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Chitosan films formulated with mintand lemongrass extracts

Brenda Yasmin Avendaño Salinas[1], Nancy Patricia Díaz Zavala[1, 2*], Beatriz Imelda Tijerina Ramos[1], Lorena Margarita Salas Ordaz[1], Laura Isela Montoya Ortiz[1], Patricia Mendoza Guzmán[1]

[1]*Tecnológico Nacional de México, Instituto Tecnológico de Ciudad Madero, Avenida Primero de Mayo Col. Los Mangos, Ciudad Madero Tamaulipas, México*

[2]*Centro de Investigación en Petroquímica, TecNM/Instituto Tecnológico de Ciudad Madero, Prol. Bahía de Aldair Avenida Las Bahías Parque Tecnia, Altamira Tamaulipas, México*

Corresponding Author:Nancy Patricia Díaz Zavala

ABSTRACT :*The antimicrobial activity of chitosan-starch films with natural extracts of mint and lemongrass was studied.The aqueous extracts were more effective than the essential oils. The extracts were added at different concentrations showing that as the concentration increases, the coloring of the films, the characteristic functional groups as well as the optical properties of the films intensify.*

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I. INTRODUCTION

The development of biodegradable films has been very useful in numerous applications due to the versatility of their properties and environmental factors. Chitosan is considered non-toxic and a polymer biologically compatible with living beings [1]. Starch is the raw material most used in the production of biodegradable films, mainly because it is renewable, abundant and relatively easy to handle. It is used mainly to reduce gas exchange between the environment and food [2]. The hydrophilic character of these films gives them a brittle appearance caused by high intermolecular forces, using plasticizers such as glycerol, to increase the flexibility of the films due to their ability to reduce the internal hydrogen bonds between the polymer chains while increasing the molecular space [3]. Natural antimicrobial agents derived from sources, such as plant extracts, have been recognized and used for centuries. However, in recent years research into the antimicrobial properties of these substances has increased. The extracts consist of the non-volatile fraction of the active principles, that is, those that are not volatile or unstable with temperature, cannot be obtained by distillation, but are obtained by various extraction techniques such as: HPLC, Extraction in supercritical fluids, steam drag, extraction by solution, extraction by centrifugation. In this research work the antimicrobial effect on bacteria and fungi was evaluated in chitosan-starch films by adding aqueous extracts and essential oils of mint and lemongrass as well as their optical and chemical properties.

II. MATERIALS AND METHODS

2.1 Chitosan-starch-natural extracts films

The chitosan solution (2%, w / v) was prepared by dispersion. The aqueous extracts were prepared by heating at 65 °C for 5 min and allowed to cool at 25 °C. Both extracts were subsequently filtered.

2.2Pre-contamination tests

This test was performed to know if the films were contaminated before starting with microbial inhibition tests, since, if so, this could affect the following tests. The procedure was carried out by placing 5 ml of distilled water in a tube, sterilized and a 1 cm^2 square of the film was placed inside it, stirred and left to rest for a minimum of 24 hours. It was planted, placing as sample 1 ml of the water contained in the tubes with the film, with the respective agar (either for mesophilic aerobes, total coliforms and fungi), observing the growth according to the norms indicated for each microorganism.

2.3 Antimicrobial tests

Once the chitosan-starch films with and without natural antioxidants were obtained, the antimicrobial efficacy of each elaborated polymer was analyzed, to which the culture method of pouring in plate was applied, using dextrose-potato agar, bile agar red violet and agar for standard method. For antimicrobial tests against total coliforms and fungi, it was necessary to make several dilutions in order to facilitate the colony count. The colony forming units (CFU) were counted with the naked eye. Previously, the handling material was sterilized in a dry oven for 2 hours at 150 °C.

2.4 Characterization methods

The analysis of the properties and characteristics of the chitosan-starch films with natural antioxidants was carried out by characterization methods such as optical properties by UV-visible Spectroscopy (UV-vis) and Fourier Transform Infrared Spectroscopy (FTIR).

III. RESULTS AND DISCUSSION

3.1 Synthesis of the films

The chitosan-starch films with natural antioxidants of mint and lemongrass showed diverse colorations and translucency. This is attributed to the coloration that each extract gives to the chitosan-starch mixture and the type of extract as well as the amount added.Films with lemongrass extract are translucent and flexible with yellow coloration, mint films are more rigid and have a brown coloration that increases in relation to the increase of the different amounts of extract.

3.2 Pre-contamination tests

Once the planting of microorganisms was finished and after the incubation period of each one (48 hours for aerobic mesophiles, 24 hours for total coliforms and 120 hours for fungi), the following results were obtained in triplicate, indicated in table 1.Contaminated films are those that comply with the following specifications:

• For aerobic mesophilics more than 25 colonies, total coliforms 1 or more colonies, fungi more than 10 colonies.

Table 1 shows that most films showed acceptable levels of previous contamination except for those of 7% extract (QSM7 and QSZL7) in total coliforms. The film that presented the best antimicrobial properties with respect to the QSB white is that of 10% lemongrass (QSZL-10).

Table 1. Previous contamination tests with aqueous extract of mint and lemon grass.

