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# Active packaging material based on buriti oil – *Mauritia flexuosa* L.f. (Arecaceae) incorporated into chitosan films

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**ABSTRACT:** Active and biodegradable materials have great potential in food packaging applications, improving the safety and quality of products. The objective of this study was to develop a new material based on buriti oil incorporated into a chitosan film. Different concentrations of buriti oil in dried films (2.1 g/m$^2$, 10.4 g/m$^2$, 20.8 g/m$^2$, and 31.3 g/m$^2$) were added into a chitosan matrix (41.7 g/m$^2$). The chitosan/buriti oil films were characterized by water-vapor barrier properties, total water-soluble matter (TSM), tensile properties, thermogravimetric analysis, microstructure, microbial permeation properties, and biodegradation estimation. The higher oil concentration improved the water-vapor barrier and the buriti oil acted largely as a plasticizer and increased the elongation at break, and decreased the tensile strength (TS) of chitosan films. The total water-soluble matter of chitosan films decreased in function of the buriti oil concentration, but the biodegradation and thermal stability increased. The chitosan films presented a microbial barrier against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 43210.

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## INTRODUCTION

Active packaging is an innovative food packaging concept, in which bioactive compounds are incorporated into packaging materials in order to improve the safety, maintain the quality, and extend the shelf life of food products.[1] Different active functions could be provided, including antioxidant activity, antimicrobial activity, and scavenging of compounds like oxygen, moisture and ethylene, emission of ethanol or flavor. In addition, the bioactive compounds could improve the mechanical barrier properties[2] and reduce the synthetic additives in food products.[3,4]

Chitosan is a deacetylated form of chitin, a natural biopolymer largely found in the exoskeleton of crustaceous, in fungal cell walls and other biological materials. Chitosan is an excellent film forming a linear polymer with a rigid backbone structure that consists of $\beta$-(1-4)−2-acetoamido-2-deoxy-D-glicose units. It is described in terms of the degree of deacetylation and average molar mass.[5] Chitosan films are resistant for handling,[6] flexible, and difficult to tear. Most of its mechanical properties are comparable to many medium-strength commercial polymers.[7] Chitosan films have great potential as food packaging materials, owing to the facilitated biodegradation and gas barrier.[8–12] The poor water-vapor barrier is associated with the hydrophilic characteristic of chitosan macromolecules.[8–13] The three-dimensional chitosan matrix incorporates different bioactive compounds such as rosemary,[14] lemon, cinnamon, and thyme essential oils.[15]

Buriti (*Mauritia flexuosa* L.f) is an endemic palm tree in the northern Brazilian region. The pulp of its fruit is orange and contains high concentrations of $\beta$-carotene,[16] vitamins A, B, C, and E, minerals (calcium, iron), and proteins. The oil is cold pressed and extracted from the fruit pulp.[17] Flavonoids are the main polyphenols present in extracts of buriti, mainly glycosylated flavonoids and anthocyanins, which are responsible for its antioxidant capacity.[18]

Buriti pulp contains a high level of 18:2 (linoleic acid), and buriti nuts have a higher content of 18:3 (linolenic acid).[19] The fatty-acid profile of the oil extracted from buriti pulp presents a high content of 18:1 (palmitic acid, approximately 73.3–78.7%) and low content of 18:2 (2.4–3.93%).[16,20,21] Batista *et al.*[22] observed antimicrobial activity of buriti oil in gram-positive and gram-negative bacteria. Nazif[23] and Silveira *et al.*[24] indicated that the saturated and unsaturated fatty acids could contribute to the antimicrobial activity against some pathogenic bacteria. The high antimicrobial activity of buriti against *S. aureus* and *P. aeruginosa* was associated with the mature

**Table I.** Composition of Chitosan Dried Films Containing Different Buriti Oil Concentrations

| | Chitosan solution | | Chitosan dried films | |
|---|---|---|---|---|
| Film formulation | Chitosan concentration (%, w/w) | Buriti oil (%, w/w) | Chitosan concentration (%, w/w) | Buriti oil (%, w/w) |
| Control | 2.0 | 0.0 | 100.0 | 0.0 |
| F0.1 | | 0.1 | 95.2 | 4.8 |
| F0.5 | | 0.5 | 80.0 | 20.0 |
| F1.0 | | 1.0 | 66.7 | 33.3 |
| F1.5 | | 1.5 | 57.1 | 42.9 |

epicarps and mesocarps, extracted with ethanol, and partitioned with hexane and ethyl acetate. The antioxidant activity of buriti oil extract was related to the high content of carotenoids.[18] Buriti oil presents high oxidative stability, owing to the high content of tocopherol.[16]

The aim of this work was to develop an active packaging material based on buriti oil (bioactive compound) incorporated into a biodegradable and renewable matrix film (chitosan). The chitosan-film system was characterized by the color parameters, water-vapor permeability (WVP), mechanical properties, total water-soluble matter (TSM), microstructure, thermal analysis, microbial permeability, and biodegradation.

