

The addition of *Jatropha* proteins and bentonite modified the mechanical and barrier properties of a soy protein film

La adición de proteínas de *Jatropha* y bentonita modificó las propiedades mecánicas y de barrera de una película de proteína de soja

Sierra-Sarmiento, M. A., Hurtado-Amado, N., Espitia-Vasquez, R. A. y Hoyos-Leyva, J. D.*

Grupo de Investigación e Innovación en Ingeniería Agroindustrial - GINNA, Facultad de Ingeniería, Fundación Universitaria Agraria de Colombia, Colombia.

Fecha de recepción: marzo de 2020 / Fecha de aceptación: julio de 2020

Abstract

The effect of the proportion of isolated protein from *Jatropha curcas* L. and *Glycine max* L. on the mechanical and barrier properties of cast films, nanoreinforced with bentonite, was tested. The cast films were prepared with different proportions of *Jatropha*/soy isolated protein (2.5%/97.5% and 5%/95%, respectively). Additionally, different concentrations of bentonite (0.1% and 0.06%) and a constant quantity of glycerol (0.4 g/g of protein) were added.

It was found that the concentration of the reinforcement and the protein significantly affect the mechanical properties of the polymeric films, the best formulation being that of 2.5% of *Jatropha* protein and 0.0625% of bentonite, with breaking stress values of 0.87 ± 0.076 MPa, Young's modulus of 48.4 ± 3.3 MPa, and a percentage of elongation of $35.1 \pm 5.2\%$.

The barrier properties were significantly improved with this same formulation, showing moisture content levels of $19.1 \pm 0.7\%$, a swelling percentage of $84.6 \pm 2.8\%$, and permeability values of $0.007 \pm 0.0001\%$. Characterization by infrared spectroscopy made it possible to identify the presence of functional groups and the interactions of the compounds present in the polymeric films. The addition of bentonite to the protein matrix significantly improves the mechanical and barrier properties of the films.

Keywords: Bentonite, polymeric, glycerol, spectroscopy, *Jatropha*, *Glycine max*.

* Correspondencia: hoyos.javier@uniagraria.edu.co

Resumen

Se evaluó el efecto de la proporción de proteína aislada de *Jatropha curcas* L. y *Glycine max* L. en las propiedades mecánicas y de barrera de películas moldeadas, nanorreforzadas con bentonita. Las películas se prepararon con diferentes proporciones de proteína aislada de *Jatropha*/soja (2,5 %/97,5 % y 5 %/95 %, respectivamente). Además, se añadieron diferentes concentraciones de bentonita (0,1 % y 0,06 %) y una cantidad constante de glicerol (0,4 g/g de proteína).

Se observó que la concentración del refuerzo y de la proteína afectan significativamente las propiedades mecánicas de las películas poliméricas. La mejor formulación fue la de 2,5 % de proteína de *Jatropha* y 0,0625 % de bentonita, obteniéndose valores de resistencia a la rotura de $0,87 \pm 0,076$ MPa, módulo de Young de $48,4 \pm 3,3$ MPa y porcentaje de elongación de $35,1 \pm 5,2$ %.

Las propiedades de barrera mejoraron significativamente con esta misma formulación, presentando niveles de humedad de $19,1 \pm 0,7$ %, un porcentaje de hinchamiento de $84,6 \pm 2,8$ % y valores de permeabilidad de $0,007 \pm 0,0001$ %. La caracterización mediante espectroscopia infrarroja permitió identificar la presencia de los grupos funcionales y las interacciones de los compuestos presentes en las películas poliméricas. La adición de bentonita a la matriz proteica mejora significativamente las propiedades mecánicas y de barrera de las películas.

Palabras clave: bentonita, polimérico, glicerol, espectroscopia, *Jatropha*, *Glycine max*.

Introduction

Conventionally, the materials used to obtain packaging in the food industry come from non-renewable petroleum resources, and this has been generating great concern due to the non-biodegradability of these materials and their significant environmental impact. In response to this problem, research on biodegradable biopolymers made from renewable resources has been recently strengthened (Medina-Jaramillo, Ochoa-Yepes, Bernal, & Famá, 2017; Panseri *et al.*, 2018).

The most used substances to obtain these biopolymer matrices include proteins, alginates, pectins, starches, cellulose derivatives, and other polysaccharides. Protein sources include wheat gluten, corn zein, soy protein, collagen, gelatin, keratin, casein, and whey proteins, which can be used as polymeric materials in the food industry through techniques such as pour plate or compression molding (Atarés & Chiralt, 2016; Garavand *et al.*, 2017).

Soy contains around 40% of protein, which makes it one of the sources with the greatest potential for obtaining biodegradable films (Lambrecht *et al.*, 2018). Other promising protein sources include *Jatropha curcas* L, which is a plant that produces non-edible seeds that have been used for oil extraction in biodiesel production. From the oil extraction process, a protein-rich cake results as a by-product, which contains between 19 and 30% of protein (Abdul-Khalil *et al.*, 2013).

Osorio-Ruiz *et al.* (2017) reported the morphological, as well as some physical and functional, properties of biofilms made with *Jatropha* and soy protein; these authors found that soy protein films had higher resistance to distortion than *jatropha* films, but the latter had lower permeability due to its amino acid composition. The mechanical and the barrier properties of the films are affected by environmental conditions such as humidity due to the hydrophilic nature of proteins (Rhim, Park, & Ha, 2013).

In this sense, there are different strategies to improve the mechanical and barrier properties of films made with biopolymers, such as the use of mixtures of different materials (protein, carbohydrates, gums, etc.), the addition of plasticizers, and nanoreinforcements, such as the addition of nanoclays (for example, bentonite), among others. Nanoclays have been used to improve the physical-mechanical characteristics of biofilms (Rhim *et al.*, 2013) due to the interactions between the nanoclay and the internal structure of the proteins that make up the polymeric matrix through hydrogen bonds (Monteiro *et al.*, 2018).

