Laboratory Experiment

Measurement of the Water Vapor Permeability of Chitosan Films: A Laboratory Experiment on Food Packaging Materials

Patricia Cazón, Eduardo Morales-Sanchez, Gonzalo Velazquez, and Manuel Vázquez*
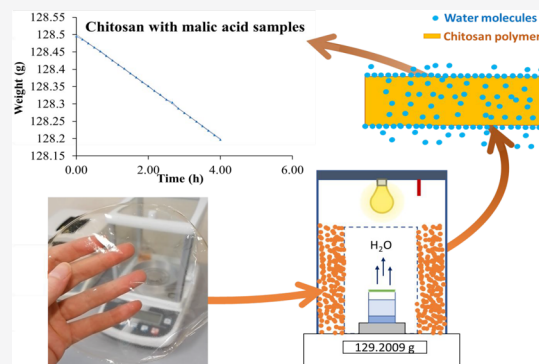
Read Online

| ACCESS | | Metrics & More | | Article Recommendations | | Supporting Information |

ABSTRACT: In recent years, the food industry has been undergoing major changes driven by new social and environmental demands. Nowadays, biodegradable packaging is perceived as the most feasible alternative for food packaging. Great efforts are being devoted to the development of new materials from agro-industrial wastes and byproducts that are opening new alternatives and trends in the biopolymer sector. The aim of this laboratory exercise is to provide a tool to motivate active learning of new technologies and trends in the chemistry of biopolymers. The presented guide introduces to undergraduate students to the basic concepts of polysaccharide-based biopolymers and the characterization of functional properties. This exercise is focused on barrier properties, specifically on water vapor permeability. Chitosan is used as an example of a biopolymeric matrix, and its synthesis and water vapor permeability measurement are described in detail. In addition, a guide for the 3D printing of a temperature-controlled permeability chamber is provided, which will allow the experiment to be carried out in less time as the equilibrium state of the system is not broken when the permeability cup is weighed. It is expected that this exercise and the complementary Supporting Information will be of great interest to biopolymer teaching and research laboratories.

KEYWORDS: Edible Films, Biopolymers, Chitosan, Polysaccharides, Water Vapor Permeability, 3D Printing

INTRODUCTION

The food and drink industry is the largest manufacturing sector in the United States in terms of employment and added value. According to the Bureau of Labor Statistics, there are nearly 1.7 million people employed in food and beverage manufacturing in the United States. The impact of this market is of such significance that the global food and beverage sector is expected to grow from US$5.9 trillion in 2019 to US$7.5 trillion in 2023 (https://agentblog.nationwide.com/commercial-insights/food-and-beverage/consumer-trends-in-food-and-beverage-industry/). Moreover, the food sector employs 4.82 million people in the European Union, generates a turnover of €1.2 trillion, and generates €266 billion in added value. Over the past 10 years, EU food and drink exports have doubled to more than €90 billion and contributed to a positive balance of almost €30 billion (https://ec.europa.eu/growth/sectors/food-and-drink-industry_en; www.fooddrinkeurope.eu).

Nevertheless, the competitiveness and sustainability of the food sector face major global challenges in adapting to current and future social and environmental demands. In the food sector, the packaging industry is one of the industries facing the greatest revolution and innovation. The main challenges for the food packaging industry derive from the environmental problems due to the uncontrolled use and management of nonbiodegradable synthetic plastics for decades. The difficulties and limitations of the recycling processes of nonbiodegradable synthetic materials have led to the need to develop alternative materials with biodegradable properties.

In recent years, research into raw materials to develop alternative packaging with low environmental impact has boosted the interest in using biomass. Biomass materials have several advantages such as they are low cost, nontoxic, edible, and biocompatible. These materials are sometimes a viable alternative to take advantage of agro-industrial wastes or byproducts. Proteins and polysaccharides are the most widely investigated biopolymers in the field of edible or biodegradable coatings and films.[1] The intrinsic properties of the aforementioned materials have opened up new research topics focused on the development of safe films and/or coatings to be applied in contact with food. By using these materials, it is also possible to control the desired migration of active agents such as antimicrobials and natural antioxidant compounds. The

potential applications of these biopolymers in the food industry have prompted a significant evolution and new trends in packaging since the first published article on food packaging materials[2] toward intelligent, active, edible, or nanotechnological packaging.[3−5]

