Vol. 19, No. 3 (2020) 1095-1101
Revista Mexicana de Ingeniería Química

Active biodegradable films based on thermoplastic starch and poly (ε-caprolactone): technological application of antioxidant extracts from rice husk

Películas biodegradables activas a base de almidón termoplástico y poli(ε-caprolactona): aplicación tecnológica de extractos antioxidantes del arroz

N. Piñeros-Guerrero[1,2], Y. Piñeros-Castro[1], R. Ortega-Toro[2]*

[1]*Departamento de Ingeniería, Programa de Ingeniería Química, Universidad Jorge Tadeo Lozano, Cra. 4 No. 22-61, Bogotá, Colombia.*

[2]*Departamento de Ingeniería, Programa de Ingeniería de Alimentos, Grupo de investigación Food Packaging and Shelf Life (FP&SL) y Grupo de Investigación Ingeniería de Fluidos Complejos y Reología de Alimentos (IFCRA), Universidad de Cartagena, Avenida el Consulado Calle 30 No. 48-152, Cartagena de Indias, Colombia*

Received: October 1, 2019; Accepted: December 15, 2019

Abstract

Films of a polymeric material based on cassava starch and poly ε-caprolactone (PCL) with antioxidant properties were made. The influence of PCL and the antioxidant extract were evaluated, over the mechanical, thermal and chemical properties of the films. They were elaborated with, and without the antioxidant extract, which is rich in polyphenolic compounds. The films were conditioned at 20°C and 53% RH. The antioxidant extract was obtained from rice husk, by the hot water methodology in a high-pressure reactor, at 160°C and 10 bar. The total phenolic content of the extract was 12.27 (mEAG/100g dry matter), and the antioxidant activity had a value of 6691.95 (mETRX/100g dry matter). The extract was incorporated into a thermoplastic starch and poly E-caprolactone matrix, and films by compression molding were obtained. The antioxidant capacity was evaluated by DPPH method, where the lesser value was 1.54 (kg film/g DPPH) from EC_{50}, it was found that the addition of PCL and antioxidant improved the general properties of the films.

Keywords: Antioxidant extract, cassava starch, poly ε-caprolactone, rice husk, thermoplastic starch.

Resumen

Se realizaron películas a base de almidón de yuca y poli ε-caprolactona (PCL), con propiedades antioxidantes. Se evaluó la influencia de la adición de PCL y el extracto antioxidante sobre las propiedades mecánicas, térmicas y químicas de las películas. Las películas fueron elaboradas con y sin extracto antioxidante rico en compuestos polifenólicos, las películas fueron acondicionadas a 20 °C y 53% HR. El extracto antioxidante se obtuvo de la cascarilla de arroz, mediante la metodología de agua caliente, en un reactor de alta presión a 160 °C y 10 bar. El contenido fenólico total del extracto fue 12.27 (mEAG/100g masa seca), y se obtuvo un valor de 6691.95 (mETRX/100g masa seca) de actividad antioxidante. El extracto se incorporó a la matriz de almidón termoplástico y poli E-caprolactona, y se obtuvieron películas por moldeo con presión. La capacidad antioxidante de las películas se evaluó mediante el método de DPPH, donde el menor valor de EC_{50} fue de 1.54 (kg película/g DPPH), se encontró que la adición de PCL y extracto antioxidante mejoró en general las propiedades de las películas.

Palabras clave: Extracto antioxidante, almidón de yuca, poli ε-caprolactona, cascarilla de arroz, almidón termoplástico.

1 Introduction

In recent years, biodegradable materials have been studied extensively, due to their appealing properties, such as high oxygen barrier, high CO_2 barrier and easy processability compared to conventional polymers (Averous, 2016). Even though, further research and development about biodegradable polymers is necessary, in order to improve their physical properties. The incorporation of polyphenols in films based on biopolymers, has been carried out in different researches (Rodríguez-Soto *et al*., 2019). Such as different formulations of edible films based on pea starch (St), chitosan (CH) and hydroxypropyl methylcellulose (HPMC), glycerol as a plasticizer and tannic acid as a cross-linking agent were obtained and its physicochemical properties were studied. The films were conditioned at 25 °C and 75% RH.