The overall performance of polymer films depends on factors such as matrix compatibility, the type of nanocomposite (Wakai & Almenar, 2015), the production method of the film, and the percentage of nanocomposite used, among others (Yun, Youn, Shin, & Yoon, 2017).

Therefore, the aim of this study was to determine the effect of the mixture of soy and *Jatropha* protein isolates on the mechanical and barrier properties of biofilms prepared by casting.

Additionally, the effect of the addition of bentonite at different concentrations on the physical-mechanical and barrier properties of polymer films obtained from mixtures of *Jatropha* and soy protein isolates was evaluated.

Materials and methods

Pre-treatment of raw materials

Initially, before film production, the raw materials were pretreated as follows. The *Jatropha* protein was isolated following the methodology proposed by Popovi, Perin, Vaštag, Lazi, & Popovi (2012) from a *Jatropha* seed cake obtained after oil extraction by extrusion. The soy protein isolate was purchased from Laboratorio Hunivor S.A. (Bogota D.C., Colombia). The bentonite was activated through a modified thermo-acid treatment proposed by Elfadly, Zeid, Yehia, Abouelela & Rabie (2017) in order to modify the surface of the clay. Briefly, the bentonite was dispersed in a solution containing citric and sulfuric acid; this solution was heated for 3 hours at a temperature of 80°C and then allowed to cool to room temperature and centrifuged to remove the activated clay.

Amino acid profile of jatropha and soy protein

The *Jatropha* and soy isolated proteins were hydrolyzed and analyzed to determine their amino acid composition, following the AOAC 994.12 method (AOAC, 2005). The chromatography was carried out using an Agilent 1100 (Agilent Technologies Inc., Santa Clara, CA, USA) series instrument.

Cast film preparation

Polymeric films were obtained by mixing *Jatropha* and soy protein concentrates (5%/95% and 2.5%/97.5%, respectively) with glycerol at a concentration of 0.4 g/g of protein isolate (Popovi *et al.*, 2012), which was used as a plasticizer, and 0.0625% (w/w) and 0.125% (w/w) of bentonite as a reinforcing agent. The cast films were made using the casting method proposed by González, Strumia, & Alvarez Igarzabal (2011).

The bentonite and glycerol were dispersed in 10 mL of deionized water and subjected to sonication for 10 min at room temperature. Simultaneously, the soy and *Jatropha* proteins were dispersed in 40 mL of deionized water; the pH was adjusted to 10 with NaOH (0.1 M). The solutions were then mixed by stirring for 10 min, after which the mixture was sonicated for 10 min at room temperature and stirred continuously for 30 min at a temperature of 75°C. The mixture was sonicated for 10 min and finally poured into molds measuring 20 cm x 4 cm x 0.05 mm, and the films were dried at room temperature for 48 hours.

Mechanical properties of the cast films

The mechanical tests of the films were performed using a Universal WDW-20E machine with a maximum load of 200 N and a strain tension velocity of 5 mm/min. The cast films were evaluated in terms of their mechanical characteristics, namely modulus of elasticity and tensile strength, according to ASTM standard D882-10 (ASTM, 2010).

Barrier properties

The steam transfer rate barrier characteristic was based on a modified method of ASTM standard E96-80; the percentage of swelling (S (%)) was determined according to the methodology proposed by Rhim *et al.* (2013).

Briefly, films were cut into square samples (25.4 mm x 50 mm) and were immersed in water. The initial and final weights were recorded, and the swelling was calculated from the following equation:

$$S(\%) = \frac{m_t - m_o}{m_o} \times 100 \quad (1)$$

Where m_o was the initial weight and m_t the final weight after film immersion.

Fourier-transform infrared spectroscopy (FTIR) analysis

The Fourier transform infrared spectroscopy (FTIR) of the polymeric films was carried out using a Cary 630 FTIR instrument (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with an ATR accessory to analyze solid samples. The wavelength range of the analysis was 650 to 4000 cm^{-1} and 4 cm^{-1} of resolution; 72 scans were performed for each sample.

Statistical analysis

All experiments were carried out in triplicate. The reported values were means $\pm$ standard error (SE). The data were analyzed by means of a one-way analysis of variance (ANOVA) using the software MATLAB R2009b; when statistically significant differences were found

(p value < 0.05), a Tukey test was applied in order to determine multiple differences between the means.