Among the available natural biodegradable materials to develop innovative packaging materials, chitosan has aroused great interest as reflected in the more than 900 articles published between 2015 and 2020 according to the Scopus database.[6] Chitosan is synthesized by deacetylation of chitin (poly(β-(1→4)-*N*-acetyl-D-glucosamine)), one of the most extended polysaccharides on earth. Chitin can be obtained from multiple renewable sources, mainly waste from the seafood industry,[6] being a cheap and commercially available polysaccharide. In the solid phase, chitosan is semicrystalline and soluble in dilute organic acids such as acetic, lactic, formic, malic, and citric, among others.[7−9] The soluble properties of this biopolymer simplify its application in food as a coating or film. The edible coating is applied by dipping the food in a chitosan film-forming solution or by spray, pulverizing the solution using an aerosol spray. The polymer as a film shape is mostly prepared following the casting method. This method basically consists of pouring the film-forming solution onto a flat surface such as a Petri dish or a mold and allowing the evaporation of the solvent. Once the solvent has been completely evaporated, a chitosan film can be easily peeled off the mold.[9]

The simple chitosan film and coating preparations and their antimicrobial properties have promoted several research studies in the reformulation of this polymer to obtain materials that allow guaranteeing food safety and extending the shelf life of food, maintaining good organoleptic properties.[6]

Despite the promising properties of this polysaccharide, it is necessary to analyze and characterize chitosan-based materials to understand the material−food interaction and its suitability for each purpose. For instance, the analyses of the barrier properties of the polymeric matrix are extremely important in the design of packages capable of protecting food quality. The measurement of these properties allows knowing the mass transfer between food and environment through the polymeric matrix of certain molecules such as gases, water vapor, or aromatic compounds. The internal−external exchange of the different components through the polymer package wall causes a continuous variation of the product quality and shortens its shelf life.[10−12]

Water vapor permeability (WVP) is the most studied property of biodegradable films, mainly because of the important role of water in spoilage reactions, keeping food fresh, crisp, or preventing dehydration, and partly because of the simple measurement method which does not require specific equipment as is the case for the measurement of permeability to gases such as O_2 or CO_2.[9] WVP quantifies the amount of water that permeates through the matrix per unit of area, time, and pressure difference (g/s·m·Pa). The standard method ASTM E96 is the most used to study the WVP following a gravimetric method. The WVP parameter will determine the suitability of a specific polymeric matrix for each food or the need to improve the polymeric formulation to extend its applications.

The simple handling and preparation of chitosan-based films can become a useful didactic tool to introduce food science and technology students to biopolymers and edible or biodegradable packaging field. It can be interesting for the

training of future professionals in food packaging. Therefore, this work deals with a laboratory practice guide on the preparation of biodegradable films and measurement of water vapor barrier properties as an introduction to polymer characterization. The aim of this laboratory exercise is to teach students the standard test method for measuring WVP that can be applied to other types of polymeric matrixes and to provide resources for correct data processing and analysis.

In addition, guidance and a design for the 3D printing of a temperature-controlled permeability chamber are provided. This can be useful for laboratories to carry out accurate and automated WVP measurements without the need for expensive equipment or other methods that can slow down experimentation time.

Overall, the purpose of this work is to provide a guide for a laboratory experiment that includes the following: (1) introduction and basic theoretical concepts on biopolymers and water vapor permeability properties, (2) detailed description of the chitosan-based film preparation process (consult the Supporting Information), (3) detailed description for the construction of a temperature-controlled permeability chamber, including the design as a .STL file (consult the Supporting Information), (4) the complete description of the WVP measurement method, (5) explanation of data processing and analysis, (6) sample data (consult the Supporting Information), and (7) the information and documents necessary to develop the exercise as a food packaging laboratory practice described in the Supporting Information.