* *Corresponding author. E-mail*: rortegap1@unicartagena.edu.co
https://doi.org/10.24275/rmiq/Poli898
ISSN 1665-2738, issn-e: 2395-8472

Then, they were characterized, measuring its mechanical properties, moisture content, solubility and antioxidant capacity. The results showed that the properties of the films were affected by the incorporation of polyphenols so that they became more extensible than those without antioxidants. Also, its solubility in water and its antioxidant capacity increased. Among them, those that presented the best characteristics were those of chitosan and tannic acid, for their mechanical properties and their greater antioxidant activity, due to the higher efficiency of encapsulation of the polyphenols and the cross-linking effect of tannic acid, which also contributed to the antioxidant power of the films obtained (Hernández-Solomando, 2015; Torres-Huerta *et al*., 2019). Polymer mixtures have been studied, in order to improve the polymer properties. For example, the addition of PCL and polyethylene glycol (PEG) to thermoplastic starch (TPS), lead to a reduction of the polymer's glass transition temperature. Meanwhile, no changes were observed in the melting properties of PCL. Starch films incorporated 5% PCL without noticeable phase separation, which leads to more elastic and stable films. TPS films with 10% PCL showed clear phase separation, without improved tensile properties, but with lower water vapor permeability. In ternary systems, PEG reduced the affinity of PCL-starch, increasing phase separation, while not improving the physical properties of the film concerning starch films (Ortega-Toro *et al*., 2016). In a homologous research, the thermal behavior of banana starch films with degraded PET found that the thermal stability of the films was lower than the individual components (Ramírez-Hernández *et al*., 2015). Other study, in which rice flour and banana films were made, different concentrations of glycerol and sodium montmorillonite (MMT) nanoparticles were evaluated, by means of a response surface analysis. The study showed that the polymer properties were improved at higher concentrations of MMT and low concentrations of glycerol (Rodriguez-Marín *et al*., 2013). The use of lignocellulosic agro-industrial waste is essential for sustainable production of biodegradable polymers. Among these, cereal residues such as rice stand out. In Colombia, more than 2 million tons of rice are produced per year, and their waste is usually thrown to the ground or incinerated, generating sources of contamination. Rice husks are high in polyphenols, which can be used as antioxidants or antimicrobials for obtaining active materials (Collazo-Bigliardi *et al*., 2019). Piñeros-Guerrero *et al*., studied the effect of the addition of an antioxidant extract of the rice husk, on the microstructural and optical properties of thermoplastic starch and poly (ε-caprolactone) films, the extract improved its morphology and provided the films with antioxidant capacity (Piñeros-guerrero *et al*., 2019). In another study the effect of a corn starch coating on the quality of the tomato crop was validated, in order to maintain the physical and chemical quality of tomatoes (Fitch-Vargas *et al*., 2019). The aim of this work was to analyze the effect of the addition of an antioxidant extract from the rice husk on the thermal and chemical mechanical properties of a matrix based on cassava starch and poly starch (ε-caprolactone).

2 Materials and methods

2.1 *Obtaining the antioxidant extract*

The rice husk was processed in a blade mill and sieved in a set of sieves (FISHER SCIENTIFIC COMPANY) with a maximum opening of 850 μm to achieve a greater surface area. The material was hydrated in distilled water for 2 hours. In a high-pressure reactor, 500 g of previously conditioned biomass and 4.5 L of distilled water were charged. The operating conditions were 160 ° C and 10 bars of pressure for a time of 30 minutes, the solid phase was separated from the liquid extract obtained. This extract was centrifuged at a speed of 536 G-force to remove the suspended solids completely, the extract was stored in an amber colored container under refrigeration conditions at 4°C.

2.2 *Total solids*

In this assay, an aliquot of 1mL of the extract was taken and placed in a Petri dish of known weight. Then, it was oven-dried at 50°C for 24 hours to remove all the water from the mixture and precipitate the total solids, weighed on a precision analytical balance of 0.1 mg by weight difference the mass of total solids was obtained (Bradley, 2010).

2.3 *Content of phenolic compounds*

The concentration of total phenolic compounds was determined by a spectrophotometric method, using the Folin-Ciocalteau reagent and the methodology described by (Singleton, 1965). The method consists of detecting the concentration of polyphenols by the formation of molybdenum salts of blue color,

quantifiable by spectrophotometry in a range of 700 to 765 nm. The results are expressed in Gallic acid equivalents (EAG), for which a standard of Gallic acid is used as a reference.

