaining values of 53 ± 5.22 µm. Finally, films were conditioned at 53 % of relative humidity with a saturated solution of Mg(NO₃)₂·6H₂O (JT Baker®) and they were maintained in a desiccator until analysis.

Physicochemical characterization

Mechanical properties

Breaking Strength (BS) and Deformation (D) of EF were evaluated with the use of an universal texture analyzer (INSTRON, Model 3342, Mass., U.S.A.) according the ASTM D-5748-95 (ASTM, 1995) methodology, with some modifications and recommendations made by Gontard *et al.* (1993). BS is the maximum force just before the break, and it is measured in Newtons (N), while D is the breakage distance from the contact with the sample until the break, and it is measured in millimeters (mm). Twenty EF samples from each treatment were used for measuring these mechanical properties.

Water Vapor Permeability (WVP)

The WVP of EF was determined using the gravimetric glass method according to McHugh *et al.* (1993). Films were placed with 15 g of calcium chloride on the top of glass containers (JT Baker®, Pa., U.S.A.). After that, the containers were placed into a desiccator (Dry Keeper, Sanplatec Corp., Osaka, Japan) with a saturated solution of sodium chloride to generate 75 % of RH. The weight gain of calcium chloride was registered every 12 hours for 4 days by quintuplicate; these data were used to generate a graph of weight gain versus time. WVP was determined according to the next equation:

$$WVP = \frac{Mp \times E}{A \times t \times \Delta p}$$

Where: *Mp* = moisture absorbed mass (g), *E* = film thickness (m), *A* = exposed film area (m²), *t* = time (s) and *Δp* = partial pressure difference through the film (Pa).

Water Solubility (WS)

The WS was determined according to the methodology reported by Gontard *et al.* (1992), as percentage of disintegrated material, as expressed in the following equation:

$$\% WS = \frac{(w_i - w_f)}{w_i} \times 100$$

Where: % WS = water solubility percent, *W_i* = initial weight of sample and *W_f* = final weight of sample.

Experimental Design

A hybrid design D416_B of the response surface methodology (RSM) with four factors was used (Roquemore, 1976): CaCO₃ nanoparticles (CCNP: 0.00 – 3.03 %), extrusion

temperature (ET, 74.82 – 105.18 °C), plasticizers content (PC: 12.41 – 27.59 %) and moisture content (MC: 16.85 – 25.19 %). All the independent variable levels were selected according to preliminary experiments and technical limitations of the study at five levels was performed. The whole design consisted of 21 experimental points carried out in random order, which included 16 factorial and axial points, 5 center points, the center of the design was used to allow for estimation of a pure error sum of squares (Table I). A second order polynomial model was used to predict the experimental behavior:

$$y_i = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{14}X_1X_4 + b_{23}X_2X_3 + b_{24}X_2X_4 + b_{34}X_3X_4 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{44}X_4^2$$

Where: *y_i* = generic response; *b₁...₄₄* = regression coefficients; *X₁* = CaCO₃ nanoparticles, *X₂* = Extrusion Temperature and *X₃* = Plasticizers Content and *X₄* = Moisture Content. The numerical method was applied as optimization technique, where the main criteria for determining the optimal treatment to produce EF were better BS and D (highest values), and WVP and S (lowest values).

Microstructural characterization

To determine the damage caused in starch and plasticizers mixture with and without extrusion process and EF during casting technique, optimal treatment was characterized according to its microstructural properties (X-ray diffraction and Scanning Electron Microscopy).

X-ray diffraction

Samples of optimal treatment, at the various stages of the process were evaluated (without processing mixture -OWPM-, processing mixture -OPM- and edible film -OEF-). This samples were packed into a glass sample holder with a depth of 0.5 mm and mounted on an X-ray diffractometer (Rigaku Model Last D/Max-2100, Rigaku Denki Co. Ltd., Japan). The diffractograms were obtained as described by Gómez *et al.* (1989) with a sweep angle of Bragg of 4 – 40° over a scale of 2θ with intervals of 0.02, operating at 30 KV and 16 mA, with CuKα radiation and a wavelength λ = 1.5406 Å. Relative crystallinity was calculated using Herman's method. The percentage of relative crystallinity of the starch was measured by separating the crystalline and amorphous areas in the X-ray diffractograms using an Origin 9.0™ software. The percentage of relative crystallinity was calculated as crystalline area/total area × 100 (Aguilar-Palazuelos *et al.*, 2006).

Scanning Electron Microscopy (SEM)

Materials obtained were evaluated with SEM analysis, according to the Rodríguez-Castellanos *et al.* (2015) procedure. Briefly, a powder sample of both, starch and OWPM, were dispersed over the SEM plate and the films were fractured by previously cutting a film piece. Then, they were immersed in liquid nitrogen for 10 seconds, breaked and placed on the copper sample plate holder with 1.2 cm diameter car-