■ MATERIALS AND METHODS

Chitosan (M_w 100,000−300,000) was purchased from Acros Organics (Geel, Belgium; CAS Number 9012-76-4), DL-malic acid extra pure (CAS Number 6915-15-7) and L-(+)-lactic acid 88−92% extra pure (CAS Number 79-33-4) were provided by Scharlau Microbiology (Barcelona, Spain), and Petri dishes of 210 mm diameter were used to contain the chitosan film-forming solutions. Low-density polyethylene (LDPE) was purchased from the Melitta Group (Germany), and silica gel 2.5−6 mm was provided by Scharlau Microbiology (Barcelona, Spain). The apparatus used were a thickness meter ET115S (Etari GmbH, Stuttgart, Germany), an electronic analytical balance (0.0001 g accuracy; Denver Instrument Co., Arvada, CO), a 3D printer model Colido M2020 (Colido, Hong Kong), ABS and TPU filaments from Colido (Colido, Hong Kong), an incandescent light bulb, and a digital temperature microcomputer thermostat 220 V XH-W3001 with a control switch (Digital Dropsipping Store, Guangdong, China).

Preparation of Chitosan-Based Films

Two chitosan solutions were prepared by using different solvents (malic acid or lactic acid) to observe the dependence of the permeability properties of the polymer on the solvent used. The film-forming solutions were prepared by dissolving 1% (w/w) chitosan in an aqueous solution of 2% (w/v) malic acid or 2% (v/v) lactic acid (Figure 1). For this purpose, 100 mL of distilled water was measured into a beaker and 2 mL of lactic acid or 2 g of malic acid was added. The solution was stirred to facilitate homogenization. Then, 1 g of chitosan was added and left under vigorous stirring for 1 h. A volume of 30 mL of the resulting film-forming solution is poured into a Petri dish and dried for 48 h at room temperature or for 3 h in an air-dryer chamber at 45 °C. This volume (100 mL) is enough to obtain three chitosan films of 210 mm diameter (diameter
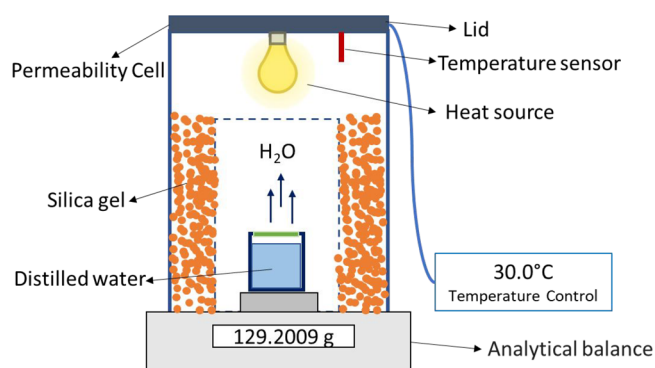
Figure 1. Scheme of the installation of the water permeability chamber designed with a 3D printer.

of the Petri dish). Once the films are completely dried, they are cut in a circular shape with a diameter of 68 mm. The thickness (millimeters) is measured at five random locations by using a thickness gauge with a readability range between 0 and 1000 μm. The average value is taken as the thickness of the sample.

Water Vapor Permeability: 3D Design and Analysis Method

One of the alternatives to carry out this experiment is to store the cups in a desiccator.[16] The main drawback of this procedure is that the desiccator must be opened to weigh the cups and the equilibrium state is broken. Once the cups have been weighed and put back into the desiccator, it is necessary to wait again for the equilibrium state to be reached.

As an alternative, a low-cost double-bottom permeability chamber with temperature control designed to be placed on an analytical balance and a cup permeability are presented in our exercise, as shown Figure 1. The handmade permeability chamber allows measuring the weight variation without opening the chamber and breaking the equilibrium state, reducing the experimental time. Complete instructions are available in the Supporting Information.

The wide-mouth cup (5×10^{-3} m^2 area) was filled with 100 mL of distilled water, taking care to leave a headspace lower than 50 mm between the surface of the water and the film. The cup is sealed with a waterproof lid to avoid water evaporation through the edges. The cup is placed in the center of the balance plate making sure that there is no contact with the chamber walls, the permeability chamber is closed, and the control temperature is set to 30 °C. The closed system is conditioned for at least 3 h to guarantee that the whole system is at the selected temperature at the time of the experiment for the permeability chamber with silica and the cup with water.

Once the system is stable at 30 °C, it is time to place the first sample. The waterproof lid is removed from the cup, the sample is placed between two gasket, and then it is closed with the screw lid to tighten everything together. It is important that the sample is correctly positioned, without cracks or wrinkles, and covering the whole mouth of the cup. The cup is placed again in the center of the analytical balance plate. Tests are run at 30 °C for at least 3 h, time enough to reach the dynamic equilibrium of the water vapor flux. If it is possible, the balance is connected to the computer via the specific software and data are automatically recorded in a spreadsheet. Otherwise, the data will be taken by hand each 10 min.