2.4 Measurement of antioxidant activity

The total antioxidant activity was determined by the DPPH method (2,2-diphenyl-1-picrylhydrazine) (Brand-Williams, Cuvelier and Berset, 1995) and expressed as Trolox equivalents (ET). 1000 μL of DPPH solution were placed in cuvettes and followed by 30 μL of extract in plastic cuvettes, allowed to react for 30 min at room temperature and in the dark. Finally, absorbance readings were taken in a UV-Visible EV-300® spectrophotometer. The results were expressed as mg of Trolox equivalent/mg dry mass.

2.5 Preparation, conditioning and incorporation of the antioxidant extract to the polymeric matrix

The thermoplastic starch (TPS) was obtained through melt blending and compression molding. Firstly, all the components (starch, glycerol, antioxidants extract, polycaprolactone and water) were hand blended in a beaker. Then, a double roll mixer was used at a temperature of 160°C and 0.004 G-force for 30 minutes until a homogeneous paste was obtained. The particle size was reduced in a manual mill and conditioned in a desiccator with oversaturated sodium chloride for 7 days. After that, the films were obtained in a hydraulic press at a temperature of 130°C with a preheating time of 3 minutes, the conditions for the melting stage were 2 minutes and 5 MPa, and the molding conditions were 3 minutes and 20 MPa, finally, a cooling stage of 3 minutes at a pressure of 5 MPa was performed. The formulations including antioxidant extract were prepared in a beaker and continuously stirred for 24h to promote the diffusion of the extract throughout the mixture. In Table 1 the mass fraction of each component in the dry blends and their identification codes are reported.

2.6 Mechanical properties

The films were conditioned at 20°C and 75% RH. A universal testing machine (TA.XTplus model, Stable Micro Systems, Haslemere, England) was used for the determination of tensile strength (TS), modulus of elasticity (YM) and elongation (E) of films according to the standard method ASTM D882 (ASTM, 2001) YM, TS and E were made from the stress-strain curves, estimated from the force-distance data obtained for the different films (2.5 cm wide and 10 cm long) (Ortega-Toro *et al*., 2016).

2.7 Thermogravimetric analysis (TGA)

The thermogravimetric analysis was carried out by heating from 25°C to 700°C with a speed of 10°C/min, in a nitrogen atmosphere with a flow of 30 mL/min, 8-10 mg of sample was taken. The initial degradation temperature (Onset) and the maximum temperature (Peak) were recorded from the first derivative of the resulting weight loss curves.

2.8 Differential scanning calorimetry (DSC)

Conditioned films were analyzed (20 °C and 75% RH). Samples of approximately 6 mg sample were placed in aluminum trays, sealed and punched to promote the loss of bound moisture during heating. The thermograms were obtained by heating from 25 to 110°C at a rate of 20°C/min. Then the samples were cooled to -80 °C at a rate of 10 °C/min, and then heated to 200 °C at a speed of 20 °C/min, in the first scan the fusion properties of PCL were obtained, in the second scan the crystallization properties of PCL were obtained and in the final scan the glass transition temperature was obtained.

Table 1. Mass fraction (X, g compound / g dried film) of the different components (Starch: S, Glycerol: Gly, Polycaprolactone: PCL, Antioxidant extract: ANT).

Formulations	$\mathbf{X_S}$	$\mathbf{X_{Gly}}$	$\mathbf{X_{PCL}}$	$\mathbf{X_{ANT}}$
F1	0.7692	0.2308	-	-
F2	0.7143	0.2143	0.0714	-
F3	0.7138	0.2141	0.0714	0.0007
F4	0.7686	0.2306	-	0.0008
F5	0.7128	0.2138	0.713	0.0021
F6	0.7675	0.2302	-	0.0023

2.9 Measurement of antioxidant capacity

The fraction of DPPH removed versus the mass ratio antioxidant and DPPH (g Antioxidant/g DPPH) was plotted, performing a polynomial adjustment of grade 3 with R2 = 0.99. For the experimental data obtained in the assay of determination of antioxidant activity of the polymer material was followed the method described by Brand-Williams, Cuvelier and Berset (1995) to define the parameter EC_{50} which describes the amount of material needed to reduce the initial concentration of DPPH by 50%. The EC_{50} value was expressed as grams of antioxidant per grams of DPPH concerning the Kg of film per g of DPPH (Talón *et al*., 2017).

3 Results and discussion

3.1 Characterization of the antioxidant extract

Table 2 shows the average values obtained from the characterization of the antioxidant extract, the total phenols content is expressed in milliequivalents of Gallic acid (mEAG) and the antioxidant activity is expressed in milliequivalents of Trolox (mETRX).