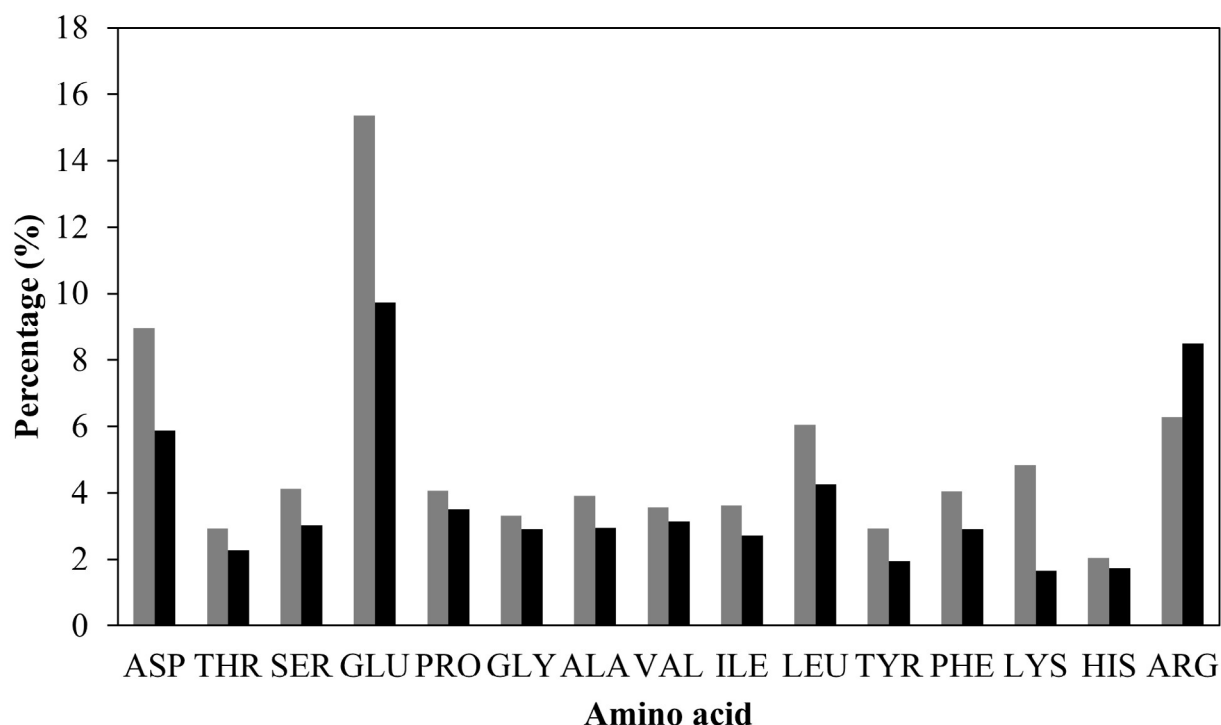
Results and discussion

Amino acid profile of soy and *Jatropha* protein isolates

According to the chromatographic analysis of the soy and *Jatropha* protein isolates, the most abundant amino acid was glutamic acid, with values of 15.36% and 9.7%, respectively (Figure 1). Other amino acids at high concentrations include aspartic acid (8.96%, 5.88%) and arginine (6.28%, 8.51%). In contrast, the lowest quantities were found for tyrosine (2.94%, 1.95%) and histidine (2.04%, 1.72%).

The amino acid composition of the proteins used for film-forming purposes has been reported to affect the mechanical and barrier properties of the final product (Guerrero *et al.*, 2010). The amino acid composition of *Jatropha* protein revealed the presence of arginine, lysine, proline, histidine, tyrosine, threonine, and serine.

These amino acids had been reported to form hydrogen bonds that allow for greater protein cross-linking within the polymer matrix in the presence of functional groups such as OH, NH, and COOH (Guerrero *et al.*, 2010). The formation of a macromolecular network depends on these amino acids, offering stability to new bonds within the polymer matrix in a 3D-network.

Figure 1. Amino acid composition of *Jatropha* (black bars) and soy (gray bars) proteins

Source. Own elaboration.

Effect of the concentration of the nanoreinforcement and percentage of *J. curcas* L. protein on the deformation characteristics of films

The breaking tension, Young's modulus, and elongation at break were studied as mechanical properties of protein films (Table 1).

As shown in Table 1, the bentonite and *Jatropha* protein concentration significantly affect the mechanical properties of the polymeric films ($p < 0.05$). The breaking tension and the modulus of elasticity increased with the addition of nanoreinforcement, up to a maximum value of 0.87 ± 0.08 MPa and 48.4 ± 3.3 MPa, respectively, in the film formed by 2.5% *Jatropha* protein and 0.0625% bentonite.

This improvement can be attributed to the increase in the stiffness of the film given by the interaction of the nanoreinforcement, as well as the high affinity and interaction between the polymeric matrix of the *Jatropha* and soy proteins, and the dispersion of the bentonite (Rhim, 2011; Saurabh *et al.*, 2015). The data agrees with previous studies where improvements were found in the mechanical characteristics by incorporating nanoreinforcements into polymeric matrices, such as chitosan (Yun *et al.*, 2017), starch (Wang *et al.*, 2018), and cellulose (Lee, Kim, & Ha, 2017).

The flexibility of a film is measured by its characteristic of elongation at break and is defined as the ability of the film to deform before breaking.

It was possible to demonstrate in this study that the nanoreinforcement and the *Jatropha* protein significantly affected the elongation of the films ($p < 0.05$); it was found that the films used as control showed elongation percentage values of 26.6 ± 5.0 and 15.9 ± 2.1 for films made with 2.5% and 5% *Jatropha* protein, respectively, whereas films obtained with 2.5% *Jatropha* protein and 0.0625% bentonite nanoreinforcement presented the maximum elongation percentage of 35.1 ± 5.2 .

However, an increase in the percentage of bentonite and *jatropha* protein decreased the film's elongation property.

The trend in the effect of bentonite addition was consistent with that reported in previous studies (Chrissafis, *et al.*, 2007; Saurabh *et al.*, 2015; Zhou *et al.*, 2016), where an increase in clay nanoreinforcements causes a less flexible film but induces a greater breaking tension.

Table 1. Mechanical properties of cast films made with a mixture of *Jatropha* (J) and soy isolated proteins, nanoreinforced with bentonite (B)

Treatment	Breaking tension (MPa)	Young's modulus	Tratamientos
2.5%J_0%B	0.53 ± 0.06^a	17.4 ± 5.2^a	26.6 ± 5.0^b
5%J_0%B	0.55 ± 0.10^a	26.8 ± 2.5^b	15.9 ± 2.1^a
2.5%J_0.0625%B	0.87 ± 0.08^c	48.4 ± 3.3	35.1 ± 5.2^c
2.5%J_0.125%B	$0.80 \pm 0.05^b^c$	22.3 ± 2.1^{ab}	21.2 ± 4.0^{ab}
5%J_0.0625%B	0.65 ± 0.13^{ab}	23.1 ± 2.3^{ab}	18.0 ± 1.7^a
5%J_0.125%B	$0.75 \pm 0.15^b^c$	15.3 ± 1.9^a	11.7 ± 2.5^a

Values are the mean $\pm$ SE. The values in columns with the same upper letter indicate they were not different statistically. J=percentage of *Jatropha* protein added; B=percentage of bentonite added to the cast films' production.

Source. Own elaboration.