Weight loss of the cup is recorded to the nearest 1×10^{-4} g and plotted as a function of time. The slope is evaluated by

linear regression ($R^2 > 0.99$). The water vapor transmission rate (WVTR), water vapor permeance, and water vapor permeability (WVP) are calculated according to eqs 1, 2, and 3, respectively:[17,18]

$$\text{WVTR}\left(\frac{g}{s \cdot m^2}\right) = \frac{\Delta w}{\Delta t \cdot A} \tag{1}$$

$$\text{permeance}\left(\frac{g}{s \cdot m^2 \cdot Pa}\right) = \frac{\text{WVTR}}{\Delta P} \tag{2}$$

$$\text{WVP}\left(\frac{g}{s \cdot m \cdot Pa}\right) = \text{permeance} \cdot \text{thickness} \tag{3}$$

where $\Delta w / \Delta t$ (g/s) is the flux measured as weight loss of the cell per unit of time and calculated as the slope of the weight loss of the cup, to the nearest 0.0001 g, versus time; A (m^2) is the actual exposed area determined by the mouth cup diameter; and ΔP (Pa) is the water vapor pressure differential calculated as 4245 Pa at 30 °C[19] assuming a full water vapor saturation in the headspace and a full-dried environment provided by the silica gel. Each test is performed in triplicate.

◾ HAZARDS

Malic and lactic acid should be handled with care. Gloves and protective eyewear are recommended. A 3D printer must be operated with care because it generates high temperatures to melt the plastic filament.

◾ RESULTS AND DISCUSSION

The measurement of the water vapor permeability allows quantifying the amount of water that diffuses through the film per unit of area, time, and pressure gradient (g/s·m·Pa). There are two processes by which gases and vapor may pass through polymeric materials: (1) the morphology of the polymer such as the pores or cracks and (2) the solubility–diffusion effect due to the combination of Fick's law of diffusion and Henry's law of gas solubility. Fick's law can be applied to the water vapor permeation process when the diffusion is assumed to be steady state and there is a linear concentration gradient across the film. On the other hand, Henry's law could be applied when the vapor pressure of the solute varies with concentration in a linear order. The permeability due to the solubility–diffusion effect is described as the real permeability.

According to the solubility–diffusion model, the permeation of a water molecule through the film occurs following a series of phenomena. First, the transport of a water molecule to the film interface takes place, and then the molecule is adsorbed at the film surface facing the zone of higher concentration of water vapor followed by the dissolution of the molecule. The dissolution of the water vapor molecule promotes the diffusion in the direction of the driving force until the dissolution of the molecule occurs in all the film matrix. Finally, the desorption of the molecule takes place on the opposite side of the film and it is transported away from the film surface.[13−15]