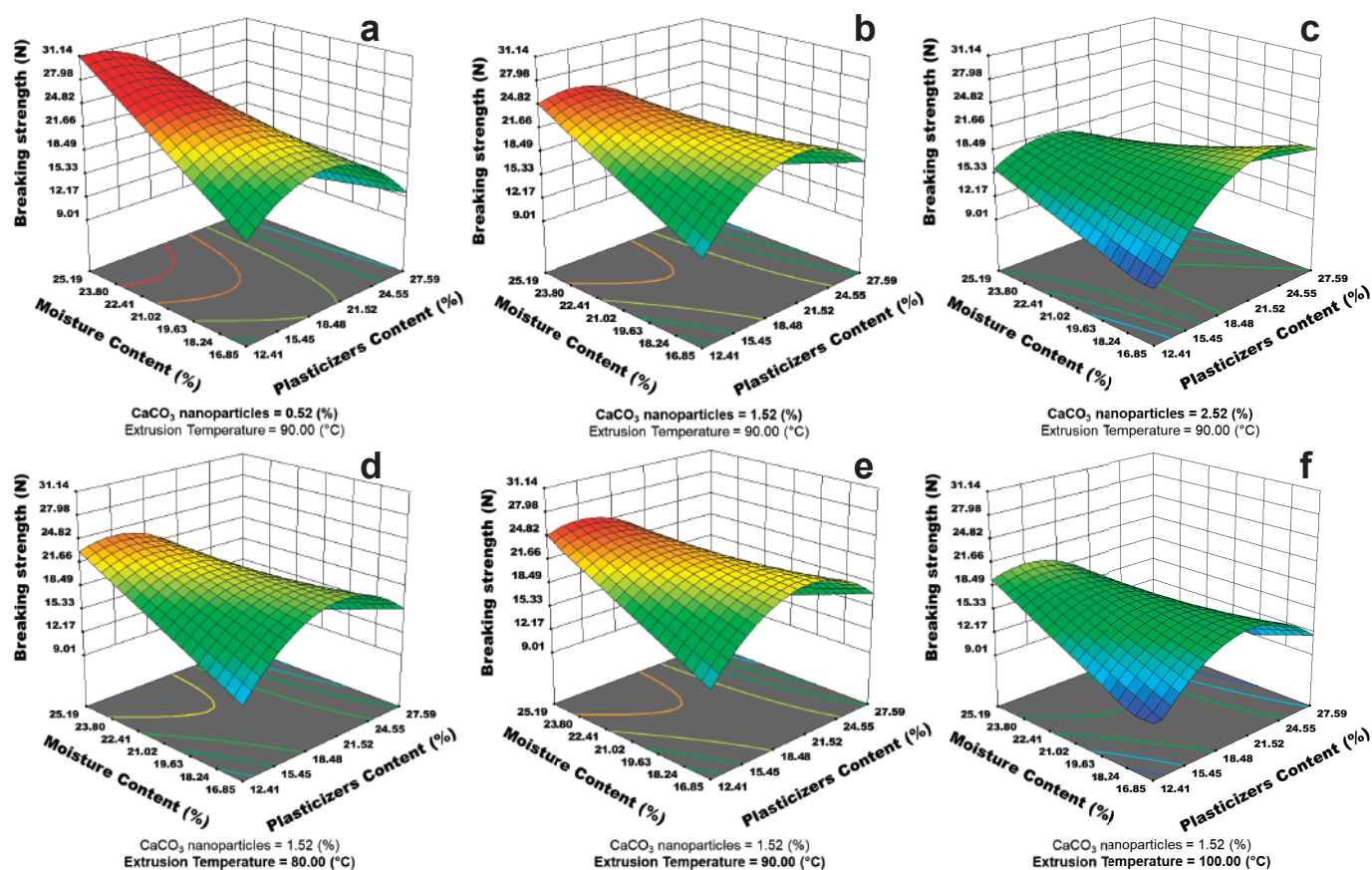


Figure 1. Effects of Calcium Carbonate Nanoparticles (a-c), Extrusion Temperature (d-f), Moisture Content and Plasticizers Content (a_2 , b_2), on Breaking Strength, of edible films of corn starch.

Figura 1. Efecto de las Nanopartículas de Carbonato de Calcio (a-c), la Temperatura de Extrusión (d-f), el Contenido de Humedad y Contenido de Plastificantes (a_2 , b_2), sobre la Resistencia a la Rotura, de películas comestibles de almidón de maíz.

bon adhesive tape. Samples were placed and covered for 10 min with a light gold layer, then submit for posterior analysis. A SEM (Philips®, Model XL30 ESEM, Eindhoven, Netherlands) was used, employing a secondary electron detector with 15 KV of acceleration. CCNP powder samples were placed on a carbon-coated copper grid and characterized in a Scanning Electron Field Emission Microscope (FEI, Nova NanoSEM 200, Shinagawa-ku, Tokio) with 15 kV acceleration and helix detector in low vacuum conditions.

Data Analysis

Quadratic model for predicting the optimal point was expressed according to the response surface methodology (RSM) The significance of the models was tested using variance analysis (F test). The significances of all terms in the polynomial were judged statistically by computing the F-value at a probability ($p \leq$) of 0.001, 0.01 or 0.05. The regression coefficients were then used to make statistical calculations to generate dimensional and contour maps from the regression models. The response surface regression (RSREG) procedure was determined with Design Expert® Software Version 11.0 (Stat-Ease, Inc., Minn., U.S.A.). The microstructural characterization of the optimal treatment was analyzed by LSD Fisher means comparison ($p \leq 0.05$) with StathGraphics Centurion XVIII.

RESULTS AND DISCUSSION

Physicochemical characterization

Breaking Strength (BS)

BS showed a significant model of regression with R^2 values of 0.82, coefficient of variation $CV = 12.10$, and p of $F = 0.0005$ and showing lack of fit (Table 2). Also, the statistical analysis (Table 3) showed the CCNP and ET in their linear terms ($p \leq 0.05$), were the factors that had the most significant effect on the BS. All quadratic terms had a significant effect, except MC. Only CCNP-PC and PC-MC interactions had a significant effect ($p \leq 0.01$).

BS values ranged from 7.69 to 26 N (Table 1), values higher when compared with those reported by Zhong y Li (2014) (7.82 N), using films elaborated of kudzu starch with glycerol (53 % RH) and employing casting technique only. Figure 1(a-c) show the combined effect of PC, MC and CCNP. The BS increased by the effect of MC, as higher MC and BS. MC produced protective effect (gelatinization delaying), resulting in least damaged structures. However, the presence of plasticizers, which are introduced inside the starch chains, probably, generate more flexible and weak structures with lower BS (Gontard *et al.*, 1993), furthermore, the CCNP presence produce a plasticizing effect, though, there is an interaction with the plasticizers generating an increase on

Table 2. Analysis of variance for the responses of BS, D, WVP and WS of edible films of corn starch.

Tabla 2. Análisis de varianza para las respuestas de RR, D, PVA y SA de películas comestibles de almidón de maíz.

Response	R ² Adjusted	CV* (%)	F Value	p of F (model)	Lack of fit
BS	0.82	12.10	10.38	0.0005	0.0141
D	0.98	4.86	93.39	<0.0001	0.070
WVP	0.96	7.59	43.87	<0.0001	0.064
WS	0.98	2.11	112.29	<0.0001	0.487

*CV = Coefficient of Variation

BS= Breaking Strength, D= Deformation, WVP= Water Vapor Permeability, WS= Water Solubility.

Table 3. Regression coefficients of the second-order polynomial equations for the BS, D, WVP and WS responses of edible films of corn starch.

Tabla 3. Coeficientes de regresión de las ecuaciones polinomiales de segundo orden para las respuestas de RR, D, PVA y SA de películas comestibles de almidón de maíz.

		BS	D	WVP	WS
Intercept		22.204	5.4752	11.348E-12	25.191
Lineal	A	-1.7516**	-0.9815***	-00.695E-12	-0.401
	B	-1.9046**	0.9746***	04.088E-12***	2.800***
	C	-1.1072	0.8128***	03.718E-12***	3.020***
	D	0.7037	-0.7593***	00.741E-12	-0.701**
Quadratic	A ²	-1.427*	0.7574***	02.442E-12***	1.289***
	B ²	-3.610***	2.1190***	04.947E-12***	4.160***
	C ²	-2.735***	1.2205***	01.635E-12**	1.695***
	D ²	-	2.3731***	01.399E-12**	1.756***
Interactions	A*B	-	-	-02.212E-12**	-1.532***
	A*C	2.0770**	-0.2716	02.742E-12***	0.870**
	A*D	-1.4907	1.3304***	01.251E-12*	0.661*
	B*C	-	-0.9084***	-00.589E-12	1.264***
	B*D	-	1.6111***	02.482E-12***	1.849***
	C*D	-2.3546**	1.9093***	03.861E-12***	2.991***

* Significant at $\alpha \leq 0.05$ level.

** Significant at $\alpha \leq 0.01$ level.

*** Significant at $\alpha \leq 0.001$ level

A= CaCO₃= Calcium Carbonate Nanoparticles, B= ET= Extrusion Temperature, C= PC= Plasticizers Content, D= MC= Moisture Content.

BS values, especially when the CCNP were higher. The effect of the plasticizers, agrees with Arvanitoyannis *et al.* (1996), who tested different plasticizers in starch-based films, and found that the BS decreased with higher plasticizers. On the other hand, Sun *et al.* (2014) made corn starch edible films with glycerol adding calcium carbonate nanoparticles. They reported that an increment in calcium carbonate nanoparticles concentration, was directly related to an increment in BS.