## MATERIALS AND METHODS

### Materials

Chitosan (Polymar, Brazil) has a degree of acetylation (DA) of 18% and molar mass of $1.47 \times 10^5$ g mol$^{-1}$. Acetic acid (Synth, Brazil) and buriti oil (Mapric®, Brazil) were also used.

**Microorganisms.** The microorganisms *Pseudomonas aeruginosa* (ATCC 15442), *Staphylococcus aureus* (ATCC 13150), and *Escherichia coli* (ATCC 11229) were purchased from the Oswaldo Cruz Foundation FIOCRUZ (Manguinhos, Rio de Janeiro, Brazil). The strains were stored in brain heart infusion broth (BHI) solution and 20% glycerol at −80°C. All media cultures were purchased from Acumedia (Brazil).

### Methods

**Preparation of Chitosan Film Containing Buriti Oil.** Chitosan suspensions were prepared based on Yoshida *et al.*[8] Chitosan (2.0 g/100 g of total suspension) was dispersed in aqueous acetic acid. The addition of acetic acid was stoichiometrically calculated to reduce the acetic acid smell in dried chitosan films. The stoichiometric amount of acetic acid was calculated taking into account the DA and the mass of chitosan, in order to achieve protonation of all $NH_2$ sites of chitosan macromolecule. The suspension was stirred with a magnetic stirrer for 1 h. The buriti oil was added in chitosan solution at different concentrations of 0.1, 0.5, 1.0, and 1.5% (w/w), which were named F0.1, F0.5, F1.0, and F1.5, respectively. The final solid content of chitosan and buriti oil in different dried films was presented in percentages in Table I. The suspension was ultra-homogenized at ambient temperature at 20,000 rpm with an UltraTurrax homogenizer (T25, IKA, Germany) for 10 min. Films without buriti oil represented the control chitosan films (41.7 g/m$^2$ of chitosan concentration in dry basis). About 0.21 g/cm$^2$ of suspension was poured into plastic Petri dishes and dried at 40°C in an air-forced oven (Tecnal, TE-394/2, Brazil) for approximately 12 h. The dried films were removed from the plane support and pre-conditioned in a desiccator at 25° ± 1°C and 75 ± 5% rh.[8]

**Film Thickness.** Film thickness was measured using a micrometer (Mitutoyo Mfg. Co., Japan) and measurements were taken at five random positions on the film. The average values were used to calculate the film properties.

**Color Parameters.** The color parameters of chitosan films were measured using a Chroma Meter CR 400 colorimeter (Konica Minolta, Japan). The calibration was carried out using a white calibration plate. Measurements were performed using the CIE-Lab system. The parameter $L^*$ represents the lightness of the colors from 0 (dark) to 100 (light), $a^*$ the greenness/redness (negative $a^*$ is green and positive $a^*$ value is red), and $b^*$ the grade of blueness/yellowness (negative $b^*$ is blue and positive $b^*$ is yellow). There were three replicates per experiment. The color difference ($\Delta E$) was calculated using eq. (1).

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

where:

$$\Delta L^* = L^* - L_0^*,\ \Delta a^* = a^* - a_0^*,\ \Delta b^* = b^* - b_0^*.$$

$L_0^*$, $a_0^*$ and $b_0^*$ were values of control chitosan films.

**Water-Vapor Permeability.** The water-vapor permeability (WVP) of chitosan films was determined using ASTM E96-02,[25] a gravimetric standard method. The films were fixed on the top of test cells containing a desiccant (silica gel). The test cells were placed in a BOD incubator (Tecnal, TE-371, Brazil) with a controlled temperature and relative humidity (25°C and 75% RH). Permeation cells were weighed before and at least four times after the incubation, and then the acquired weight was used to calculate the WVP. Five specimens were tested for each film type.

**Moisture Content.** The moisture content of the films was determined by measuring the weight loss of films upon drying in an oven (Tecnal, TE 394/1, Brazil) at 105°C until a constant weight was reached (dry sample weight).[11]

**Total Water-Soluble Matter.** The percentage of total water-soluble matter (TSM) was determined according to the method

adapted of Hosseini *et al.*[26] Samples (2.0 cm in diameter) were immersed in 100 mL of distilled water and kept under orbital agitation (40 rpm) at 25°C (Infors® HT, Multitron Standard, Switzerland). After 24 h, the samples were removed from the solution and dried in a forced-air oven (Tecnal, TE 394/1, Brazil) at 105°C for 24 h, and then they were re-weighed. The analyses were carried out in triplicate. The TSM was calculated as follows:

$$TSM = 100 \times \left( \frac{S_o - S}{S_o} \right) \quad (2)$$

where $S_0$ is the initial dry matter (g) and $S$ the insoluble dry matter (g).