Films	Aerobic Mesophilic(CFU)	TotalColiforms (CFU)	Fungi (CFU)
QSB-1	13	0	3
QSB-2	10	0	5
QSB-3	20	0	5
QSM5-1	7	0	7
QSM5-2	4	0	5
QSM5-3	6	0	0
QSM7-1	3	0	4
QSM7-2	6	1	4
QSM7-3	4	0	5
QSM10-1	20	0	9
QSM10-2	4	0	2
QSM10-3	15	0	2
QSZL5-1	0	0	3
QSZL5-2	0	0	4
QSZL5-3	10	0	6
QSZL7-1	4	0	5
QSZL7-2	0	1	3
QSZL7-3	0	0	3
QSZL10-1	0	0	1
QSZL10-2	0	0	0
QSZL10-3	15	0	6

Q: chitosan **S**:starch **M**: menthe **ZL**: lemongrass **5,7,10**: percentage of added extract (v/v)

In Table 2 can be observed that the films containing natural extract of mint and lemongrass generally inhibit aerobic mesophilic, coliforms and fungi in comparison to the chitosan-starch film without extract used as reference. The film QSM3eo does not act on fungi but on aerobic mesophilic and coliforms. The film with 0.5% aqueous mint extract works against the three types of microorganisms compared to the QSA white and those made with essential oil.

Table 2. Antimicrobial tests with aqueous extracts and essential oils of mint and lemongrass.

Film	Aerobic Mesophilic		Coliforms		Fungi	
	CFU	Inhibition halo (mm)	CFU	Inhibition halo (mm)	CFU	Inhibition halo (mm)
QSA	>25	0	70	0.5	7	0
QSZL5eo	>25	0.5	14	1	2	0
QSM3eo	>25	1	3	1	11	0.5
QSM0.5	>25	1	0	1.5	7	0.5

3.3 Optical properties

In most cases, as shown in Table 3, the higher the concentration of the extract, the lower the transparency value, except for 7% in both extracts. It was observed that the addition of extracts increases the opacity value which indicates that the addition of natural extracts of 5% and 10% mint (QSM5 and QSM10) and 10% lemongrass (QSZL10) improves the light barrier properties.

Tabla 3. Optical properties of films with aqueous extract of mint and lemongrass.

Film	% T at 280 nm	% T at 400 nm	Transparency	Opacity
QSZL5-1	13.51895618	33.38127899	10.1	0.0648317
QSZL5-2	7.423197746	23.73913002	9.46	
QSZL7-1	8.791810036	32.88636398	9.19	0.0622202
QSZL7-2	10.72001839	35.79272079	9.34	
QSZL10-1	6.318483353	16.85806465	8.82	0.0926009
QSZL10-2	7.079891205	26.81677055	10.104	
QSM5-1	0.178687841	8.861348152	8.597	0.1091273
QSM5-2	0.268797189	11.41510582	8.76	
QSM7-1	0.038203247	12.58364964	10.899	0.0659185
QSM7-2	0.002899084	10.24201679	10.95	
QSM10-1	0.002663952	3.601636648	8.86	0.1019776
QSM10-2	0.026661236	3.660832167	8.88	

3.4 Fourier Transform Infrared Spectroscopy

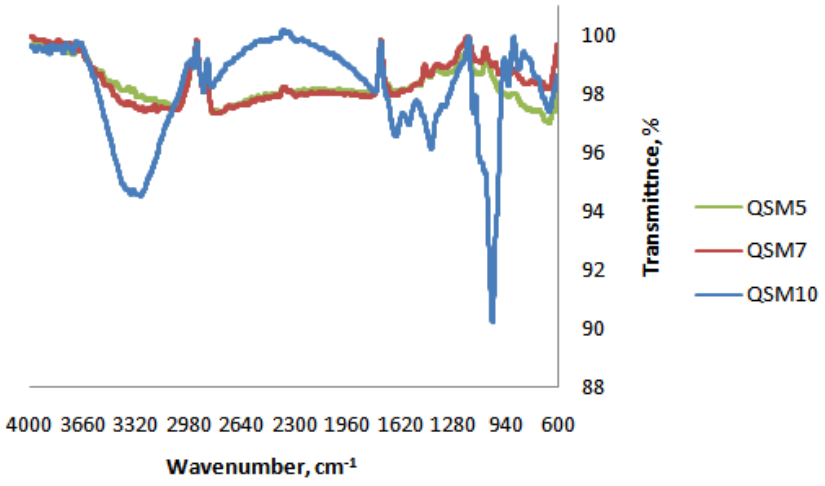

Figure 1. FTIR spectrum of the chitosan-starch film with aqueous extract of mint.

In Figure 1 it was considered that the band at 3500-3200 cm^{-1} is attributed to compounds containing OH bonds. In 1660 cm^{-1} the stretching C = O was observed; in 1575 cm^{-1} the flexion of the N-H group of the primary amines was presented; at 1360 cm-1 the C-N stretch of the amide group III of the chitosan; in 1160 cm^{-1} the glucosidic bond C-O-C was observed; in 1080 cm^{-1} the stretch C-O; and the stretching of the C-O-C group at 1000 cm^{-1}.

IV. CONCLUSIONS

The films with natural aqueous extracts of mint and lemongrass with contents of 5 and 10% extract (v / v)showed better antimicrobial properties in the preliminary and optical tests. The films made with essential oil showed greater contamination in the antimicrobial tests, therefore the mint aqueous extract has greater biocidal power at a lower concentration (QSM0.5). Based on the results obtained in the FTIR analysis it is possible to deduce the presence of antioxidants on the films attributed to the phenolic groups in the hydroxyl group region due to the broadening of the band.

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