The value obtained from the phenolic compounds from dried rice husk is similar to the report in materials from Taiwan Huang and Ng (2012). In addition, there is evidence that the rice husk extract has a high antioxidant activity, when compared with other materials such as rice bran extract which has a high amount of phenolic compounds, thus employing a lower amount of extract to obtain the same antioxidant capacity.

Table 2. Concentration of total solids (g/mL), total phenols (mEAG/100g dry matter) and antioxidant activity (mETRX/100g dry matter) of extract.

	Antioxidant extract
Total solids	0.0314 ± 0.0007
Phenolic compounds	12.270 ± 0.0003
Antioxidant activity	6691.9 ± 0.0003

3.2 Mechanical properties

According to Table 3, the tensile strength (TS) decreases significantly ($p < 0.05$) with the addition of the antioxidant extract. For the formulations with 0.1% ANT this parameter decreases approximately 30% and, for the formulations with 0.3% ANT, it is reduced about 25%. The addition of PCL decreases the TS by 6%; this could be due to the low proportion of PCL (Ortega-Toro, Rodrigo *et al*., 2015). The percentage of deformation (%E) at the break-point and elastic modulus (YM) are reduced by the addition of the antioxidant extract generating less rigid polymers. That could mean that the antioxidant extract had the capability of hydrolyzing the PLC slightly, according to their acid nature of the polyphenols.

This phenomenon can be justified by the structural change promoted by the addition of these phenolic components, generating discontinuities in the matrix that promotes the breakage of the film at lower levels of deformation as reported by Pastor *et al*., (2013). An opposite case reported by Talón *et al*., (2017) where the addition of tannic acid (TA) to chitosan (CH) films produced a significant increase in modulus and tensile strength, producing more rigid and resistant films. In this case, the tannic acid acts as a crosslinking agent.

Table 3. Mean values and standard deviations of the mechanical properties of the films stored at 53% relative humidity and 20 °C for 1 week, comparative data obtained from Talón *et al*. (2017) and Pastor *et al*. (2013).

Formulations	**Thickness (μm)**	**YM (Mpa)**	**TS (Mpa)**	**E (%)**
F1	244 ± 4^{a}	127 ± 29^{a}	4 ± 2^{b}	16 ± 2^{ab}
F2	409 ± 4^{a}	120 ± 6^{a}	3.46 ± 0.08^{ab}	120 ± 5^{a}
F3	399 ± 1^{a}	94 ± 9^{a}	2.3 ± 0.4^{a}	15.9 ± 0.2^{b}
F4	254 ± 0.9^{a}	122 ± 17^{a}	2.6 ± 0.2^{a}	15.4 ± 0.4^{b}
F5	373 ± 7^{a}	109 ± 6^{a}	2.74 ± 0.12^{a}	14.4 ± 0.9^{a}
F6	268 ± 3^{a}	104 ± 6^{a}	2.79 ± 0.15^{a}	14.6 ± 0.8^{a}
CH:St:TA (Talón)	40 ± 3	82 ± 31	11 ± 3	36 ± 6
CH + R100 (Pastor)	74 ± 5	1550 ± 195	43 ± 5	5.1 ± 0.4

Different superscript letters within the same column indicate significant differences among formulations ($p<0.05$)

Table 4. Mass loss values and initial and maximum degradation temperatures.

Formulations	Weight loss (%) (25-150ºC)	Starch		PCL	
		Tonset(ºC)	Tpeak (ºC)	Tonset(ºC)	Tpeak (ºC)
F1	10.6 ± 0.2	294.1 ± 0.3	320.1 ± 0.5	-	-
F2	-	-	-	393.2 ± 0.4	405.2 ± 0.5
F3	10.5 ± 0.4	296.1 ± 1.1	317.2 ± 0.6	370.1 ± 1.2	402.3 ± 0.4

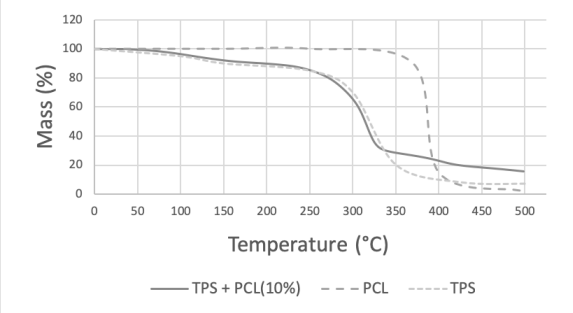

Fig. 1. TGA plots of some studied formulations.