**Thermogravimetric Analysis.** Thermogravimetric analyses were applied to the chitosan films using a Thermogravimetric analysis (TGA) analyzer (model DTG-60, Shimadzu®). Samples of chitosan films (10–20 mg) were heated from 30 to 700°C at 10°C $min^{-1}$ under a nitrogen flow (50 mL/min).

**Mechanical Properties.** The tensile strength (TS), Young's modulus ($E$), and percentage elongation at break ($\varepsilon_r$) were determined as specified in ASTM-D882.[27] Films were cut into 25.4 × 100.0 mm strips and then pre-conditioned at 75% RH and 25°C for 48 h. The TS and $E$ values were measured with a TexturePro CT V1.2 (Brookfield®, CT3 50K Texture Analyzer). The initial grip separation was set at 50 mm and the crosshead speed at 1 mm/s. There were at least 10 replicates per experiment.

**Scanning Electron Microscopy.** Scanning electron microscopy (SEM) analysis was performed on fractured cross-sections of gold-sputtered films using a LEO 440i scanning electron microscope (LEO Electron Microscopy) with 10 kV and 100 pcA.

**Infrared Spectroscopy.** Fourier-transformed infrared (FTIR) spectra of chitosan films were obtained using an FTIR spectrometer (IRPrestige21, Shimadzu) with an attenuated total reflection (ATR) device, which allows us to obtain information about the chemical structure of the surface of films. The range of the wave number was from 650 to 4000 $cm^{-1}$, and each sample was scanned 32 times.

**Antimicrobial Properties.** ***Microbial permeation.*** Film discs (2.5 cm in diameter) were sterilized using exposition in UV light in sterile Petri dishes for 5 min on each side. The samples were fixed at the top of permeation cells with sterile TSB (Tryptic Soy Broth, Oxoid®) media. The positive control was the permeation device completely sealed with Parafilm. The negative control was the permeation device without a seal. The permeation devices were incubated at room temperature (25±5°C) for 10 days. The visual turbidity of TSB media indicated the microbial permeation through the chitosan film. There were five replicates per formulation film.

***Induced microbial permeation.*** The induced microbial permeation method was similar to that described in the Microbial permeation section, but the microorganisms (*Escherichia coli, Staphylococcus aureus*, and *Pseudomonas aeruginosa*) were inoculated directly at the chitosan film surfaces. The bacteria were diluted to obtain a stock solution with a working concentration of the $10^6$ UFC/mL. Sample suspensions of 200 μL were directly applied to the chitosan film (1.5% of buriti oil and control film) at the top of the permeation device. The flasks were incubated at room temperature (25° ± 5°C) for 10 days. The turbidity of the TSB media indicated the microbial permeation through the chitosan film. There were three replicates per formulation film.

**Biodegradation Test in Soil.** The methodology of the biodegradation test in soil was adapted from Kato-Jr *et al.*[28] Dried samples (1.0 ± 0.2 g) of chitosan films were pre-conditioned at 75 ± 5% RH for 24 h. The samples were enclosed separately in nylon bags (10 cm × 10 cm), and then they were buried to about 10 cm in sand latosol (Coxim, MS, Brazil) inside a plastic recipient. The moisture content of the soil was controlled by sprinkling a controlled volume of water. Periodic gravimetric measurements (24 h) were performed over 35 days using an analytical balance (Shimadzu®, AY220, Japan). The biodegradation was calculated using eq. (3). There were five replicates per treatment.

$$B = \left[ \frac{m_i - m_f}{m_i} \right] \times 100 \quad (3)$$

where $B$ is the biodegradation after 35 days (%), $m_i$ is the initial weight of chitosan films (g), and $m_f$ is the final weight of chitosan films (g).

**Statistical Analysis.** Statistical analysis was carried out using Action 2.6 software (Estatcamp®, São Carlos, Brazil). The differences between the mean values were detected by the Tukey multiple comparison test.

## RESULTS AND DISCUSSION

Chitosan films with different contents of buriti oil were homogeneous, flexible, and had no visible defects (macropores) (Figure 1).

The color parameters ($L^*$, $a^*$, and $b^*$) were measured (Table II). Control chitosan films (without buriti oil) were naturally yellow. Increasing the buriti oil in the filmogenic matrix formulation caused the films to become more yellow, as shown by the higher values of $b^*$.The presence of buriti oil changed the luminosity of chitosan films. Buriti oil presents high concentrations of carotenoids, mainly β-carotene.[29] A similar result was obtained by Rubilar *et al.*[11] by incorporating carvacrol and grape seed extract into a chitosan film matrix. In comparison to the control film, the highest color difference ($\Delta E$) of the chitosan films was associated with the presence of β-carotene in buriti oil.[16]