Barrier properties and swelling of films with *Jatropha* and soy protein nanoreinforced with bentonite

To evaluate the barrier properties, the polymeric film that showed the best mechanical results was selected, which was obtained with 2.5% of the protein isolate and 0.0625% of bentonite nanoreinforcement and

was compared with the film obtained with 2.5% of the isolated protein of *J. curcas* L., without the addition of nanoreinforcement. In general, it was possible to observe that the nanoreinforcement significantly affected ($p < 0.05$) the characteristics of moisture content (%), swelling (%), and permeability; however, in table 2 it can be observed that the addition of nanoclay does not influence the water vapor transfer rate (WVTR).

The results obtained agree with previous studies that demonstrate that the use of bentonite and clay materials increases the tortuosity in the polymer structure, therefore decreasing the speed of the water vapor transfer rate and gas permeation (Liu *et al.*, 2016; Monteiro *et al.*, 2018; Peelman *et al.*, 2013). Likewise, this study showed evidence that the incorporation of bentonite in the

polymeric matrix made it possible to reduce the film's capacity to retain moisture and swell, which in turn is in line with previous studies (Monteiro *et al.*, 2018; Wakai & Almenar, 2015) where the levels of swelling and moisture content were reduced by up to 35% in films obtained from wheat protein isolate reinforced with montmorillonite.

Table 2. Swelling and barrier properties of the *Jatropha* and soy protein films with and without bentonite nanoreinforcement

Sample	Swelling (%)	WVTR	CO ₂ permeability (%)
With bentonite	84.6 ± 2.8	132.8 ± 31.5	0.007 ± 0.0001
Without bentonite	146.5 ± 5.5	178.8 ± 17.5	12.4 ± 0.01

The average of the values accompanied by different letters present significant differences ($p < 0.05$).

Source. Own elaboration.

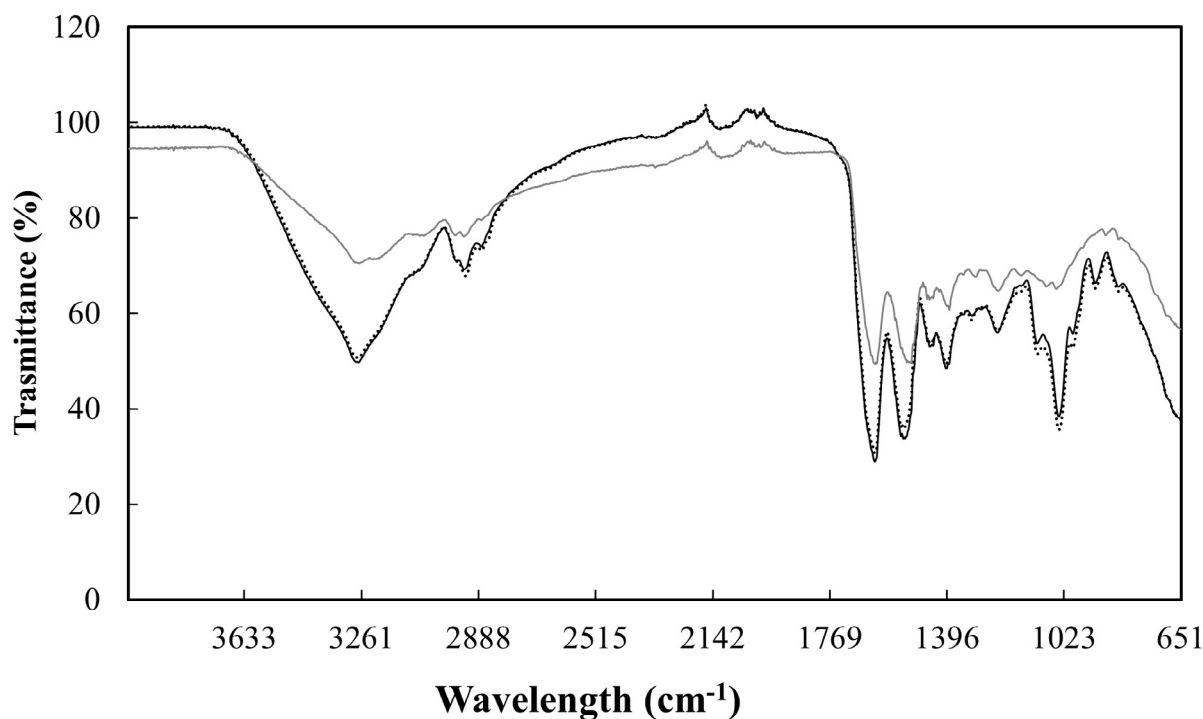
Fourier transformed infrared spectroscopy

The infrared spectra of the films made with and without nanoreinforcement can be observed in Figure 2. The peaks at 3622.4 and 3416.0 cm^{-1} , attributed to vibrations of the hydroxyl group present in the bentonite, cannot be seen in the spectrum (Wang, Zhang, Han, & Bai, 2009); this is because their intensity is too low compared to the values of the O-H and N-H groups (3500 to 3300 cm^{-1}) present in the polymeric films of *Jatropha* and soy proteins, which are capable of forming hydrogen bonds

with the carbonyl groups of peptides present in the proteins (Karnnet, Potiyaraj, & Pimpan, 2005).

The presence of absorption bands can be observed in the range of 800 to 1500 cm^{-1} , related to five peaks corresponding to the vibrations of C-C and C-O present in the glycerol used as a plasticizer in the formation of the films (Guerrero *et al.*, 2010). On the other hand, the peak present in the 1020-995 cm^{-1} interval indicates the presence of the Si-O group of the bentonite nanoreinforcement in the films (Iman & Maji, 2012).

Figure 2. FTIR spectrum of the protein-isolate films with and without bentonite reinforcement



Note. 2.5% jatropha protein (gray line), 2.5% jatropha protein+0.125% bentonite (black line), 5% jatropha protein+0.125% bentonite (dashed line).

Source. Own elaboration.

