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Physical and antimicrobial properties of quinoa flour-based films incorporated with essential oil

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ABSTRACT: Films of quinoa flour (*Chenopodium quinoa*, W) incorporated with oregano (*Origanum vulgare* L.) and thyme (*Thymus vulgaris* L.) essential oils (EOs) at 0.5%, 1%, and 2% p/p were prepared to examine their physical and mechanical properties and antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*. The type of EO was not significant for the physical and barrier parameters of the films. The increase in the EOs concentration led to an increase in the elongation at break, but decrease in the tensile strength, Young's modulus, solubility and water vapor permeability. Films containing 1% and 2% EOs exhibited an inhibitory effect on the growth of *S. aureus* and *E. coli*. However, *S. aureus* was more sensitive to both EOs and the oregano was more efficient in the inactivation to both microorganisms. The incorporation of EOs as natural antibacterial agents may lead to their potential application as active packaging materials to increase food safety. © 2016 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 43311.

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INTRODUCTION

The majority of packaging used by industry comes from nondegradable polymers, the increase in waste and the difficulty in recycling most of the available synthetic packaging, has led to increased research into the development of new biodegradable materials that are suitable for packaging.[1–3] The use of agricultural biopolymers for the development of edible and/or biodegradable films could be an alternative that could create new markets.[4]

Proteins and polysaccharides are well known for being good film formers. Protein films are characterized by good mechanical properties, although they are usually quite permeable to water and gases.[5] Starch is the most widely employed polysaccharide for film production because it is naturally abundant and inexpensive. Starch films present good mechanical properties, but their sensitivity to moisture is a major drawback.[6,7] To overcome this problem, researchers have turned to natural mixtures of starch, protein, lipids, and fibers, which can be obtained in the form of flour from raw plant materials, such as cereals and legumes.[3,7–9]

Several authors have reported the potential application of flours from whole materials, such as amaranth,[5,8,10,11] soy,[2,12] wheat,[13,14] rice,[7,15] and achira flour,[3] for film production.

The quinoa seed (*Chenopodium quinoa*, W) is a small grain (~3 mm diameter) found typically in the South American Andean highlands, and it is composed of significant amounts of starch (~80%), which has an amylose content of 10–21% (depending on the variety).[16,17] Quinoa is described as a seed with a high protein content of 12–23% (depending on the variety) and a highly recommendable amino acid balance for human consumption.[18] Moreover, quinoa presents a balanced content of lipids and fiber, making this grain highly promising for the development of biodegradable edible films for applications in the food industry.

In addition to the significant use of nonbiodegradable packaging, another problem confronting the food industry is food spoilage, which is caused by microbial contamination. Studies have demonstrated that antimicrobial agents could be effective for reducing the levels of foodborne organisms when incorporated into packaging films.[19] Packaging that contain biodegradable polymers have the possibility of being carriers of different additives, such as antimicrobial, antioxidant, nutraceuticals and flavorings agents.[20] The use of biodegradable edible films containing antimicrobials has been demonstrated as a useful stress factor tool to protect foodstuff against spoilage flora and to decrease the risk of pathogen growth.[21]

Essential oils (EOs) from many aromatic spices (oregano, thyme, salvia, parsley, clove, coriander, garlic, and onion) have natural antimicrobial and antioxidant properties that could potentially extend the shelf life of food.[19,20,22–25] However, the effect of these active components on the structure and functionality of the films should be evaluated.[26] Therefore, the combination of production technologies for biodegradable packaging with active antibacterial technologies may be a good alternative to lengthen the shelf life and improve the quality and safety of food.[24]

The aim of this work is to develop and characterize biodegradable/edible quinoa flour films and to analyze the effect of essential oil incorporation on the mechanical, optical, and barrier properties and on the antimicrobial activity.

MATERIALS AND METHODS

Materials

The films were formulated with flour Quinoa (*Chenopodium quinoa* W), which was acquired in the local market and was originally from Bolivia, glycerol (MERCK®) as plasticizer and essential oils of were rosemary (*Rosmarinus officinalis*), cloves (*Eugenia* caryophyllata), oregano (*Origanum vulgare* L.), sage (*Salvia officinalis*) and thyme (*Thymus vulgaris* L.) that were acquired from Verbhena® from Rio Grande do Sul, Brazil.

Quinoa Flour Production and Proximate Composition

The grains were first washed at least four times in an excess of deionized water to remove the saponins that cover the quinoa seeds, which are totally soluble in water.[16] Afterward, the grains were dried in an oven with forced air circulation (DeLeo B5AFD, Brazil) at 40°C for 24 h. The quinoa flour was produced by grinding the seeds using a benchtop mill (Arbel, model MGR90, Brazil). The resulting flour was sifted on 150 mesh sieves and used for the preparation of the films. The protein, ash, lipid, total fiber, and water contents were determined using standard AOAC methods (AOAC, 1995). The carbohydrate content was determined by the difference. The moisture content was determined by desiccation of the sample in an oven at 105°C (DeLeo, model TLK 48, Porto Alegre, Brazil). All of the analyses were performed in triplicate, and the results are expressed as grams per 100 g of flour in dry matter (DM).