The most widely used method to measure the water vapor permeability is ASTM Standard Test Method E96 (https://www.astm.org/Standards/E96.htm), also well-known as the cup method. This method is based on calculating the WVP of a sample by gravimetry. In this method, the selected film sample covers the mouth of a cup. Inside the cup there is distilled water which creates a specific vapor pressure depending on the temperature. The sealed cup is placed in an airtight chamber or
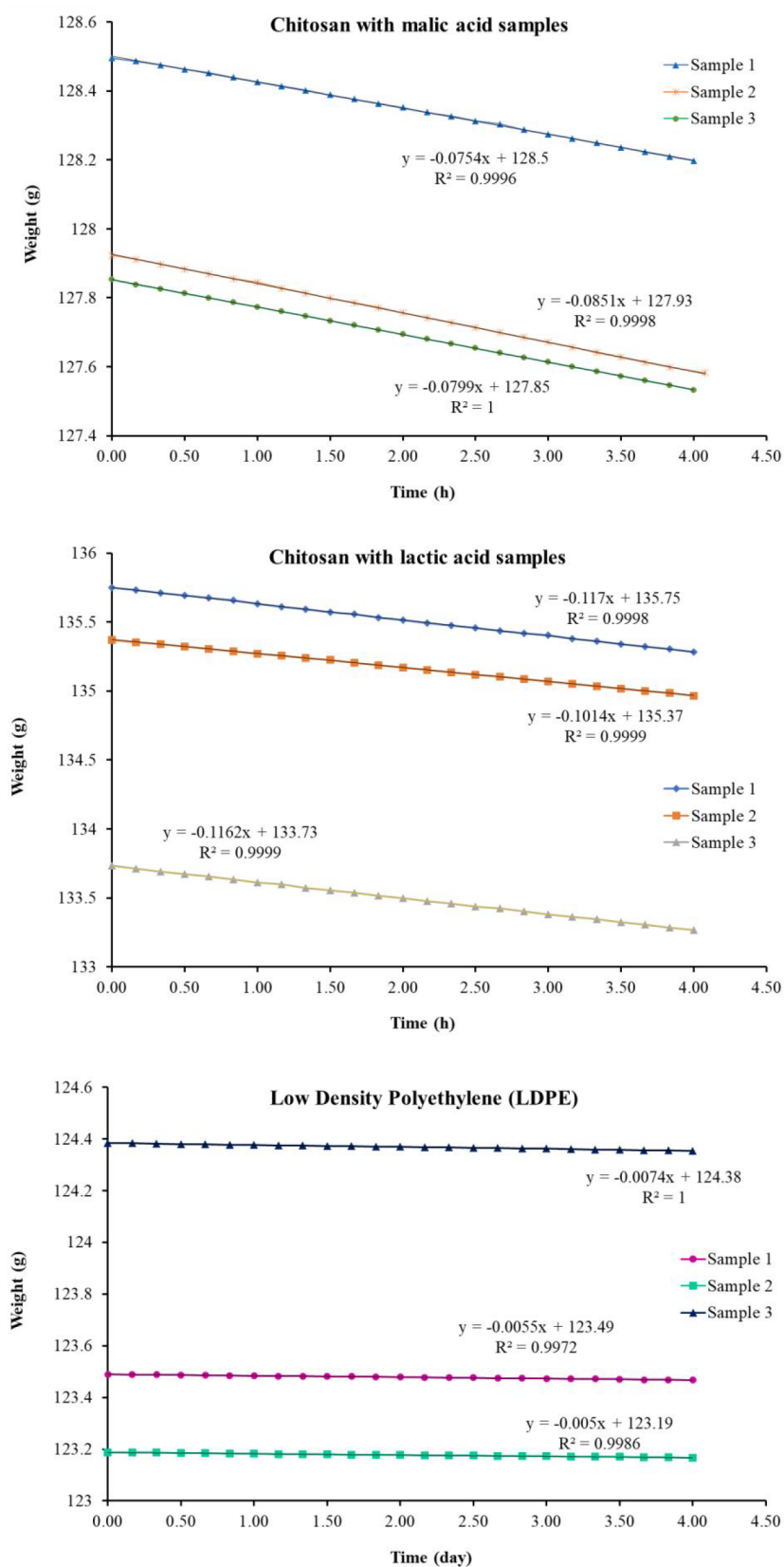
Figure 2. Weight loss versus time data from the water vapor permeability measurement experiment for chitosan samples and low-density polyethylene films as control.

desiccator with silica gel and stored at constant temperature. The partial pressure gradient between both sides of the film due to the different relative humidity produces a driving force that promotes the flow of water through the film. As the cup contains distilled water, the flow of water causes the decrease of the cup weight. Under dynamic equilibrium conditions, the

weight loss of the cup will be constant, and it will be used to calculate the permeability of the film at the given temperature.

In this exercise the water vapor permeability of chitosan films prepared from malic acid or lactic acid solutions as well as low-density polyethylene films have been evaluated to analyze the permeability variation as a function of the used solvent and origin of the polymer. In addition, the results can be discussed by comparing biodegradable polysaccharide-based films with nonbiodegradable synthetic films. From the experimental data obtained, the calculation of the WVP value of each film is detailed step by step.

With the use of a spreadsheet, the cell weight loss over time for each of the samples analyzed was plotted (Figure 2). A template file is provided as Supporting Information. The regression line and the coefficient of determination (R^2) were calculated. The R^2 value must be higher than 0.990.

For the calculation of the permeability parameters, it is necessary to consider the average thickness of each of the samples (m), the ΔP inside and out of the cup at 30 °C (4245 Pa),[19] and the mouth cup area (m^2) that is equal to the film area through which the permeation of the water molecules takes place.