Conclusions

The incorporation of bentonite nanoreinforcement significantly improved the deformation characteristics of the films made from protein isolates of soy and *Jatropha* cake. The formulation that showed the highest values for mechanical properties was the film obtained with 2.5% *Jatropha* protein isolate, 97.5% soy protein isolate, and 0.0625% bentonite. The interaction of the constituent compounds of the films was verified using infrared spectroscopy. The addition of nanoreinforcement to the film contributed to a significant improvement in barrier characteristics, such as moisture

content (%), swelling (%) and permeability. Although a certain effect on the speed of water vapor transfer was seen, this was not statistically significant with respect to the incorporation of the bentonite nanoreinforcement.

Acknowledgements

Sierra-Sarmiento acknowledges the financial support from UNIAGRARIA. Additionally, the authors acknowledge the Instrumental Analysis Laboratory of the Universidad Nacional de Colombia, Medellín campus, and CENIVAM for the HPLC and FTIR experiments.

Conflicts of interest

The authors declare no conflict of interest.

References

- Abdul-Khalil, H. P. S., Sri-Aprilia, N. A., Bhat, A. H., Jawaid, M., Paridah, M. T., Rudi, D., & ahin-Nadeem, H. (2013). A Jatropa biomass as renewable materials for biocomposites and its applications. *Renewable and Sustainable Energy Reviews*, 22, 667–685. <https://doi.org/10.1016/j.rser.2012.12.036>
- AOAC. (2005). Official Methods of Analysis; Association of Oficial Analytical Chemist. Washington, DC.
- ASTM. (2010). Standard Test metohd for tensile properties of thin plastic sheeting.
- Atarés, L., & Chiralt, A. (2016). Essential oils as additives in biodegradable films and coatings for active food packaging. *Trends in Food Science and Technology*, 48, 51–62. <https://doi.org/10.1016/j.tifs.2015.12.001>
- Chrissafis, K., Antoniadis, G., Paraskevopoulos, K. M., Vassiliou, A., & Bikiaris, D. N. (2007). Comparativestudy of the effect of different nanoparticles on the mechanical properties and thermal degradation mechanism of in situ prepared poly(-caprolactone) nanocomposites. *Composites Science and Technology*, 67 (10), 2165–2174. <https://doi.org/10.1016/j.compscitech.2006.10.027>
- Elfadly, A. M., Zeid, I. F., Yehia, F. Z., Abouelela, M. M., & Rabie, A. M. (2017). Production of aromatic hydrocarbons from catalytic pyrolysis of lignin over acid-activated bentonite clay. *Fuel Processing Technology*, 163, 1–7. <https://doi.org/10.1016/j.fuproc.2017.03.033>
- Garavand, F., Rouhi, M., Razavi, S. H., Cacciotti, I., & Mohammadi, R. (2017). Improving the integrity of natural biopolymer films used in food packaging by crosslinking approach: A review. *International Journal of Biological Macromolecules*, 104, 687–707. <https://doi.org/10.1016/j.ijbiomac.2017.06.093>
- González, A., Strumia, M. C., & Alvarez Igarzabal, C. I. (2011). Cross-linked soy protein as material for biodegradable films: Synthesis, characterization and biodegradation. *Journal of Food Engineering*, 106(4), 331–338. <https://doi.org/10.1016/j.jfoodeng.2011.05.030>
- Guerrero, P., Retegi, A., Gabilondo, N., & De La Caba, K. (2010). Mechanical and thermal properties of soy protein films processed by casting and compression. *Journal of Food Engineering*, 100(1), 145–151. <https://doi.org/10.1016/j.jfoodeng.2010.03.039>
- Iman, M., & Maji, T. K. (2012). Effect of crosslinker and nanoclay on starch and jute fabric based green nanocomposites. *Carbohydrate Polymers*, 89(1), 290–297. <https://doi.org/10.1016/j.carbpol.2012.03.012>

- Karnnet, S., Potiyaraj, P., & Pimpan, V. (2005). Preparation and properties of biodegradable stearic acid-modified gelatin films. *Polymer Degradation and Stability*, 90(1), 106–110. <https://doi.org/10.1016/j.polymdegradstab.2005.02.016>
- Lambrecht, M. A., Deleu, L. J., Rombouts, I., & Delcour, J. A. (2018). Heat-induced network formation between proteins of different sources in model systems, wheat-based noodles and pound cakes. *Food Hydrocolloids*, 79, 352–370. <https://doi.org/10.1016/j.foodhyd.2017.12.032>
- Lee, H. G., Kim, G. H., & Ha, C. S. (2017). Polyimide/amine-functionalized cellulose nanocrystal nanocomposite films. *Materials Today Communications*, 13(August), 275–281. <https://doi.org/10.1016/j.mtcomm.2017.10.010>
- Liu, S., Cai, P., Li, X., Chen, L., Li, L., & Li, B. (2016). Effect of film multi-scale structure on the water vapor permeability in hydroxypropyl starch (HPS)/Na-MMT nanocomposites. *Carbohydrate Polymers*, 154, 186–193. <https://doi.org/10.1016/j.carbpol.2016.08.006>
- Medina-Jaramillo, C., Ochoa-Yepes, O., Bernal, C., & Famá, L. (2017). Active and smart biodegradable packaging based on starch and natural extracts. *Carbohydrate Polymers*, 176(August), 187–194. <https://doi.org/10.1016/j.carbpol.2017.08.079>
- Monteiro, M. K. S., Oliveira, V. R. L., Santos, F. K. G., Barros Neto, E. L., Leite, R. H. L., Aroucha, E. M. M., Silva, R. R., Silva, K. N. O. (2018). Incorporation of bentonite clay in cassava starch films for the reduction of water vapor permeability. *Food Research International*, 105, 637–644. <https://doi.org/10.1016/j.foodres.2017.11.030>
- Osorio-Ruiz, A., Solorza-Feria, J., Chiou, B. Sen, Wood, D. F., Williams, T. G., Avena-Bustillos, R. J., & Martínez-Ayala, A. L. (2017). Effect of montmorillonite clay addition on the morphological and physical properties of *Jatropha curcas* L. and *Glycine max* L. protein concentrate films. *Journal of Applied Polymer Science*, 134(7), 1–11. <https://doi.org/10.1002/app.44459>
- Panseri, S., Martino, P. A., Cagnardi, P., Celano, G., Tedesco, D., Castrica, M., Balzaretto, C., Chiesa, L. M. (2018). Feasibility of biodegradable based packaging used for red meat storage during shelf-life: A pilot study. *Food Chemistry*, 249(August 2017), 22–29. <https://doi.org/10.1016/j.foodchem.2017.12.067>
- Peelman, N., Ragaert, P., De Maulenaer, B., Adons, D., Peeters, R., Cardon, L., Van Impe, F., Devlieghere, F. (2013). Application of bioplastics for food packaging. *Trends in Food Science and Technology*, 32, 128–141. <https://doi.org/10.1016/j.tifs.2013.06.003>