Chitosan films were quite insoluble after immersion in water represented by lower total water-soluble matter (TSM) values. At the beginning of the swelling process, chitosan films maintained their original form. After 24 h immersion, the films seemed like gels. The solubility of the biopolymer could predict the biodegradability of a film and its application in food products.[30,31] It is related to the hydrophilicity of the biopolymer material.[32]


**Figure 1.** Chitosan films with different contents of buriti oil in dry compositions (g/m$^2$): (a) control, (b) 2.1, (c) 10.4, (d) 20.8, and (e) 31.3. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The average thickness of the chitosan films, TSM (total water-soluble matter), water-vapor permeability (WVP), and moisture content (X) are presented in Table III. The TSM values of chitosan films were lower than 9%, which is a low value for biopolymer films. Pereda *et al.*[33] found 100% TSM of a gelatin film and Bourtoom and Chinnan[30] found about 33% TSM for a starch/chitosan film (ratio 2:1). Buriti oil addition increased the hydrophobicity characteristic of chitosan films, reducing the affinity for water molecules, thereby reducing the TSM values. Krochta and Mulder-Johnson[34] also obtained low water solubility for chitosan films.

The moisture content of chitosan films reduced significantly in F1.0 and F1.5 samples. Higher concentrations of buriti oil increased the hydrophobicity of chitosan film, which reduced the moisture content. It could be associated to the less available regions to the water sorption of the polymer matrix.

According to Krochta and Mulder-Johnson,[34] the flexible films are classified as a function of WVP values. Films that present a WVP value between 0.4 and 4.2 g mm/m$^2$ h kPa are considered poor, whereas they are considered moderate between 0.004 and 0.4 g mm/m$^2$ h kPa and good between $4 \times 10^{-4}$ and $4 \times 10^{-3}$ g mm/m$^2$ h kPa. In this work, chitosan films are classified to have a moderate water-vapor barrier. The WVP was reduced by about 12% for the F1.5 samples when compared to the control films.

The thermograms of chitosan films presented at least two thermal events (Figure 2). The first peak (ca. 80°C) resulted from an evaporation of water process, related to the hydrophilic nature of the polysaccharide matrix. Control films presented a higher weight loss than chitosan/buriti oil films, that it could be associated to a more hydrophobic chitosan/buriti oil matrix films than control film. Water molecules are associated with chitosan macromolecules through hydrogen bonds between the hydroxyl and amino groups.[35] The second peak (270–350°C) was related to dehydration, depolymerization, and pyrolytic decomposition of the chitosan chain.[36] Chitosan film F0.1 presented a similar thermal behavior to the control film. Abdelrazek *et al.*[37] identified a melt temperature of the chitosan films (170–190°C) in the second stage of the thermal profile. Lin and Pascall[38] indicated that the chitosan decomposition occurs between 270 and 320°C.

Chitosan films F0.5, F1.0, and F1.5 showed a third peak (peak 3 at around 430°C) associated with the decomposition of the phenolic compounds present in the buriti oil composition. Schlemmer and Sales[39] observed that the buriti oil showed decomposition at 420°C and an endothermic peak at 407°C.

As the buriti oil concentration increased, the plasticity of the films improved, the elongation at break ($\varepsilon_r$) increased, and the tensile strength (TS) is reduced (Table IV). This could suggest that buriti oil acts as a plasticizer. The control films presented a higher rigidity ($E$) as compared to chitosan/buriti oil.

Mechanical properties are related to the chemical structure of biopolymer films.[40] The chitosan film had higher TS and lower elongation at break. However, adding plasticizer (glycerol), the films became more flexible, decreasing the TS, and increasing $\varepsilon_r$.[41] Plasticizer penetrates through the biopolymer matrix, interfering with the chitosan chains. This could decrease the intermolecular attraction and increase the mobility of chitosan chains.[41]

**Table II.** Color Parameters (L*, a*, and b*) and Color Difference (ΔE) of Chitosan Films Containing Different Buriti Oil Concentrations in Dried Films

| Film formulation | Buriti oil concentration in dried films (g/m$^2$) | L* | a* | b* | ΔE |
|---|---|---|---|---|---|
| Control | 0.0 | $93.43 \pm 1.24^a$ | $-0.99 \pm 0.28^a$ | $7.09 \pm 1.66^a$ | - |
| F0.1 | 2.1 | $93.03 \pm 0.68^a$ | $-0.82 \pm 0.48^a$ | $7.58 \pm 0.86^a$ | $1.09 \pm 0.78^a$ |
| F0.5 | 10.4 | $92.47 \pm 0.96^a$ | $-3.71 \pm 0.63^b$ | $17.99 \pm 2.90^b$ | $11.31 \pm 3.00^b$ |
| F1.0 | 20.8 | $90.71 \pm 1.45^b$ | $-6.56 \pm 0.48^c$ | $33.62 \pm 3.85^c$ | $27.27 \pm 3.96^c$ |
| F1.5 | 31.3 | $88.39 \pm 1.73^c$ | $-6.26 \pm 0.45^c$ | $40.99 \pm 3.09^d$ | $34.75 \pm 4.23^d$ |

Values are expressed as means ± standard deviation. Different letters in the same column indicate significant differences ($P<0.05$) according Tukey Test.