Figure 1(d-f) shows the temperature effect over BS. BS increased when temperature was ~ 90 °C, nevertheless, increment in temperature to ~ 100 °C, caused a decrease in the BS. These changes in BS may be due to the following: at low temperature could be present some amorphous areas (starch areas without gelatinized), generating less stiffness and causing weak film formation. On the other hand, with intermediate temperatures (~ 90 °C), structures are opened good enough to have an interaction with the components producing a low resistance; though, high temperatures increase the percent of gelatinized starch, being possible that the structure shows damage resulting in a BS decrease (Bhattacharya y Hanna, 1987). Yan *et al.* (2012) mentioned that high temperature and strong shear promoted the plasticizing efficiency and can cause the BS decrease.

Deformation (D)

The statistical analysis of the D data for EF showed a significant regression model ($R^2 = 0.98$, $CV = 4.86$, p of $F < 0.0001$) and did not show lack-of-fit ($p \geq 0.07$) (Table 2). All linear and quadratic terms had a significant effect ($p \leq 0.001$) (Table 3), as well as all interactions terms ($p \leq 0.001$), with the exception of CCNP-ET and CCNP-PC. The D values in the present study ranged from 4.86 to 14.83 mm (Table 1), values slightly high than those reported by Zhong y Li (2014), who reported a value of 4.11 ± 0.02 mm in kudzu starch edible films. Also, compared with the amilomaize EF values (11.68 mm) of Calderón-Castro *et al.* (2018), are slightly higher. Figure 2 (a-c) shows PC, MC and CCNP combined effect, in which can be observed that at lowest MC values, a more rigid and less deformable EF were obtained. A low D value could kept structures without damage. On the other hand, the presence of plasticizers induced the formation of more flexible films, generating an increase in D and a decrease in BS. This effect is avoided by adding CCNP, as reported by Sun *et al.* (2014). Furthermore, high MC and PC values, showed an increment on D. This highest effect may be due to the interaction between the 3 components (MC-PC-CCNP), since, all these variables are having a plasticizing effect under these conditions.

Figure 2(d-f) shows the temperature effect on D. An increase in D was observed, perhaps caused by damaged structures. This damage produced fewer rigid structures and generated greater deformation. However, a low MC and an increased in PC, caused a decrease on D. This effect could be attributed to the increment in PC and temperature, which could generate structures that are physically weaker and therefore less deformable. These results are different than those reported by Calderón-Castro *et al.* (2018), who found no significant effect of extrusion temperature on deformation.

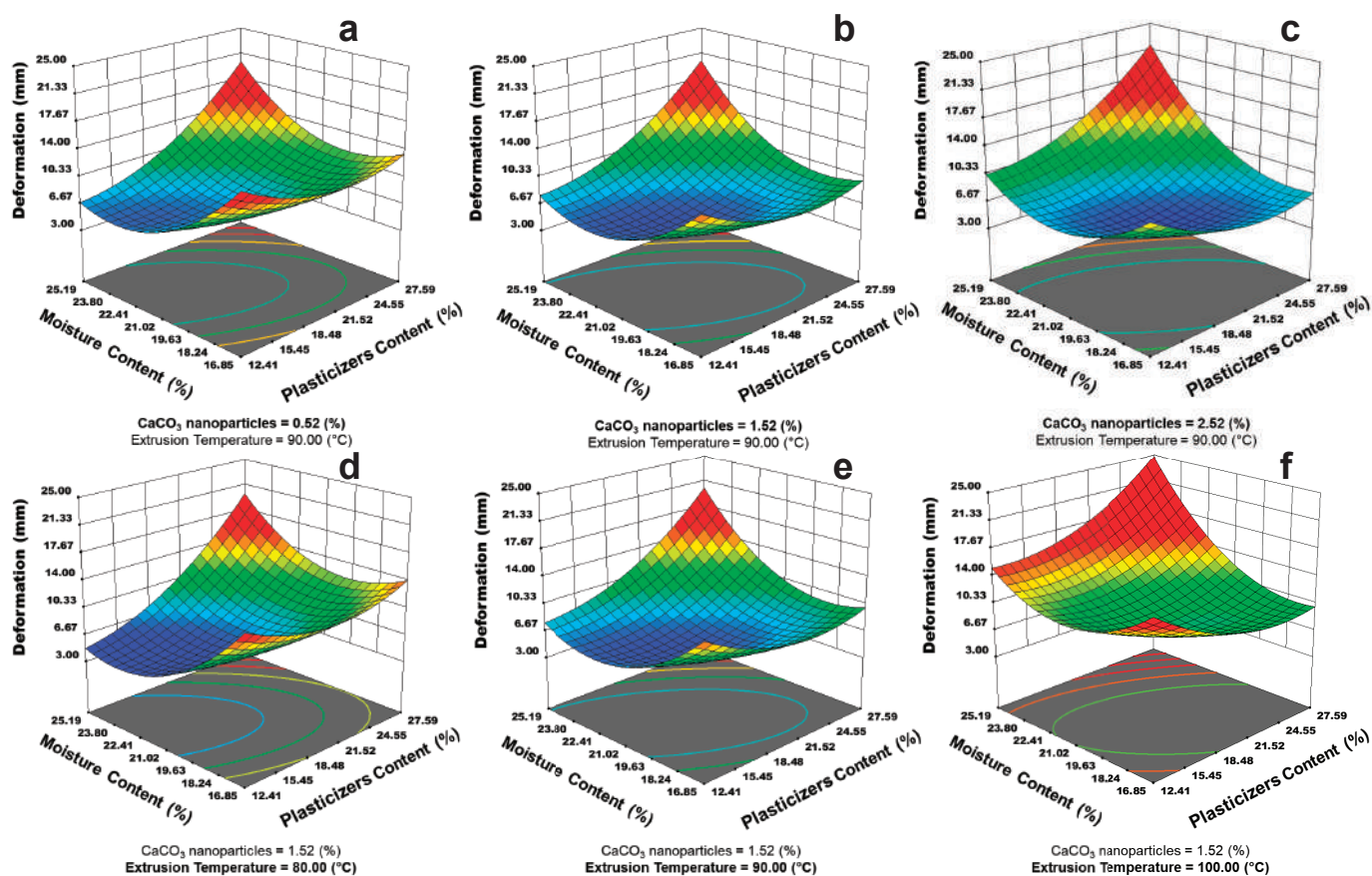


Figure 2. Effects of Calcium Carbonate Nanoparticles (a-c), Extrusion Temperature (d-f), Moisture Content and Plasticizers Content (a2; b2), on Deformation of edible films of corn starch.

Figura 2. Efecto de las Nanopartículas de Carbonato de Calcio (a-c), la Temperatura de Extrusión (d-f), el Contenido de Humedad y Contenido de Plastificantes (a_2 ; b_2), sobre la Deformación de películas comestibles de almidón de maíz.