- Popovi, S., Perić, D., Vaštag, Ž., Lazić, V., & Popović, L. (2012). Pumpkin oil cake protein isolate films as potential gas barrier coating. *Journal of Food Engineering*, 110(3), 374–379. <https://doi.org/10.1016/j.jfoodeng.2011.12.035>
- Rhim, J. W. (2011). Effect of clay contents on mechanical and water vapor barrier properties of agar-based nanocomposite films. *Carbohydrate Polymers*, 86(2), 691–699. <https://doi.org/10.1016/j.carbpol.2011.05.010>
- Rhim, J. W., Park, H. M., & Ha, C. S. (2013). Bionanocomposites for Food Packaging Applications. *Progress in Polymer Science*, 38(10–11), 1629–1652. <https://doi.org/10.1016/B978-0-12-811942-6.00018-2>
- Saurabh, C. K., Gupta, S., Bahadur, J., Mazumder, S., Variyar, P. S., & Sharma, A. (2015). Mechanical and barrier properties of guar gum based nano-composite films. *Carbohydrate Polymers*, 124, 77–84. <https://doi.org/10.1016/j.carbpol.2015.02.004>
- Wakai, M., & Almenar, E. (2015). Effect of the presence of montmorillonite on the solubility of whey protein isolate films in food model systems with different compositions and pH. *Food Hydrocolloids*, 43, 612–621. <https://doi.org/10.1016/j.foodhyd.2014.07.022>
- Wang, N., Zhang, X., Han, N., & Bai, S. (2009). Effect of citric acid and processing on the performance of thermoplastic starch/montmorillonite nanocomposites. *Carbohydrate Polymers*, 76(1), 68–73. <https://doi.org/10.1016/j.carbpol.2008.09.021>
- Wang, W., Zhang, H., Jia, R., Dai, Y., Dong, H., Hou, H., & Guo, Q. (2018). High performance extrusion blown starch/polyvinyl alcohol/clay nanocomposite films. *Food Hydrocolloids*, 79, 534–543. <https://doi.org/10.1016/j.foodhyd.2017.12.013>
- Yun, Y. H., Youn, H. G., Shin, J. Y., & Yoon, S. Do. (2017). Preparation of functional chitosan-based nanocomposite films containing ZnS nanoparticles. *International Journal of Biological Macromolecules*, 104, 1150–1157. <https://doi.org/10.3969/j.issn.2095-4344.2017.32.010>
- Zhou, M., Liu, Q., Wu, S., Gou, Z., Wu, X., & Xu, D. (2016). Starch/chitosan films reinforced with polydopamine modified MMT: Effects of dopamine concentration. *Food Hydrocolloids*, 61, 678–684. <https://doi.org/10.1016/j.foodhyd.2016.06.030>

Radish flour obtained by combined osmotic dehydration and microwave drying: Process optimization and application in cookies

Harina de rábano obtenida mediante deshidratación osmótica combinada con secado por microondas: optimización del proceso y aplicación en galletas

Cifuentes-Cruz, L. M. y Villamil-Galindo, J. E.*

Departamento de Ingeniería de Alimentos, Fundación Universitaria Agraria de Colombia, Colombia.

Fecha de recepción: marzo de 2020 / Fecha de aceptación: julio de 2020

Abstract

Radish production in Colombia has increased in recent years; however, its use remains limited to fresh consumption, leading to significant postharvest losses. This study evaluated the optimization of radish flour production through combined osmotic dehydration and microwave drying and its application in cookie formulation. Radish slices were osmotically dehydrated using sodium chloride solutions (10, 20, and 30% w/w) and subsequently dried by microwave radiation. Mass transfer kinetics, color changes, and sensory acceptance were analyzed.

The 20% NaCl treatment showed the most balanced performance in terms of moisture reduction, solid gain, and color preservation. Radish flour was incorporated into cookies at substitution levels of 10, 20, and 30%, with the 10% formulation achieving the highest level of sensory acceptance. Shelf-life evaluation demonstrated superior stability in metallized packaging compared to transparent packaging. These results support radish flour as a viable ingredient for sustainable bakery applications.

Keywords: Radish flour, osmotic dehydration, microwave drying, food valorization, bakery products.

* Correspondencia: Villamilg.johan@uniagraria.edu.co

Resumen

La producción de rábano en Colombia ha aumentado en los últimos años; sin embargo, su uso sigue limitado al consumo en fresco, lo que genera pérdidas significativas en poscosecha. Este estudio evaluó la optimización de la producción de harina de rábano mediante la combinación de deshidratación osmótica y secado por microondas, así como su aplicación en la formulación de galletas. Rodajas de rábano fueron sometidas a deshidratación osmótica utilizando soluciones de cloruro de sodio (10, 20 y 30 % p/p) y posteriormente secadas mediante radiación de microondas. Se analizaron las cinéticas de transferencia de masa, los cambios de color y la aceptación sensorial.

El tratamiento con 20 % de NaCl mostró el mejor desempeño equilibrado en términos de reducción de humedad, ganancia de sólidos y preservación del color. La harina de rábano se incorporó en galletas a niveles de sustitución del 10, 20 y 30 %, siendo la formulación con 10 % la que alcanzó el mayor nivel de aceptación sensorial. La evaluación de vida útil demostró una mayor estabilidad en empaques metalizados en comparación con empaques transparentes. Estos resultados respaldan la harina de rábano como un ingrediente viable para aplicaciones sostenibles en panificación.