3.3 Thermogravimetric analysis (TGA)

Figure 1 shows a typical thermogram for a polymer, showing the percentage of weight loss versus the increase in temperature:

Table 4 shows the mean values and standard deviations of mass loss and thermal degradation temperatures for TPS and PCL, formulations conditioned at 53% relative humidity and 20 °C.

The thermograms show three separate sections of mass loss. The first section shows the evaporation of the free and bound water between 25°C and 150°C. The second section is assigned to the degradation of the starch between 293°C and 300°C. Finally, the last section shows the degradation of PCL at a temperature of 390°C. Formulation F2 shows a slight decrease in the mass loss with regard to the TPS and the initial degradation temperature (Tonset) of the PCL decreases significantly with the addition of TPS, no significant change in the degradation temperature was evidenced of formulation F1.

3.4 Differential Scanning Calorimetry (DSC)

Table 5 shows the mean values and standard deviations of the melt temperature (Tm), melt enthalpies (ΔHm) and degree of crystallization (% Xcm), these values were obtained in the first heating. The crystallization temperature (Tc), crystallization enthalpy (ΔHc) and degree of crystallization (% Xcc) were determined in the cooling step and, the glass transition temperatures (Tg) and the melt enthalpy (ΔHmc) obtained during the second heating. The formulations studied were conditioned at 53% relative humidity and 20 °C.

Table 5. Mean values and standard deviation of melt temperatures, melt enthalpies and degree of crystallisation.

Formulations	First heating scan		
	Tm (°C)	ΔHm (J/gPCL)	Xcm (%)
F1	-	-	-
F2	56.2 ± 0.5	53.2 ± 1.4	39.1 ± 1.0
F3	60.8 ± 0.7	68.3 ± 0.3	50.2 ± 0.2
	Cooling		
	Tc (°C)	ΔHc (J/gPCL)	Xcc (%)
F1	-	-	-
F2	27.3 ± 0.2	46.5 ± 0.8	34.2 ± 0.6
F3	26.7 ± 0.3	56.1 ± 0.9	41.3 ± 0.7
	Second heating scan		
	Tg PCL	Tg S	ΔHmc (J/gPCL)
F1	-	100.3 ± 0.2	-
F2	-70.1 ± 0.3	97.1 ± 0.1	53.4 ± 0.9
F3	-75.1 ± 0.3	120.0 ± 0.2	59.2 ± 1.3

Table 6. EC_{50} values of the films, comparative data obtained from Talón *et al*. (2017) and Pastor *et al*. (2013).

Formulations	EC_{50} (g soluble solids / g DPPH)	EC_{50} (kg film / g DPPH)
F1	-	-
F2	-	-
F3	1.3553	1.9362
F4	3.3717	42,147
F5	3.3266	1.5412
F6	5.6764	2.468
CH:St:TA (Talón)	-	0.003
CH + R100 (Pastor)	-	0.005

The melting temperature (Tm), enthalpy of fusion (ΔHm) and the degree of crystallisation (% Xcm) were calculated from the thermograms where it is observed that the blend of PCL-TPS has lower values of Tm, ΔHm, Xcm, ΔHc, Xcc, Tg PCL and ΔHmc compared to pure PCL. This phenomenon is due to the partial miscibility of PCL in the starch matrix. The decrease in Tg, as well as the higher enthalpy values, are consistent with the lower molecular weight of PCL, which reduces the average molecular weight of the starch-rich phase. This fact promotes the plasticization of the starch phase, since it increases its fluidity during the thermal processing. A crystallisation temperature of the TPS + PCL (10%) blend of 27°C, and a degree of crystallisation of 34% is reported.

3.5 Measurement of antioxidant capacity

The fractions of the films were cut of the same size and immersed in 1 mL of ethanol at 25 °C under constant stirring for 24 h to perform the extraction of the antioxidant compounds. From each extract was taken 90μL and added to 1 mL of DPPH (0.041 g/ L). The reaction was performed in the dark for 40 min, subsequently, its absorbance was read at a wavelength of 517 nm.

The formulations containing PCL have a lower value of EC_{50} parameter compared to the films without PLC, which indicates that a smaller amount of antioxidant or film is required to reduce the concentration of DPPH by 50%. A tendency of the formulations containing PCL is observed where a more efficient release of the antioxidant extract, the high amount of film necessary to remove 50% concentration of DPPH is due to the low proportion of the antioxidant present in the film. Formulations 1 and 2 did not present an EC_{50} value because their matrix does not contain antioxidants.