Water Vapor Permeability (WVP)

The statistical analysis of WVP data for EF showed a significant regression model (RSM) $R^2 = 0.96$, $CV = 7.59$ and p of $F < 0.0001$, and did not show lack-of-fit ($p \geq 0.064$) (Table 2). The regression coefficients of the model show (Table 3) that only linear terms of CCNP and PC had significant effect ($p \leq 0.001$) as well as all quadratic terms ($p \leq 0.001$); regarding interactions terms all had significant effect ($p \leq 0.001$), except CCNP-MC ($p \leq 0.01$) and ET-PC (no significant). The WVP values in the present study range from 0.87 to $3.39 \times 10^{-11} \text{ g m Pa}^{-1} \text{ s}^{-1} \text{ m}^{-2}$ (Table 1), which are smaller than those reported by Younis y Zhao (2019), who reported minimum values at $3.3 \times 10^{-10} \text{ g m Pa}^{-1} \text{ s}^{-1} \text{ m}^{-2}$, in films elaborated with pectin and chitosan by the casting method. Figure 3(a-c) shows the effect of PC, MC and CCNP, on the WVP response. It was mentioned before that, MC has a protective effect during the extrusion process, and it was observed that when MC is increased, it produces structures in the starch matrix which are more integrated and compact, reducing the WVP. Likewise, the presence of CCNP decreases the WVP, which could be due to the CCNP-generated polymer structure, more compact and therefore less permeable, and this is observed especially at low plasticizer content. Moreover, the incorporation of plasticizers, causes the opening of the polymer chains structure

generating an increase in the WVP. Dash *et al.* (2019) evaluated the effect of titanium dioxide (TiO_2) nanoparticles (0.5 % - 4 %) on different properties of sweet potato starch and lemon pectin-based EF. They found that WVP diminish with TiO_2 nanoparticles increased.

Figure 3(d-f) shows the temperature effect. An increment in temperature produce an increase of WVP. This could be due to a major gelatinization producing molecular disruption of starch chains, increasing the presence of short chains of starch structures, with higher water affinity. These results agrees with Fitch-Vargas *et al.* (2016) who reported changes in macromolecular matrix with the temperature and therefore an increase in WVP.

Wang *et al.* (2019) prepared corn starch bio-active EF packaging based on zein incorporated with orange-peel oil; they found WVP values of 2.15 to $6.62 \times 10^{-11} \text{ g m Pa}^{-1} \text{ s}^{-1} \text{ m}^{-2}$ (0.122 to 154 mm thickness film), similar to this study, with a difference in film thickness ($0.053 \pm 0.005 \text{ mm}$). Although permeability is independent of thickness, the permeance is related with the performance of the film when used as packaging materials is dependent of thickness (Buso-Ríos *et al.*, 2020), additionally, in case of EF marketed, less materials for their elaboration will be use.

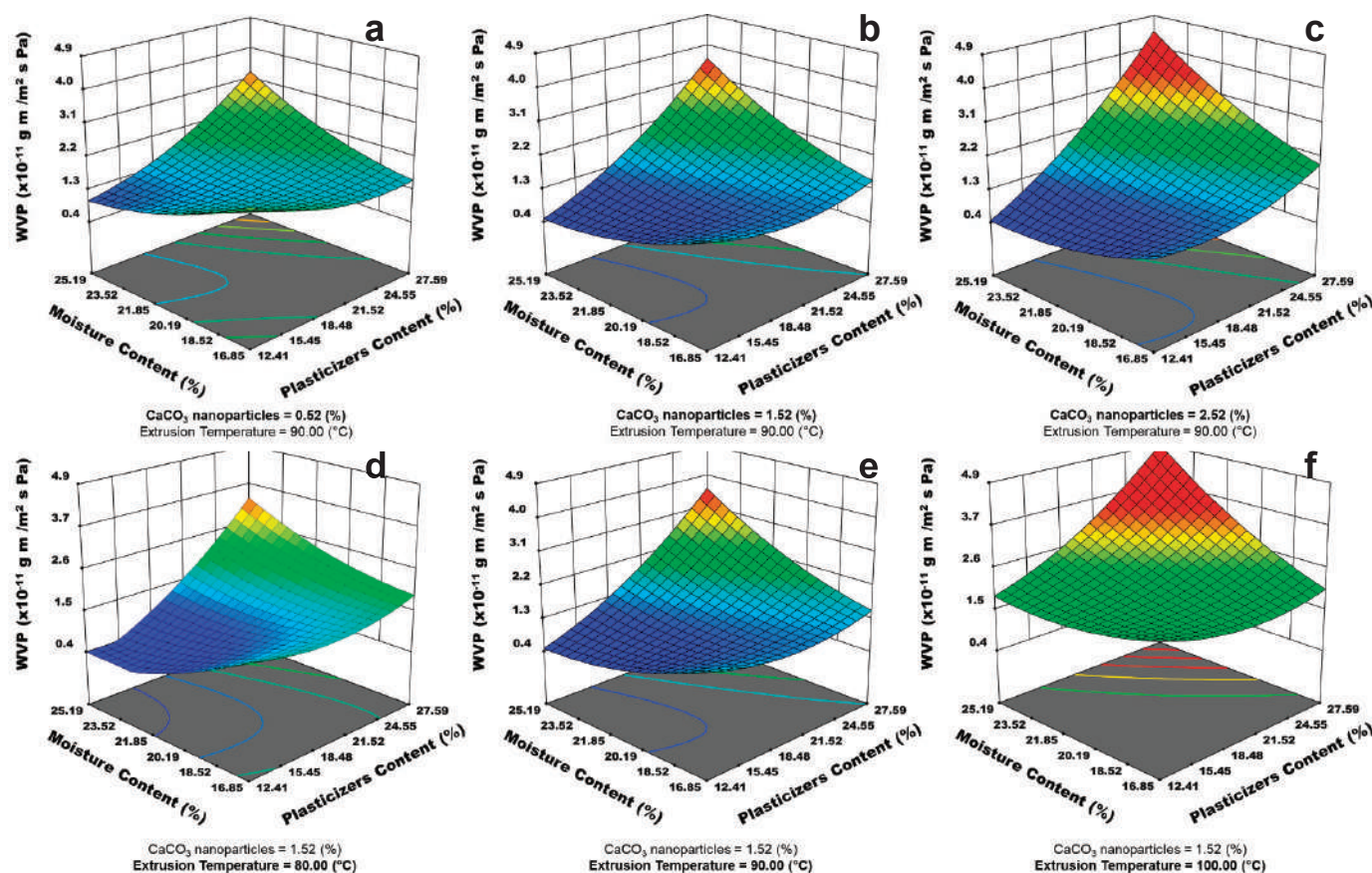


Figure 3. Effects of Calcium Carbonate Nanoparticles (a-c), Extrusion Temperature (d-f), Moisture Content and Plasticizers Content (a2; b2), on Water Vapor Permeability (WVP) of edible films of corn starch.

Figura 3. Efecto de las Nanopartículas de Carbonato de Calcio (a-c), la Temperatura de Extrusión (d-f), el Contenido de Humedad y el Contenido de Plastificantes (a2; b2), sobre la Permeabilidad al Vapor de Agua (PVA) de películas comestibles de almidón de maíz.