Palabras clave: harina de rábano, deshidratación osmótica, secado por microondas, valorización de alimentos, productos de panadería.

Introduction

Radish (*Raphanus sativus* L.) is a widely cultivated horticultural crop whose production in Colombia has shown a progressive increase in recent years. Despite its nutritional value and agronomic relevance, radish utilization remains largely limited to fresh consumption, mainly in salads. Commercial standards based on size, shape, and visual appearance result in the rejection of a considerable proportion of harvested roots, which are commonly discarded as waste. This situation contributes to postharvest losses and environmental impacts associated with organic residue disposal, highlighting the need for processing alternatives that enable value addition and resource efficiency (Asohofructol, 2020).

Dehydration is one of the most widely used preservation techniques to extend shelf life and reduce postharvest losses. Osmotic dehydration is a mild process that partially removes water by immersing food in a hypertonic solution, promoting mass transfer while preserving structural and sensory attributes (Rahman, 2003; Azoubel and Murr, 2004). The efficiency of this process depends on factors such as solute type, concentration, temperature, and immersion time (Rastogi *et al.*, 2000). When osmotic dehydration is combined with microwave drying, the overall process time can be significantly reduced, enabling rapid moisture removal with lower energy consumption and minimizing the risk of surface hardening, especially in products requiring the elimination of relatively small amounts of water (Scaman, 2005; Della *et al.*, 2010).

In parallel, the bakery industry in Colombia relies heavily on imported wheat flour, generating economic dependence and motivating the exploration of alternative flours derived from locally available crops. Partial substitution of wheat flour with vegetable-based flours represents a promising strategy to diversify raw materials while improving nutritional profiles and sustainability (FENALCE, 2019). In this context, radish flour obtained from dehydrated radish offers potential applications in baked goods such as cookies. Therefore, this study aimed to optimize osmotic dehydration and microwave drying conditions for radish flour production and to evaluate its technological performance, sensory acceptance, and storage stability when applied as a partial substitute for wheat flour in cookie formulations.

Materials and methods

Fresh radishes were selected, washed, and sliced into discs of 4 mm thickness. Osmotic dehydration was performed by immersing the slices in sodium chloride solutions at concentrations of 10, 20, and 30% (w/w) for 3 h at ambient temperature (20°C).

Mass transfer parameters, including moisture loss, solid gain, and weight reduction, were determined using a completely randomized design. After osmotic treatment, radish slices were dried using a domestic microwave oven (700 W) for 15 min. Color parameters (L^* , a^* , b^*) were measured using a colorimeter, and sensory evaluation of dehydrated radish samples was conducted using a hedonic scale.

The dried radish was milled to obtain flour, which was incorporated into chocolate chip cookies at substitution levels of 10, 20, and 30% (w/w) of wheat flour.

Sensory evaluation of the cookies was carried out to determine consumer acceptance. Shelf-life studies were performed over 28 days at 16, 25, and 37°C using transparent and metallized packaging, assessing moisture content, fracturability, color changes, and sensory attributes.

Results and discussion

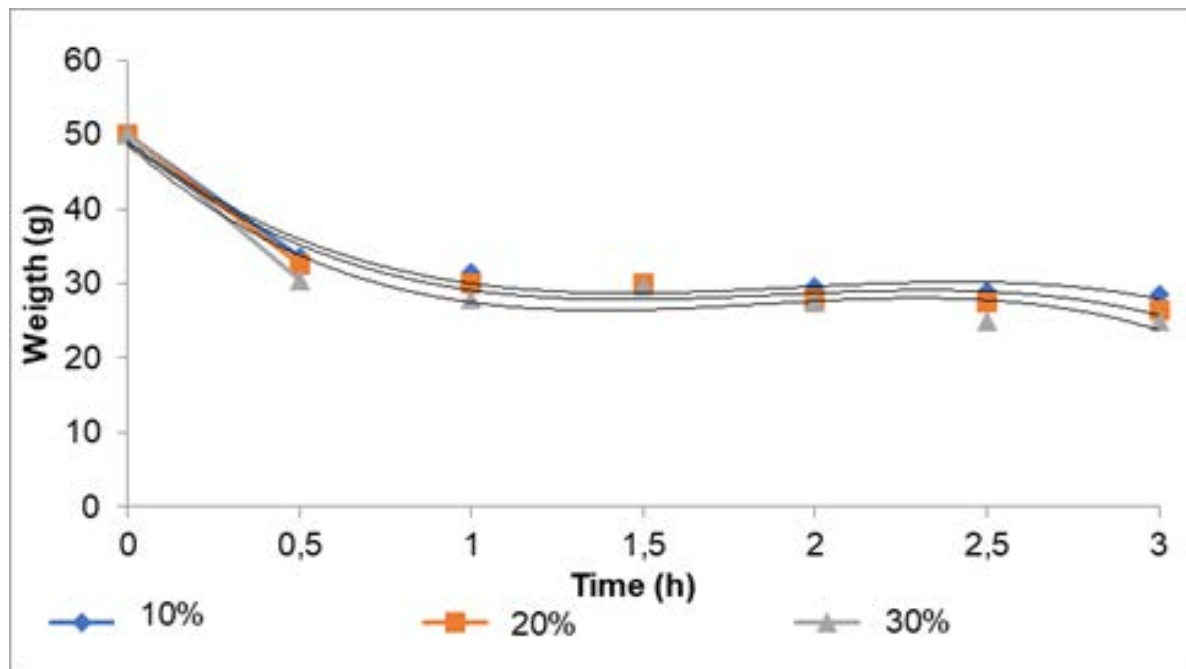
The osmotic dehydration kinetics demonstrated a clear influence of sodium chloride concentration on mass transfer

phenomena. As shown in Figure 1, increasing NaCl concentration promoted higher moisture loss and solid gain during the osmotic process, in agreement with the principles described for hypertonic dehydration systems (Rahman, 2003; Azoubel and Murr, 2004).