The spreadsheet template with the calculations of the WVP values from the experimental sample data is provided as Supporting Information. First, the slope of the weight loss-time representation is calculated using the formula =ABS(SLOPE-(known_ys; known_xs)) for each sample. Absolute values of slope are used as we are interested only in the variation of weight as a function of time. The values of the last 2 h of the experiment are taken to ensure that the calculations are made with the values of the system at the dynamic equilibrium state. Then, WVTR is calculated by dividing the slope value by the area of the cup mouth. From the WVTR value, the permeance of each sample is calculated by dividing WVTR by ΔP. Finally, WVP is calculated by multiplying the permeance by the thickness of the measured sample.

The sample permeability value shall be presented as the mean of the three samples for each film type and its standard deviation.

In the example, the obtained WVP values were 3.87×10^{-11} $\pm$ 1.14×10^{-12} (g/m·s·Pa) for chitosan samples with malic acid and $5.10 \times 10^{-11} \pm 5.91 \times 10^{-12}$ (g/m·s·Pa) for chitosan samples with lactic acid. The low-density polyethylene films showed WVP values of $6.97 \times 10^{-12} \pm 7.74 \times 10^{-13}$ (g/m·s·Pa). As mentioned, the WVP values were calculated taking into account the data from the last 2 h of the experiment. Overall, the chitosan-based films showed higher WVP values than the synthetic LDPE films, about 1 order of magnitude higher. The WVP difference is explained by the fact that chitosan films, like many other edible polysaccharide films, exhibited relatively low water barrier properties due to their high hydrophilic nature compared to synthetic materials. Chitosan matrix possesses high contents of hydroxyl and amino groups that can interact with water molecules, aiding the permeation of the water molecules through the film matrix.[12]

In this case study, the films produced from lactic acid solutions showed slightly higher WVP values than chitosan film using malic acid as a solvent. Due to the properties of malic acid, there are greater interactions between chitosan and malic acid than between chitosan and lactic acid.[8] Leceta et al.[11] observed that a lower degree of interaction between the components of the matrix caused easier migration of water vapor molecules through the film.

The values reported in the literature must be compared with caution. The WVP values depend on the properties of films such as the molecular weight, degree of deacetylation, and content of chitosan. Besides, these values are affected by various external factors such as the measuring method, the measurement conditions (relative humidity and temperature), correction of the air gap effect, the storage time, and experimental conditions.

■ CONCLUSIONS

This work shows a detailed guide to develop a laboratory experiment for undergraduate students. With this exercise, basic concepts of biodegradable polysaccharide-based polymers can be taught in a dynamic way, taking chitosan-based films as an example. In this way, the preparation of biodegradable polymers from biomass can be taught in a simple way. Besides, this exercise provides knowledge and hands-on training in the initial characterization of permeability properties following the standard method that can be extended to the characterization of other polymers.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.2c00449.

Notes for instructors (PDF, DOCX)

Student handout (PDF, DOCX)

Laboratory report guidelines (PDF, DOCX)

Laboratory timeline (PDF, DOCX)

Questionnaire for students (PDF, DOCX)

Spreadsheet template (XLSX)

■ AUTHOR INFORMATION

Corresponding Author

Manuel Vázquez − *Department of Analytical Chemistry, Faculty of Veterinary, University of Santiago de Compostela, 27002 Lugo, Spain;* ⓞ orcid.org/0000-0002-0392-1724; Email: manuel.vazquez@usc.es

Authors

Patricia Cazón − *Department of Analytical Chemistry, Faculty of Veterinary, University of Santiago de Compostela, 27002 Lugo, Spain*

Eduardo Morales-Sanchez − *Instituto Politécnico Nacional, CICATA unidad Querétaro, Querétaro 76090, México*

Gonzalo Velazquez − *Instituto Politécnico Nacional, CICATA unidad Querétaro, Querétaro 76090, México*

Complete contact information is available at:
https://pubs.acs.org/10.1021/acs.jchemed.2c00449

Author Contributions

Patricia Cazón: writing original draft. Eduardo Morales-Sanchez: design; review editing. Gonzalo Velazquez: methodology; writing review editing. Manuel Vázquez: methodology; writing review.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

■ REFERENCES

(1) Falguera, V.; Quintero, J. P.; Jiménez, A.; Muñoz, J. A.; Ibarz, A. Edible films and coatings: Structures, active functions and trends in their use. *Trends Food Sci. Technol.* **2011**, *22* (6), 292−303.