Water Solubility (WS)

WS showed a significant model of regression ($R^2 = 0.98$, $CV = 2.11$, and p of $F < 0.0001$) and did not show lack-of-fit ($p \geq 0.487$) (Table 2). The regression coefficients of the model show (Table 3) that all linear terms had significant effect ($p \leq 0.001$) except CCNP (no significant), all quadratic terms had significant effect ($p \leq 0.001$), as did all interactions terms ($p \leq 0.05$). The WS values in the present study range from 23.97 to 43.48 % (Table 1), which are smaller than those reported by Rostami y Esfahani (2019), who made EF with *Melissa officinalis* seed gum/Montmorillonite (5 %) / curcumin. They mentioned values higher than 45.39 % of WS and they were alike those reported by Mehryar y Han (2004) for rice (32.0 % soluble matter) and pea (44.4 % soluble matter) starches.

Figure 4 (a-c) shows the PC, MC and CCNP effect on WS. Higher WS values are related to high MC values, which could be due to the presence of more integral and compact structures, resulting in lower WS values. However, these values show an increase with the PC increment. The incorporation of these plasticizers could be generating structures more susceptible to solubilization, although, the presence of CCNP caused a decrease in solubility at low PC and MC. Similar results were reported by Dash *et al.* (2019), where they estimated WS between 24.27 to 20.92 % in edible films with TiO_2 nanoparticle added.

Figure 4 (d-f) shows the temperature effect on WS. In general, an increase in temperature produces higher solubility values, especially at high PC. This behavior agrees with that reported by Mercier y Feillet (1975) and Calderón-Castro *et al.* (2018).

Numerical Optimization

The best extrusion conditions were determined using the numerical optimization. The aim was to determine the best conditions of ET, CCNP, PC and MC to obtain EF with the highest BS and D values, as well as lowest WVP and WS values. According to the optimization results, the best process conditions were: CCNP = 0.77 %; ET = 84.23 °C, PC = 21.18 % and MC = 17 %. The following predicted values, corresponding to mathematical models, were obtained: BS = 20.56 N, D = 11.82 mm, WVP = $1.42 \times 10^{-11} \text{ g m Pa}^{-1} \text{ s}^{-1} \text{ m}^{-2}$ and WS = 29.12 %.

The EF produced with optimal conditions was physicochemically characterized, having the following results: BS = $19.40 \pm 1.21 \text{ N}$, D = $6.98 \pm 0.73 \text{ mm}$, WVP = $1.77 \times 10^{-11} \pm 8.6 \times 10^{-13} \text{ g m Pa}^{-1} \text{ s}^{-1} \text{ m}^{-2}$ and WS = $23.44 \pm 1.12 \%$; observing that experimental values to BS and WVP did not showed a significant difference ($P < 0.05$) regarding to the predicted values with the mathematical models. However, D and WS values were lower than the predicted values of the model used experimentally. The results found in the optimal EF,

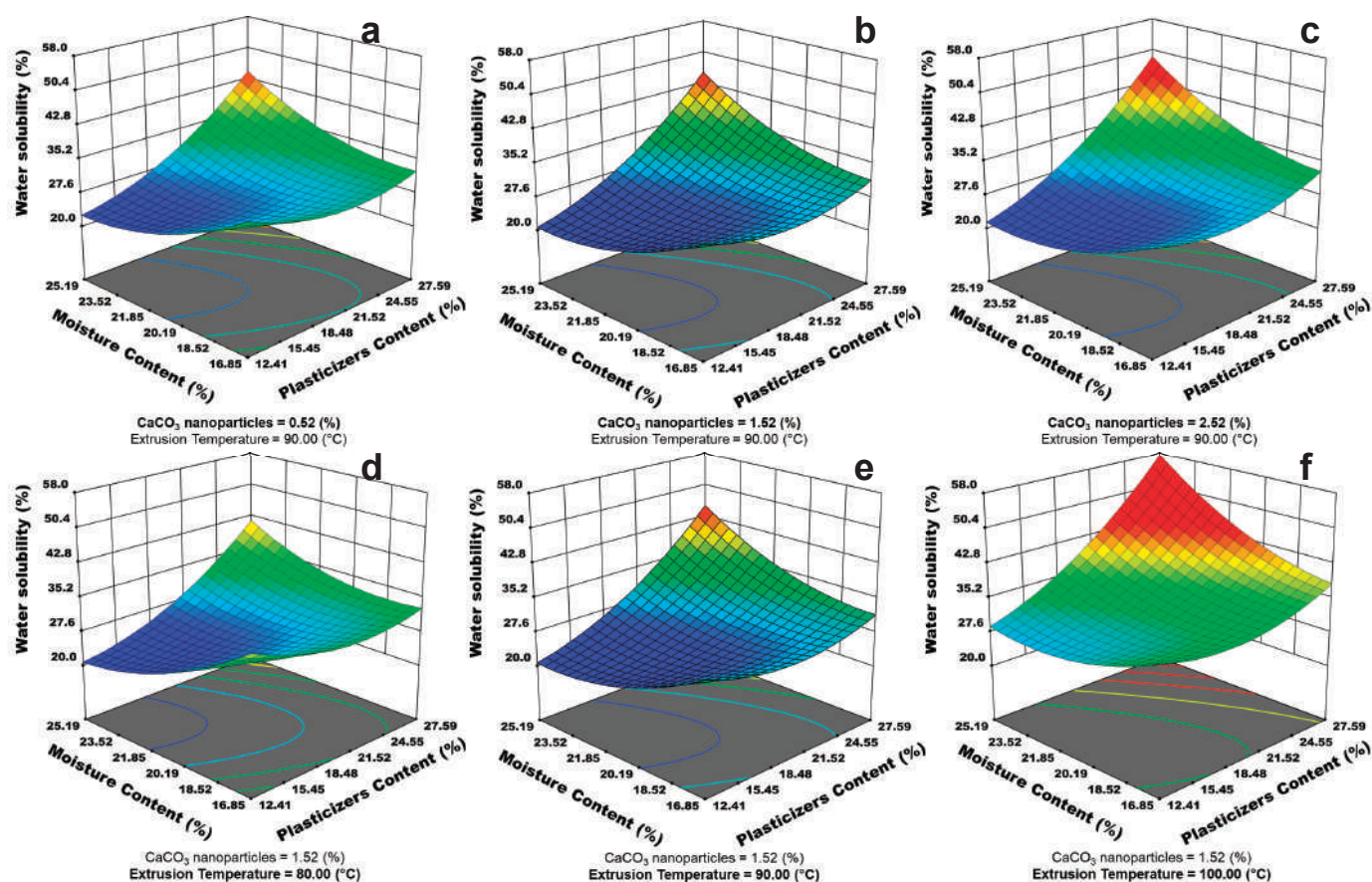


Figure 4. Effects of Calcium Carbonate Nanoparticles (a-c), Extrusion Temperature (d-f), Moisture Content and Plasticizers Content (a2; b2), on Water Solubility (WS) of edible films of corn starch.

Figura 4. Efecto de las Nanopartículas de Carbonato de Calcio (a-c), la Temperatura de Extrusión (d-f), el Contenido de Humedad y el Contenido de Plastificantes (a2; b2), sobre la Solubilidad en Agua (SA) de las películas comestibles de almidón de maíz.

showed to have a good fit with the model, finding the best conditions of extrusion process and CCNP, ET, PC and MC in the manufacture of EF with good mechanical and barrier properties.