Conclusions

The rice husk extract has a high antioxidant activity despite the low content of total phenols, which indicates that the phenolic groups present in the extract are highly reactive. The addition of the antioxidant extract to the matrices with and without PCL allows obtaining films with a lower modulus of elasticity, more stretchable and less brittle. The thermal properties of TPS are affected by the addition of PCL which indicates good compatibility of both polymers. The addition of antioxidant extract appears to improve the miscibility between TPS and PCL, according their thermal properties.

Acknowledgements

The authors thank the Universidad de Bogotá Jorge Tadeo Lozano and Universidad de Cartagena for their support in the development of this work.

References

Averous, L. and Boquillon, N. (2004). Biocomposites based on plasticized starch: Thermal and mechanical behaviours. *Carbohydrate Polymers 56*, 111-122.

Bradley, R. (2010). Moisture and total solids analysis. *Food Analysis*, 85-104.

Brand-Williams, W., Cuvelier, M. E. and Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology 28*, 25-30.

Collazo-Bigliardi, S., Ortega-Toro, R. and Chiralt, A. (2019). Improving properties of thermoplastic starch films by incorporating active extracts and cellulose fibres isolated from rice or coffee husk, *Food Packaging and Shelf Life 22*, 100383.

Fitch-Vargas, P. R. *et al*. (2019). Effect of a corn starch coating obtained by the combination of extrusion process and casting technique on the postharvest quality of tomato. *Revista Mexicana de Inginiería Química 18*, 789-801.

Hernández-Solomando, R. M. (2015). Incorporación de polifenoles a films a base de biopolímeros: propiedades físicas y antioxidantes. *Thesis in Food Science and Technology, Polytechnic University of Valencia*, 1-34.

Huang, S.H. and Ng, L.T. (2012). Quantification of polyphenolic content and bioactive constituents of some commercial rice varieties in Taiwan. *Journal of Food Composition and Analysis 26*, 122-127.

Ortega-Toro, R., Bonilla J., Talens P., and Chiralt, A. (2015). Future of starch-based materials in food packaging. *Academic Press 1*, 257-312.

Ortega-Toro, R., Muñoz A., Talens P. and Chiralt A. (2016). Improvement of properties of glycerol plasticized starch films by blending with a low ratio of polycaprolactone and/or polyethylene glycol. *Food Hydrocolloids 56*, 9-19.

Pastor, C., Sánchez-González L., Chiralt A., Cháfer M. and González-Martínez C. (2013). Physical and antioxidant properties of chitosan and methylcellulose based films containing resveratrol. *Food Hydrocolloids 30*, 272-280.

Piñeros-Guerrero, N., Sierra-Barahona, F.D., Piñeros-Castro, Y. and Ortega-Toro, R. (2019). Propiedades microestructurales y ópticas de películas biodegradables a base de almidón termoplástico y poli (ε-Caprolactona) con actividad antioxidante. *Información Tecnológica 30*, 293-300.

Ramírez-Hernández, A., Valera-Zaragoza, M., Aparicio-Saguilán A. and Conde-Acevedo J.C. (2015). Comportamiento térmico de películas de almidón de plátano con poli(etileno tereftalato) degradado. *Revista Mexicana de Ingeniería Química 14*, 513-521.

Rodriguez-Marín, M.L., Bello-Perez, L.A., Yee-Madeira, H. and González-Soto R.A. (2013). Propiedades mecánicas y de barrera de la película elaborada con arroz y suelo de banano reforzado con nanoparticulas: estudio con superficie de respuesta. *Revista Mexicana de Ingeniería Química 12*, 1-12.

Rodríguez-Soto, K.X., Piñeros-Castro, N.Y. and Ortega-Toro, R. (2019). Laminated composites reinforced with chemically modified sheets-stalk of *Musa Cavendish. Revista Mexicana de Ingeniería Química 18*, 749-758.

Singleton, V.L. and Rossi, J. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture 16*, 144-158.

Torres-Huerta, A.M., Domínguez-Crespo M.A., Palma-Ramírez, D., Flores-Vela, A.I., Castellanos-Alvarez, E. and Angel-Lopez D. (2019). preparation and degradation study of HDPE / PLA polymer blends for packaging applications. *Revista Mexicana de Inginiería Química 18*, 251-271.