(2) Morgan, W. L. Retarding Rancidity Colored Transparent Cellulose Wrappers. *Ind. Eng. Chem.* **1935**, *27* (11), 1287−1290.

(3) Ozdemir, M.; Floros, J. D. Active food packaging technologies. *Crit. Rev. Food Sci. Nutr.* **2004**, *44* (3), 185−193.

(4) Embuscado, M. E.; Kerry, C. H. *Edible Films and Coatings for Food Applications*; Huber, K. C., Embuscado, M. E., Eds.; Springer New York: New York, NY, 2009.

(5) Rodríguez-Rojas, A.; Arango Ospina, A.; Rodríguez-Vélez, P.; Arana-Florez, R. What is the new about food packaging material? A bibliometric review during 1996−2016. *Trends Food Sci. Technol.* **2019**, *85*, 252−261.

(6) Flórez, M.; Guerra-Rodríguez, E.; Cazón, P.; Vázquez, M. Chitosan for food packaging: Recent advances in active and intelligent films. *Food Hydrocoll.* **2022**, *124*, 107328.

(7) Rhim, J.-W.; Weller, C.; Ham, K. S. Characteristics of chitosan films as affected by the type of solvent acid. *Food Sci. Biotechnol.* **1998**, *7*, 263−268.

(8) Cazón, P.; Antoniewska, A.; Rutkowska, J.; Vázquez, M. Evaluation of easy-removing antioxidant films of chitosan with Melaleuca alternifolia oil. *Int. J. Biol. Macromol.* **2021**, *186*, 365−376.

(9) Cazon, P.; Vázquez, M. Applications of Chitosan as Food Packaging Materials. In *Chitin and Chitosan: Applications in Food, Agriculture, Pharmacy, Medicine and Wastewater Treatment*; Crini, G., Lichtfouse, E., Eds.; Sustainable Agriculture Reviews 36: Springer International Publishing: Cham, Switerland, 2019; pp 81−123..

(10) Siracusa, V.; Rocculi, P.; Romani, S.; Rosa, M. D. Biodegradable polymers for food packaging: a review. *Trends Food Sci. Technol.* **2008**, *19* (12), 634−643.

(11) Leceta, I.; Guerrero, P.; De La Caba, K. Functional properties of chitosan-based films. *Carbohydr. Polym.* **2013**, *93* (1), 339−346.

(12) Aider, M. Chitosan application for active bio-based films production and potential in the food industry: Review. *LWT - Food Sci. Technol.* **2010**, *43* (6), 837−842.

(13) Figura, L.; Teixeira, A. A. *Food Physics: Physical Properties-Measurement and Applications*; Springer Science & Business Media: 2007.

(14) Robertson, G. L. *Food Packaging. Principles and Practice*, 3rd ed.; CRC Press: Boca Raton, FL, 2012.

(15) Chinnan, M. S.; Park, H. J. Effect of plasticizer level and temperature on water vapor transmission of cellulose-based films. *J. Food Process Eng.* **1995**, *18* (4), 417−429.

(16) Nazan Turhan, K.; Şahbaz, F. Water vapor permeability, tensile properties and solubility of methylcellulose-based edible films. *J. Food Eng.* **2004**, *61* (3), 459−466.

(17) McHugh, T. H.; Avena-Bustillos, F. L.; Krochta, J. M. Hydrophilic Edible Films : Modified Procedure for Water Vapor Permeability and Explanation of Thickness Effects. *J. Food Sci.* **1993**, *58* (4), 899.

(18) Gennadios, A.; Weller, C. L.; Gooding, C. H. Measurement Errors in Water-Vapor Permeability of Highly Permeable, Hydrophilic Edible Films. *J. Food Eng.* **1994**, *21* (4), 395−409.

(19) Wexler, A. Vapor Pressure Equation for Water in Range 3 To 100 Degrees C. *J. Res. Natl. Bur. Stand. - A. Phys. Chem.* **1976**, *80A* (3), 775−785.

▢ Recommended by ACS