Microstructural Analysis

X-Ray Diffraction

The loss effect of organized crystalline structure by extrusion process and casting technique on X-ray diffraction patterns is shown in Figure 5A. The Optimum Without Processing Mixture (OWPM) and Optimized Processing Mixture (OPM), showed a diffraction pattern type A, which is characteristic of cereals (predominant corn starch in mixture), and both, OWPM and OPM, with two main similar peaks, of $\approx 17.7^\circ$ and $\approx 22.8^\circ$ values 2θ , respectively. These results agree with those reported by Koo *et al.* (2010), who reported values 2θ of $\approx 17.18^\circ$ and $\approx 22.73^\circ$; also Guimarães *et al.* (2010) reported values 2θ of $\approx 17.7^\circ$ and $\approx 23.3^\circ$, all of them for corn starch. The patterns of OWPM and OPM were similar between them, meaning that the damage in the corn starch was low during the extrusion process. Finally, both OWPM and OPM produced a lightly fragmentation and gelatinization of the starch granules due to the interaction of ET, PC and MC. Oliveira *et al.* (2017) found changes in diffraction patterns caused by extrusion process from A-type in unprocessed, to

V-type in processed materials. Nevertheless, Optimized Edible Film (OEF) showed a V-type pattern with a main peak at 2θ of $\approx 20.7^\circ$, appearing an additional small peak at $2\theta \approx 30.4^\circ$ which can be related to the presence of calcium carbonate nanoparticles as described by Takabait *et al.* (2016). Zhong y Li (2014) reported that the peak 2θ at $\approx 20^\circ$ correspond to the single helical crystal structure of V-type pattern, and it could indicate the formation of amylose – glycerol complexes during processing.

Changes in X ray diffraction patterns affect relative crystallinity in materials processed. Figure 5B shows the relative crystallinity values of the materials studied. OWPM crystallinity was higher than the crystallinity of OPM, showing that extrusion process and plasticizers contributed lightly to starch granules fragmentation, reducing crystalline regions. Figure (5B) also shows that the OEF registered the lowest crystallinity value, which could be due to the casting technique, where starches gelatinization is complete, considerably reducing the crystalline regions. Furthermore, addition of plasticizers contributed to crystallinity decrease. Oliveira *et al.* (2017) studied the effect of temperature, feed moisture, and whole grain wheat flour on extrusion process severity and degree of starch transformation; they found that during extrusion, loss of crystallinity is caused by mechanical disruption of the molecular bonds within the extruder, due to the

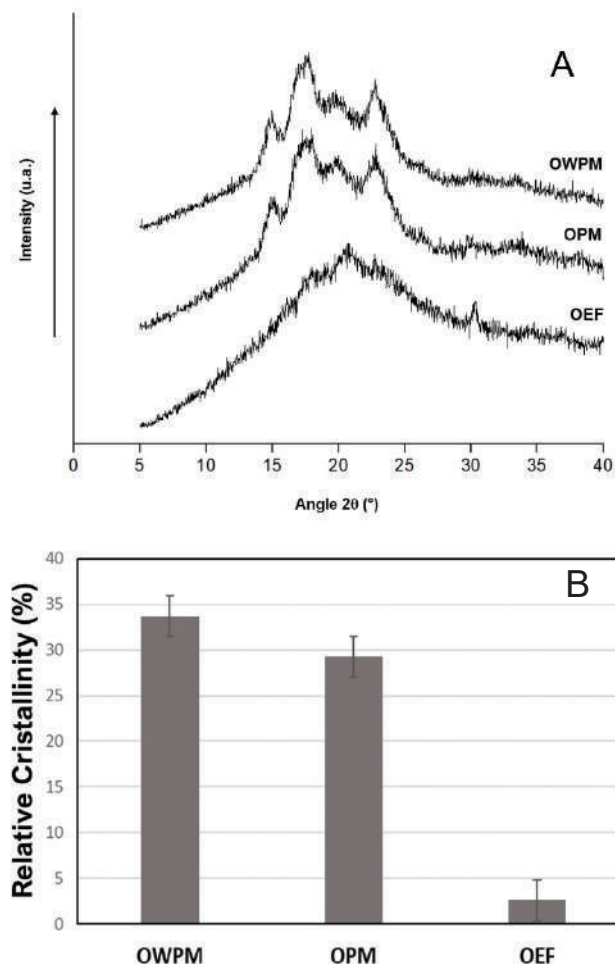


Figure 5. (A) X-ray diffractograms and (B) Relative crystallinity of Optimized Without Processing Mixture of Optimized Without Processing Mixture (OWPM), Optimized Processing Mixture (OPM) and Optimized Edible Film (OEF). Vertical bars indicate LSD= 4.48 ($p \leq 0.05$).

Figura 5. (A) Difractogramas de rayos X y (B) Cristalinidad Relativa de la Mezcla Optimizada sin Procesamiento (MOsP), Mezcla Optimizada Procesada (MOP) y Película Comestible Optimizada (PCO). Las barras verticales indican LSD= 4.48 ($p \leq 0.05$).

intense shear fields. Also, they found relative crystallinities around 25-34 % in raw materials, while processed material was 16-23 %. These results are consistent with those found in the present work.

Scanning Electron Microscopy

Figure 6A shows the CCNP. It is possible to appreciate spherical and semispherical forms with sizes of 26-99 nm. Figure 6B shows the OWPM microstructure. Corn starch granules shows spherical and polygonal shapes and diameters between 5 and 25 μm , similar to those reported by Koo *et al.* (2010) and Limón-Valenzuela *et al.* (2017). The image (6B) shows that the main component of the film forming mixture, corn starch, had not changes on its native composition when it was combined with a mixture of plasticizers; nevertheless, it is observed that the starch granules are agglomerated, which could be attributed to the plasticizer's presence.

Figure 6C shows the extrusion process effect on OPM sample. Starch granules presented an irregular shape, which are semi-open and semimolten; which may be a consequence of the processing effect. Likewise, starch agglomeration was observed indicating that some starch granules were intact after processing. This result provide that the conditions used (ET, PC and MC) in the extrusion process, could be generated by partial breaking of starch granules which allowed its fragmentation and pregelatinizing, maintaining its thermoplastic ability for future application in film forma-