**Table III.** Average Thickness, Water-Vapor Permeability (WVP), Total Water-Soluble Matter (TSM), and Moisture Content (X) of Chitosan Films with Different Buriti Oil Concentrations

| Film formulation | Average thickness (mm) | WVP (g mm/m$^2$ h kPa) | TSM (%) | X (%) |
|---|---|---|---|---|
| Control | $0.06 \pm 2.40^a$ | $0.33 \pm 0.01^a$ | $8.79 \pm 0.62^a$ | $22.38 \pm 2.91^a$ |
| F0.1 | $0.06 \pm 4.52^a$ | $0.33 \pm 0.02^a$ | $6.83 \pm 1.00^b$ | $21.88 \pm 3.45^a$ |
| F0.5 | $0.07 \pm 5.49^a$ | $0.33 \pm 0.01^a$ | $6.22 \pm 1.00^b$ | $20.87 \pm 2.88^a$ |
| F1.0 | $0.07 \pm 4.91^a$ | $0.31 \pm 0.02^a$ | $6.06 \pm 1.04^b$ | $15.71 \pm 2.68^b$ |
| F1.5 | $0.08 \pm 8.97^b$ | $0.29 \pm 0.01^a$ | $5.85 \pm 0.79^b$ | $13.32 \pm 0.89^b$ |

Values are expressed as means ± standard deviation. Different letters in the same column indicate significant difference ($P<0.05$) according Tukey Test.

The study of the microstructure was conducted using SEM in the cross-section topography of the chitosan films (Figure 3). The images showed a homogeneous, compact, continuous structure of the chitosan films (control) without any large pores. The buriti oil microparticles were uniformly distributed in the filmogenic matrix. No microphases or bilayer films were observed. The emulsion process was performed by an ultra-homogenizer, so it is possible that the microparticles of buriti oil reduced the interactions between the chitosan chains. This could decrease the intermolecular bonds and promote the mobility of the filmogenic matrix, increasing the flexibility of chitosan films.

The FTIR spectra of buriti oil and chitosan films are shown in Figure 4. When chitosan and buriti oil were homogenized, it was not possible observe new bands on FTIR spectra, indicating no chemical interactions between components. It is possible that the buriti oil droplets were entrapped into chitosan matrix film, which was also observed in Figure 3. The buriti oil composition is a mixture of fatty acids, tocopherols, and carotenoids. The spectrum of buriti oil showed a band at approximately at 1749 cm$^{-1}$, corresponding to carboxylic group stretching (C=O), which is around the same frequency as methylic esters and triglycerides. At 1449 cm$^{-1}$, a band was associated to deformation and vibration of $CH_2$ was observed. The broad band ranging from 1290 to 1040 cm$^{-1}$ was attributed to fatty acids with chemical structure $[CH_3(CH_2)_n]COOH$, with $n$ value between 14 and 16, like palmitic acid, which is the second more abundant fatty acid in buriti oil. Figure 4 presents the corresponding peaks of the FTIR spectra of buriti oil incorporated into the chitosan matrix film.

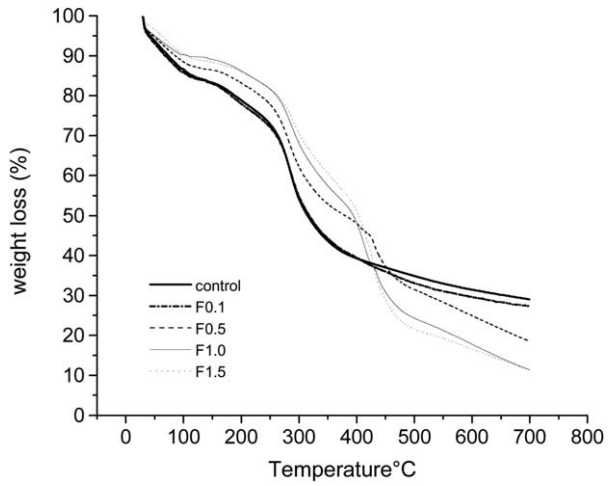


**Figure 2.** Thermograms analysis of buriti oil/chitosan film in an $N_2$ atmosphere.

Typical bands of chitosan films were observed, according to previously published data. Comparing the chitosan film (control) spectrum with the spectra of buriti oil/chitosan films, it was possible to verify an increase in the intensity of the band at 2925–2857 cm$^{-1}$. It was attributed to the stretching of C–H ($CH_2$ and $CH_3$), indicating a higher content of ester groups, which are provided by the buriti oil. The same behavior was observed at 1749 cm$^{-1}$, which was associated with the methyl esters and triglycerides present in buriti oil. A variation in the ester-group band intensity was observed in the spectra of F0.1, F0.5, F1.0, and F1.5 when compared to the control film spectra.