However, excessive solute concentration intensified osmotic stress, which negatively affected color attributes, particularly luminosity (L^*).

Among the evaluated treatments, the 20% NaCl solution provided the most balanced performance, achieving efficient water removal while limiting undesirable color degradation, which is a factor strongly associated with consumer perception and acceptance.

Figure 1. Moisture loss vs. time for 10, 20, and 30% NaCl



Source. Own elaboration.

Microwave drying proved to be an effective complementary technique, allowing a rapid reduction of moisture content to values close to 3% for all treatments (Table 1). This result confirms the suitability of microwave technology for products requiring short drying times and low final moisture levels, as previously reported by Scaman (2005). Although samples treated with higher NaCl concentrations exhibited slightly lower residual moisture after microwave drying,

noticeable color deterioration was observed in samples treated with 10% and 30% NaCl. Similar effects have been associated with intensified solute uptake and structural changes caused by osmotic stress, which can exacerbate thermal sensitivity during microwave exposure (Rastogi *et al.*, 2000; Della *et al.*, 2010). These findings further support the selection of intermediate NaCl concentration as the optimal processing condition.

Table 1. Radish moisture content during process

% NaCl	Treatment	% Moisture
0%	Fresh	93.81
10%	Osmotic dehydration	83.3
20%		76.3
30%		70.1
10%	Microwave	3.08
20%		3.01
30%		2.50

Source. Own elaboration.

Regarding product application, cookies formulated with 10% radish flour achieved the highest sensory scores for appearance, flavor, texture, and overall acceptability. Higher substitution levels negatively affected sensory attributes, likely due to increased fiber content and residual salt perception. Shelf-life evaluation showed that cookies packaged in transparent material experienced greater moisture gain, increased fracturability, and more pronounced color changes compared to those stored in metallized packaging (Table 2).

Color stability of cookies containing 10% radish flour was strongly influenced by storage time, temperature, and packaging material over the 28-day evaluation period.

At day 0, cookies exhibited initial CIELAB values of $L^* = 49.17$, $a^* = 7.38$, and $b^* = 20.27$. For samples packaged in transparent material, a progressive reduction in lightness was observed, particularly at elevated temperatures. At 37°C, L^* values decreased from 49.17 to 36.41 after 7 days and remained below 39.0 by day 28, indicating pronounced darkening.

Table 2. Effect of packaging material on the quality attributes of cookies containing 10% radish flour during storage

Packaging material	Moisture change	Fracturability	Color stability (L*, a*, b*)	Sensory acceptance	Overall stability
Transparent	High moisture gain	Significant increase	Noticeable color changes	Decreased over storage	Low
Metallized	Low moisture gain	Minor changes	High color stability	Maintained	High

Source. Own elaboration.

This behavior was accompanied by a reduction in b^* values, from 20.27 to 13.34 at day 28, suggesting loss of yellowness and chromatic instability.

In contrast, cookies stored in metallized packaging showed greater color preservation, with L^* values remaining within the range of 38.0–43.7 throughout storage, even at 25°C and 37°C. Changes in a^* and b^* coordinates were also less pronounced in metallized samples, which maintained b^* values above 16.0 across all storage conditions. These results demonstrate that metallized packaging effectively limited light-induced and moisture-related color degradation.

Considering the combined stability of L^* , a^* , and b^* coordinates, together with supporting moisture, texture, and sensory data, the estimated shelf life of cookies formulated with 10% radish flour was 28 days under ambient storage conditions when metallized packaging was used. In contrast, samples packaged in transparent material exhibited noticeable color deterioration, particularly at higher temperatures, before completing the storage period. This behavior is consistent with the higher light and moisture permeability of transparent materials, which accelerates physicochemical deterioration during storage (Rahman, 2003).

Overall, these results demonstrate that radish flour obtained through optimized osmotic dehydration and microwave drying can be successfully incorporated into bakery products, contributing to raw material diversification and the sustainable valorization of horticultural crops.

Conclusions

Combined osmotic dehydration and microwave drying represent an effective strategy for producing radish flour with suitable physicochemical and sensory characteristics. A sodium chloride concentration of 20% during osmotic dehydration provided the optimal process performance.

Radish flour can successfully replace up to 10% of wheat flour in cookies without compromising consumer acceptance. Metallized packaging is recommended to ensure product stability during storage. This approach contributes to food waste reduction and promotes the valorization of horticultural by-products.

References

- Azoubel, P. M., & Murr, F. E. X. (2004). Mass transfer kinetics of osmotic dehydration of cherry tomato. *Journal of Food Engineering*, 61(3), 291–295. [https://doi.org/10.1016/S0260-8774\(03\)00132-8](https://doi.org/10.1016/S0260-8774(03)00132-8)
- Della, R. F., Serio, A., & de Figueirêdo, R. M. F. (2010). Influence of osmotic dehydration and microwave drying on quality attributes of fruits and vegetables. *Food and Bioprocess Processing*, 88(2–3), 133–141
- ASOHOFRUCOL. (2020). *Plan Hortícola Nacional y diagnóstico del sector hortofrutícola en Colombia*. Bogotá, Colombia.
- FENALCE. (2019). *Panorama productivo y agroindustrial del sector agrícola colombiano*. Bogotá, Colombia.
- Rahman, M. S. (2003). *Handbook of food preservation*. New York: Marcel Dekker.
- Rastogi, N. K., Raghavarao, K. S. M. S., Niranjana, K., & Knorr, D. (2000). Recent developments in osmotic dehydration: methods to enhance mass transfer. *Trends in Food Science & Technology*, 11(2), 48–59.
- Scaman, C. H. (2005). Microwave processing of foods. In D.R. Heldman & D.B. Lund (Eds.), *Handbook of food engineering* (2nd ed., pp. 599–632). Boca Raton: CRC Press.