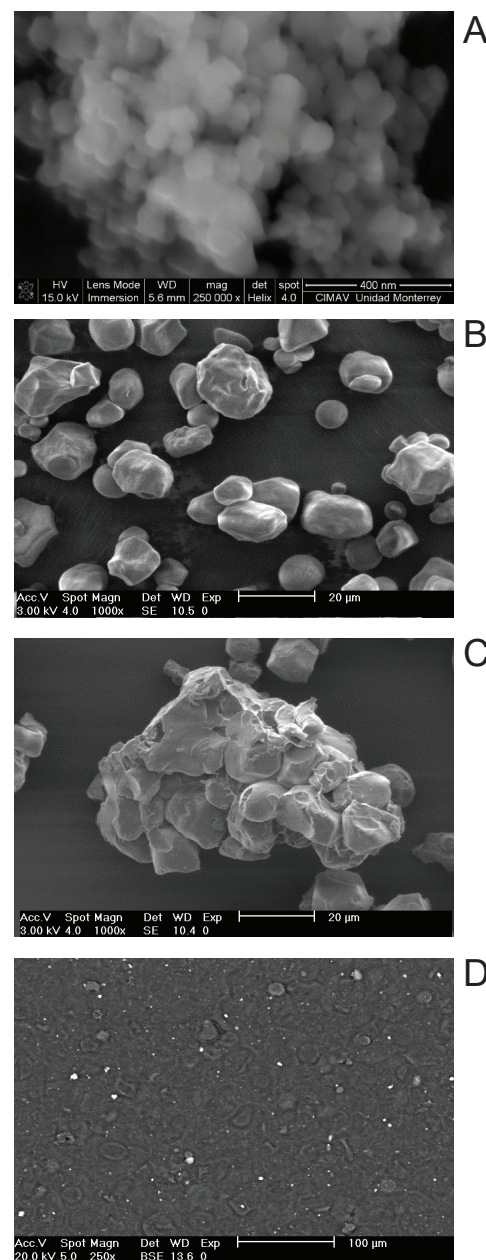


Figure 6. Scanning electron micrographs of: (A) CaCO₃ nanoparticles (B) Optimized Without Processing Mixture (OWPM), (C) Optimized Processing Mixture (OPM) and (D) Optimized Edible Film (OEF).

Figura 6. Microfotografías electrónicas de barrido de: (A) Nanopartículas de CaCO₃ (B) Mezcla Optimizada sin Procesamiento (MOsP), (C) Mezcla Optimizada Procesada (MOP) y (D) Película Comestible Optimizada (PCO).

tion. Aguilar-Palazuelos *et al.* (2007) observed that fragmentation of the granular structure and partial plasticization of the material took place during the preparation of corn starch, fiber and glycerol pellets employing the extrusion process; although, the pellets were suitable for its posterior processing by injection molding. Figure 6D shows the OEF. It is possible to observe an irregular surface with the presence of circular and semicircular forms, which are, probably, gelatinized starch granules, which were not destroyed by casting technique, probably it induces the decrement in WS values. Furthermore, it could be appreciated the presence of disperse white flashes with lower intensity, which are the calcium from CCNP embedded on this film, these brilliant points are absent in films without CCNP (data not shown).

CONCLUSIONS

Calcium carbonate nanoparticles improved the mechanical and barrier properties of EF, elaborated with extrusion-casting technologies, reinforcing, and plasticizing materials, which were enhanced by the presence of moisture content. The optimal combination allowed us to obtain edible films of corn starch with a mixture of plasticizers with greater BS and D as well as lower WVP and WS values, which could provide light fragmentation, damage and gelatinization of the starch granules, due to the interaction of ET, PC and MC, which were shown with X ray diffraction patterns and relative crystallinity changes in materials OWPM, OPM and OEF. SEM allowed to identify the size and shape of both nanoparticles and processed materials and films.

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REFERENCES

- Aguilar-Palazuelos, E., Martínez-Bustos, F., Jiménez-Arévalo, O. A., Galicia-García, T. y Delgado-Rangel, J. 2007. Potentiality of some natural fibres and native starch for making biodegradable materials. In: KLENING, T. P. (ed.) *Food Engineering Research Developments*. Nova Science Publishers, Inc.
- Aguilar-Palazuelos, E., Zazueta-Morales, J. d. J. y Martínez-Bustos, F. 2006. Preparation of high-quality protein-based extruded pellets expanded by microwave oven. *Cereal Chemistry*, 83(4): 363-369.
- Aguirre-Joya, J. A., Cerqueira, M. A., Ventura-Sobrevilla, J., Aguilar-Gonzalez, M. A., Carbó-Argibay, E., Castro, L. P. y Aguilar, C. N. 2019. Candelilla Wax-Based Coatings and Films: Functional and Physicochemical Characterization. *Food and Bioprocess Technology*, 12(10): 1787-1797.
- Arvanitoyannis, I., Psomiadou, E. y Nakayama, A. 1996. Edible films made from sodium caseinate, starches, sugars or glycerol. Part 1. *Carbohydrate Polymers*, 31(4): 179-192.
- ASTM, A. S. F. T. A. M. 1995. Standard Test Method for Protrusion Puncture Resistance of Stretch Wrap Film. Vol 14.02 West Conshohocken, PA: ASTM International.
- Bhattacharya, M. y Hanna, M. 1987. Kinetics of starch gelatinization during extrusion cooking. *Journal of Food science*, 52(3): 764-766.
- Buri, M., Gane, P. A. y Blum, R. V. 2012. Composites of inorganic and/or organic microparticles and nano-calcium carbonate particles. Google Patents.
- Buso-Ríos, O. I., Velázquez, G., Járuquín-Enríquez, L. y Flores-Marín, N. L. 2020. Efecto de la concentración de almidón y aceite esencial de clavo en las propiedades fisicoquímicas de las películas biodegradables. *Revista Mexicana de Ingeniería Química*, 19(3): 1315-1326.
- Calderón-Castro, A., Vega-García, M. O., de Jesús Zazueta-Morales, J., Fitch-Vargas, P. R., Carrillo-López, A., Gutiérrez-Dorado, R., Limón-Valenzuela, V. y Aguilar-Palazuelos, E. 2018. Effect of extrusion process on the functional properties of high amylose corn starch edible films and its application in mango (*Mangifera indica* L.) cv. Tommy Atkins. *Journal of Food Science and Technology*, 55(3): 905-914.
- Chiumarelli, M. y Hubinger, M. D. 2014. Evaluation of edible films and coatings formulated with cassava starch, glycerol, carnauba wax and stearic acid. *Food Hydrocolloids*, 38: 20-27.
- Dash, K. K., Ali, N. A., Das, D. y Mohanta, D. 2019. Thorough evaluation of sweet potato starch and lemon-waste pectin based-edible films with nano-titania inclusions for food packaging applications. *International Journal of Biological Macromolecules*, 139: 449-458.
- Duncan, T. V. 2011. Applications of nanotechnology in food packaging and food safety: barrier materials, antimicrobials and sensors. *Journal of colloid and interface science*, 363(1): 1-24.
- Fakhouri, F. M., Martelli, S. M., Caon, T., Velasco, J. I. y Mei, L. H. I. 2015. Edible films and coatings based on starch/gelatin: Film properties and effect of coatings on quality of refrigerated Red Crimson grapes. *Postharvest Biology and Technology*, 109: 57-64.
- Falguera, V., Quintero, J. P., Jiménez, A., Muñoz, J. A. y Ibarz, A. 2011. Edible films and coatings: Structures, active functions and trends in their use. *Trends in Food Science & Technology*, 22(6): 292-303.
- Fitch-Vargas, P. R., Aguilar-Palazuelos, E., de Jesús Zazueta-Morales, J., Vega-García, M. O., Valdez-Morales, J. E., Martínez-Bustos, F. y Jacobo-Valenzuela, N. 2016. Physicochemical and microstructural characterization of corn starch edible films obtained by a combination of extrusion technology and casting technique. *Journal of Food Science*, 81(9): E2224-E2232.
- Galindez, A., Daza, L. D., Homez-Jara, A., Eim, V. S. y Váquiro, H. A. 2019. Characterization of ulluco starch and its potential for use in edible films prepared at low drying temperature. *Carbohydrate Polymers*, 215: 143-150.
- Ghadam, A. G. J., Idrees, M. y Naqvi, S. A. H. 2012. Morphological and Thermal Properties of CaCO₃ Nanopowders Synthesized by Reverse Microemulsion Technique. *International Journal of Advances in Engineering Sciences*, 2(2): 1-6.
- Gómez, M., McDonough, C., Rooney, L. y Waniska, R. 1989. Changes in corn and sorghum during nixtamalization and tortilla baking. *Journal of Food Science*, 54(2): 330-336.