The microbial barrier indicated that all of the films act as a total barrier against microorganism permeation. The total permeability to microorganism diffusion was evaluated over 10 days. The positive control (without barrier) presented medium turbidity after 4 days of exposure; after the day 8, initial fungal formation on the media surface was observed. The negative control (Parafilm barrier, totally sealed) did not show any media contamination.

The total barrier of the chitosan films to microorganisms was confirmed by inoculating the microorganisms (*E. coli, S. aureus*, and *P. aeruginosa*) on the films surfaces (F1.5) as barrier and TSB media inside the permeation device (Figure 5). No contamination on the media surface was observed over 10 days for any of the chitosan films used. The microbial impermeability was

**Table IV.** Mechanical Properties of Chitosan Films Containing Different Concentrations of Buriti Oil

| Film formulation | TS (MPa) | $\varepsilon_r$ (%) | E (MPa) |
|---|---|---|---|
| Control | $21.85 \pm 3.40^a$ | $10.56 \pm 2.62^a$ | $1.03 \pm 0.13^a$ |
| F0.1 | $20.28 \pm 5.48^a$ | $12.79 \pm 1.92^a$ | $1.02 \pm 0.12^a$ |
| F0.5 | $20.30 \pm 2.00^a$ | $17.52 \pm 2.20^b$ | $0.79 \pm 0.43^b$ |
| F1.0 | $20.21 \pm 1.45^a$ | $17.97 \pm 3.11^b$ | $0.36 \pm 0.08^b$ |
| F1.5 | $16.92 \pm 1.67^b$ | $22.39 \pm 4.10^c$ | $0.15 \pm 0.08^c$ |

Values are expressed as means ± standard deviation. Different letters in the same column indicate significant differences ($P<0.05$) according Tukey Test.