- Gontard, N., Guilbert, S. y Cuq, J. L. 1992. Edible wheat gluten films: influence of the main process variables on film properties using response surface methodology. *Journal of Food Science*, 57(1): 190-195.
- Gontard, N., Guilbert, S. y Cuq, J. L. 1993. Water and glycerol as plasticizers affect mechanical and water vapor barrier properties of an edible wheat gluten film. *Journal of Food Science*, 58(1): 206-211.
- Guimarães, J., Wypych, F., Saul, C., Ramos, L. y Satyanarayana, K. 2010. Studies of the processing and characterization of corn starch and its composites with banana and sugarcane fibers from Brazil. *Carbohydrate Polymers*, 80(1): 130-138.
- Hu, L., Dong, P. y Zhen, G. 2009. Preparation of active CaCO₃ nanoparticles and mechanical properties of the composite materials. *Materials Letters*, 63(3-4): 373-375.
- Kim, H.-Y., Lamsal, B., Jane, J.-I. y Grewell, D. 2019. Sheet-extruded films from blends of hydroxypropylated and native corn starches, and their characterization. *Journal of Food Process Engineering*, n/a(n/a): e13216.
- Koo, S. H., Lee, K. Y. y Lee, H. G. 2010. Effect of cross-linking on the physicochemical and physiological properties of corn starch. *Food Hydrocolloids*, 24(6): 619-625.
- Li, Y., Chen, S., Li, X., Wu, M. y Sun, J. 2015. Highly Transparent, Nanofiller-Reinforced Scratch-Resistant Polymeric Composite Films Capable of Healing Scratches. *ACS Nano*, 9(10): 10055-65.
- Limón-Valenzuela, V., Aguilar-Palazuelos, E., Zazueta-Morales, J. d. J. y Martínez-Bustos, F. 2017. Propiedades microestructurales y de formación de pasta de pellets extrudidos elaborados a partir de almidón de maíz enriquecidos con MCP y concentrado proteínico de leche. *Revista Mexicana de Ingeniería Química*, 16(1): 193-205.
- Maftoonazad, N. y Ramaswamy, H. 2005. Postharvest shelf-life extension of avocados using methyl cellulose-based coating. *LWT-Food Science and Technology*, 38(6): 617-624.
- McHugh, T. H. 2000. Protein-lipid interactions in edible films and coatings. *Food/Nahrung*, 44(3): 148-151.
- McHugh, T. H., Avena-Bustillos, R. y Krochta, J. 1993. Hydrophilic edible films: modified procedure for water vapor permeability and explanation of thickness effects. *Journal of Food Science*, 58(4): 899-903.
- Mehyar, G. F. y Han, J. H. 2004. Physical and Mechanical Properties of High-amylose Rice and Pea Starch Films as Affected by Relative Humidity and Plasticizer. *Journal of Food Science*, 69(9): E449-E454.
- Mercier, C. y Feillet, P. 1975. Modification of carbohydrate components by extrusion-cooking of cereal products. *Cereal Chemistry*, 52: 283-297.
- Oliveira, L. C., Barros, J. H. T., Rosell, C. M. y Steel, C. J. 2017. Physical and thermal properties and X-ray diffraction of corn flour systems as affected by whole grain wheat flour and extrusion conditions. *Starch - Stärke*, 69(9-10): 1600299.
- Rodríguez-Castellanos, W., Rodrigue, D., Martínez-Bustos, F., Jiménez-Arévalo, O. y Stevanovic, T. 2015. Production and characterization of gelatin-starch polymer matrix reinforced with cellulose fibers. *Polymers from Renewable Resources*, 6(3): 105-118.
- Roquemore, K. G. 1976. Hybrid Designs for Quadratic Response Surfaces. *Technometrics*, 18(4): 419-423.
- Rostami, H. y Esfahani, A. A. 2019. Development a smart edible nanocomposite based on mucilage of *Melissa officinalis* seed/montmorillonite (MMT)/curcumin. *International Journal of Biological Macromolecules*, 141: 171-177.
- Sahebian, S., Zebarjad, S. M., Khaki, J. V. y Sajjadi, S. A. 2009. The effect of nano-sized calcium carbonate on thermodynamic parameters of HDPE. *Journal of Materials Processing Technology*, 209(3): 1310-1317.
- Sun, Q., Xi, T., Li, Y. y Xiong, L. 2014. Characterization of corn starch films reinforced with CaCO₃ nanoparticles. *PLoS One*, 9(9): e106727.
- Takabait, F., Mahtout, L., Pérez Villarejo, L., Carrasco Hurtado, B. y Sánchez Soto, P. J. 2016. Obtención de nanopartículas de carbonato de calcio a partir de precursores inorgánicos y sacarosa como aditivo con potencial utilización como biomaterial. *Boletín de la Sociedad Española de Cerámica y Vidrio*, 55(5): 179-184.
- Teixeira, E. d. M., Curvelo, A. A. S., Corrêa, A. C., Marconcini, J. M., Glenn, G. M. y Mattoso, L. H. C. 2012. Properties of thermoplastic starch from cassava bagasse and cassava starch and their blends with poly (lactic acid). *Industrial Crops and Products*, 37(1): 61-68.
- Treviño-Garza, M. Z., García, S., del Socorro Flores-González, M. y Arévalo-Niño, K. 2015. Edible Active Coatings Based on Pectin, Pullulan, and Chitosan Increase Quality and Shelf Life of Strawberries (*Fragaria ananassa*). *Journal of Food Science*, 80(8): M1823-M1830.
- Wang, Y., Zhang, R., Ahmed, S., Qin, W. y Liu, Y. 2019. Preparation and Characterization of Corn Starch Bio-Active Edible Packaging Films Based on Zein Incorporated with Orange-Peel Oil. *Antioxidants (Basel)*, 8(9).
- Yan, Q., Hou, H., Guo, P. y Dong, H. 2012. Effects of extrusion and glycerol content on properties of oxidized and acetylated corn starch-based films. *Carbohydrate Polymers*, 87(1): 707-712.
- Yildirim-Yalcin, M., Seker, M. y Sadikoglu, H. 2019. Development and characterization of edible films based on modified corn starch and grape juice. *Food Chemistry*, 292: 6-13.
- Younis, H. G. R. y Zhao, G. 2019. Physicochemical properties of the edible films from the blends of high methoxyl apple pectin and chitosan. *International Journal of Biological Macromolecules*, 131: 1057-1066.
- Zhong, Y. y Li, Y. 2014. Effects of glycerol and storage relative humidity on the properties of kudzu starch-based edible films. *Starch - Stärke*, 66(5-6): 524-532.