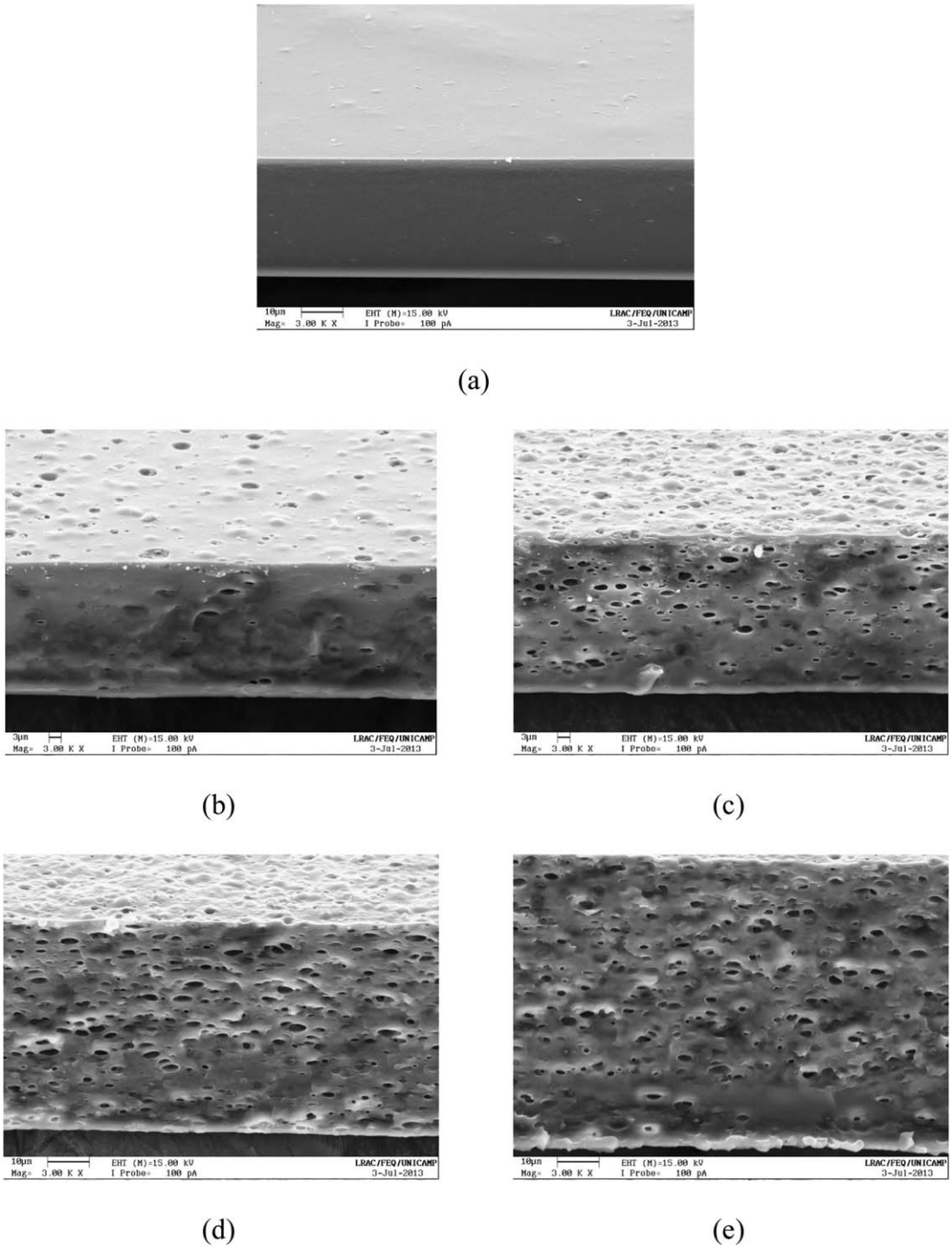


**Figure 3.** SEM micrographs of cryo-fractured cross-section of chitosan films viewed at a magnification of 3000×: (a) control, (b) F0.1, (c) F0.5, (d) F1.0, and (e) F1.5.

confirmed in this experiment. Chitosan has stronger bactericidal effects for gram-positive bacteria than gram-negative bacteria.[42]

This phenomenon was associated with the compact and cohesive structure of the chitosan films observed in the SEM images. In this methodology and in the buriti oil concentrations applied in our work, it was not possible to verify an additional antimicrobial barrier related to the buriti oil presence.

The antimicrobial activity of buriti against the phenolic compounds showed weak and moderate activities against the tested bacteria *S. aureus* and *P. Aeruginosa*.[18]

Chitosan/buriti oil films present a great potential for applications as food packaging materials, which require a total barrier (impermeability) to gram-negative bacteria such as *E. coli* and *P. aeruginosa*, as well as gram-positive bacteria such as *S. aureus*.

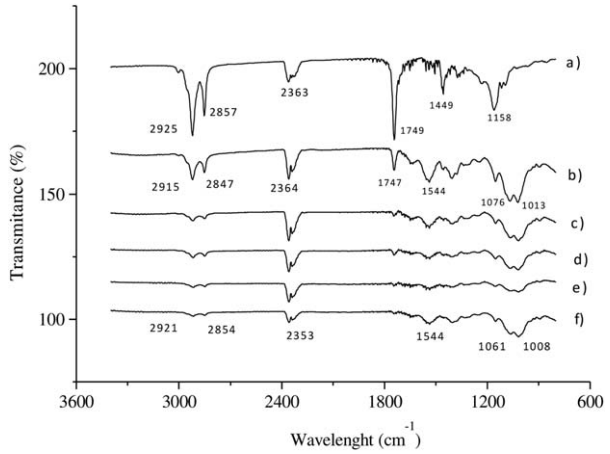


**Figure 4.** FTIR spectra of (a) buriti oil and chitosan films: (b) F1.5, (c) F1.0, (d) F0.5, (e) F0.1, and (f) control.

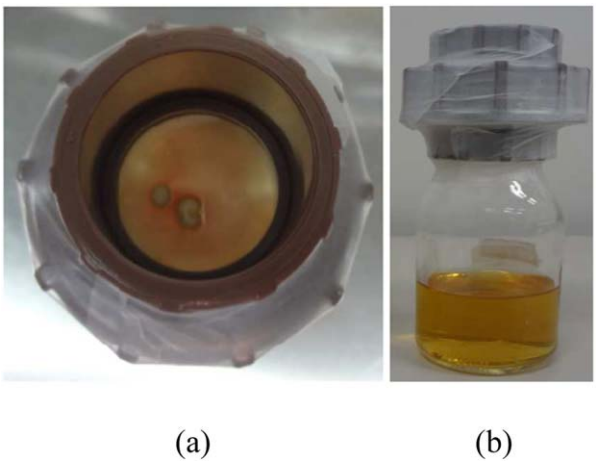


**Figure 5.** Images of microorganism permeation experiments: (a) Inoculated chitosan film (F1.5) applied on the system of microbial barrier experiment and (b) F1.5 system after 10 days of inoculated chitosan film experiment. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

In general, the initial biological contamination occurs at the surface of food products.

The biodegradation study was conducted *in vitro* using a simple method (variation of film weight and visual aspect). The main objective of the biodegradation study was to verify the behavior of this new material buried in soil and to identify whether it is possible to classify it as a biodegradable material. After 24 h, all of the chitosan films studied shrank and crinkled, which was associated with water absorption from the moisture of soil that increased the weight of the films samples.

The weight gain was observed in all chitosan film formulations over 10 days of being buried in soil. Although, it was lower in chitosan films containing buriti oil. The hydrophobic compound present in the filmogenic matrix reduced the water affinity of chitosan/buriti oil films. After the day 10, a weight-loss process of chitosan films was observed. The control, F0.1, F0.5, and F1.0 films presented the highest weight loss compared with F1.5. Fungi growth on the film surface was also observed in the control and F0.1 films. Dean *et al.*[43] verified white mycelium growth (fungi or actinobacteria) in chitosan films (1%, w/v) after 2 weeks of soil incubation. Figure 6 illustrates the visual aspect of chitosan films over 20 days of the biodegradation process.

The biodegradation of chitosan films with or without buriti oil could be considered fast compared to synthetic polymeric films. The influence of the buriti oil concentration was observed based on the degradation behavior of chitosan films. After 35 days of burial in soil, the biodegradation processes of the control and F0.1 (contains 4.8% (w/w) of buriti oil in chitosan matrix dried film) were similar (Figure 7).

The biodegradation of F0.5, F1.0, and F1.5 were significantly different. The film F1.5 (contains 57.1% (w/w) of buriti oil in chitosan filmogenic dried matrix) presented the lowest biodegradation (8% of the initial weight) after 35 days of being buried in soil, as compared to other film formulations (control film: 40% of the initial weight). The thickness of F1.5 was statistically higher than the other formulations. The lowest biodegradation could be due to its larger thickness.

The relative humidity and temperature have great importance in microorganism activity, which can attack and degrade the biopolymers.[44] Mostafa *et al.*[45] studied the biodegradation of chitosan films (50% deacetylated) on different soil types. After 30 days on sandy soil, the weight reduction of the chitosan film was around 33% and, after 4 months, the film was 100% biodegraded in all soil types. Under loamy soil, chitosan films showed 48% biodegradation after the first month and, in sandy-loam soil, the degradation was 39% after the first month of study,

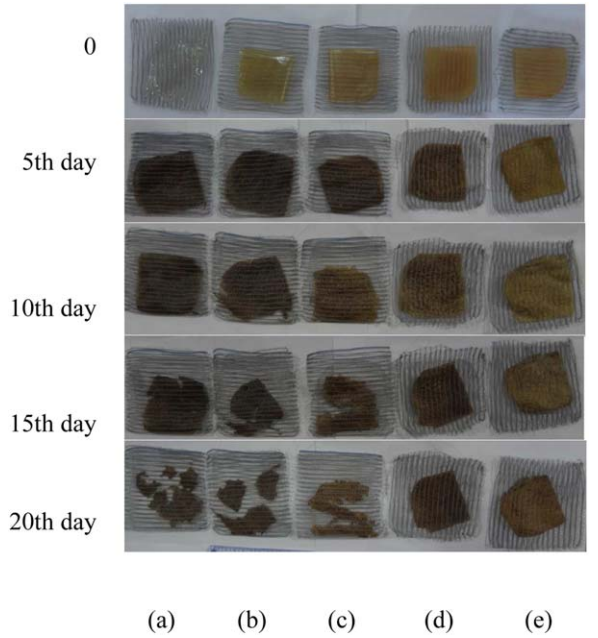


**Figure 6.** Visual aspect of chitosan films during twenty days of biodegradation process in soil: (a) control, (b) F0.1 (c) F0.5 (d) F1.0, and (e) F1.5. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

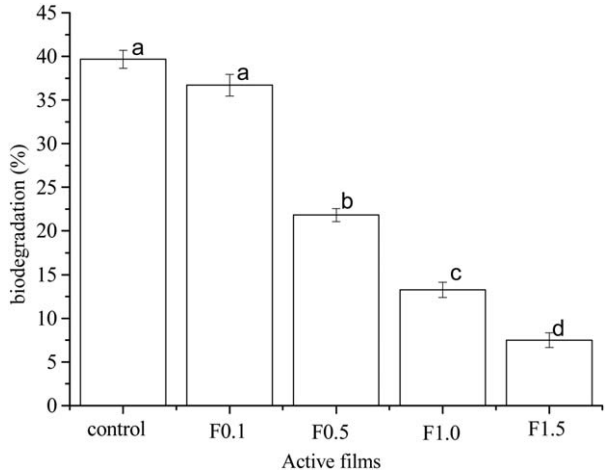


**Figure 7.** Biodegradation of chitosan films after 35 days buried in soil.

which is close to the result and conditions observed in the 35-day period of this work.

## CONCLUSIONS

Chitosan films containing different buriti oil concentrations were characterized as a new material for application in active food packaging. Chitosan/buriti oil films presented homogeneous and cohesive structures with a uniform distribution of buriti oil particles. The buriti oil presence promoted film flexibility and higher WVP and lower water solubility values. The chitosan/buriti oil films presented a total barrier for gram-negative and gram-positive microorganism permeation. The easy biodegradation of chitosan/buriti oil films in soil was also observed.

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