Selection of Essential Oils

The selection of EOs to be used in the films was determined by preliminary tests. The tested EOs were rosemary (*Rosmarinus officinalis*), cloves (*Eugenia caryophyllata*), oregano (*Origanum vulgare* L.), sage (*Salvia officinalis*) and thyme (*Thymus vulgaris* L.). Antimicrobial activity of the oils was tested by the filter paper disc diffusion method,[23] culture of *Staphylococcus aureus* (ATCC 1901) with 10^6 CFU mL^{-1} was spread on *Trypticase Soy Agar (TSA,* HiMedia, India) (0.1 mL/plate). One sterile 10 mm diameter filter paper disc was placed in the center of inoculated plate and 10 μL of EOs was carefully pipetted onto it. All of the plates were incubated at 37°C for 24 h, and the results were visually observed for the zone of inhibition.

Film Preparation

The films were prepared using the *casting* method. The film-forming solution was prepared with a suspension of 6% quinoa flour (6 g 100 g^{-1} of film-forming solution), and then it was homogenized for 30 min at 50°C to solubilize the protein. The solution was then heated to the processing temperature of 82°C with gentle stirring for 30 min in a water bath for gelatinization of starch. The film-forming solution was cooled to 40°C, and the plasticizer (1% w/w film solution) and essential oils (selected according to preliminary test) in concentrations of 0% (control), 0.5%, 1%, and 2% (w/w), was added stirred for 5 min in homogenizer (Ultra Turrax T25 DS1, disperser S25N - 25G, IKA® Germany/Deutschland). Then, 0.24 g cm^{-2} of the film-forming solution was poured evenly onto acrylic plates. The films were dried in an oven with forced air circulation (DeLeo B5AFD, Brazil) at 35°C for 16 h.

Film Characterization

The thickness of the films was measured with a digital micrometer (DIGIMESS Precision: 0.001 mm, resolution: 0 mm ~25 mm, Brazil). To determine the mechanical properties, solubility and water vapor permeability, the film samples were preconditioned at 25°C and 58% relative humidity.[1]

Mechanical Properties. The films were cut into strips (80 mm × 25 mm), and the thickness was measured using a micrometer (Precision: 0.001 mm, resolution: 0 mm–25 mm) at five random positions on each strip. The tensile strength (TS) [MPa], percent elongation at break (EB) [%] and Young's modulus (YM) [MPa] were evaluated using a tensile test performed on a texture analyzer (TA.XT2i e Stable Micro Systems, UK) with a load cell of 5 kg and using an A/TGT self-tightening roller grip fixture, according to ASTM D882-09 (2009). Ten strips were cut, and each one was mounted between the grips of the equipment for testing. The initial distance between the grips and the set test speed were 50 mm and 0.8 mm s^{-1}, respectively.

Solubility. The moisture content (MC) of films was determined by oven-drying at 105 ± 2°C for 24 h, and it is expressed as the percentage of the initial film weight lost during drying. The solubility was calculated as the percentage of dry matter in the film that was solubilized after immersion in water. Film discs (2 cm in diameter) were cut and weighed in metal capsules, and then added to 30 mL of distilled water, and placed in shaking water bath (Mark DGM, São Paulo, Brazil) and slowly and periodically agitated in speed 1 (30 cycles per minute), for 24 h at 25°C. The amounts of the dry matter in the initial and final samples were determined by drying the samples at 105°C for 24 h. The solubility was calculated using eq. (1).[1]

$$S\,\% = 100[wi - wf/wi] \quad (1)$$

where w_i is the initial dry weight of the sample (g) and w_f is the final dry weight of the sample (g).

Water Vapor Permeability (WVP). The samples were placed in permeation cells (inner diameter: 63 mm, height: 25 mm), filled with granular anhydrous calcium chloride and hermetically sealed. The permeation cells were placed in a glass chamber with a saturated sodium chloride solution, providing RH gradients of 0 to75% at 25°C. The mass gain was monitored for 24 hours by weighing the permeation cell on an analytical balance (AY 220, Shimadzu). The water vapor permeability of the samples was determined in triplicate using eq. (2).[27]

$$WVP = w.L/A.t.\Delta p \quad (2)$$

where w is the weight of the water the permeated through the film (g), L is the thickness of the film (m), A is the permeation area (m^2), t is the time of permeation (s), and Δp the water vapor pressure difference between the two sides of the film (3167,46 Pa a 25°C). The WVP is expressed in g m^{-1} s^{-1} Pa^{-1}

Optical Properties (Color and Opacity)

The opacity was determined by measuring the film absorbance at 210 nm (UV region UV) and 500 nm (visible region) using a UV spectrophotometer (Shimadzu UV-1800). The films were cut into a rectangle and directly placed in a spectrophotometer test cell. An empty test cell was used as the reference. The opacity of the films was calculated by dividing the values of the absorbance (nm) by the thickness of the film (mm).[28]

The color change of the films was determined by the color difference (ΔE) with a colorimeter (Hunter Lab system, model Miniscan XE, USA) operated with D65 (daylight) and using the CIELab color parameters. The parameters L^* (luminosity), a^* (red-green), and b^* (yellow-blue) were determined. A white disk ($L_0^* = 97.5$; $a_0^* = 0.13$; and $b_0^* = 1.7$) was used as the standard. The ΔE was calculated using eq. (3).[29]

$$\text{Eq. (3)} \quad \Delta E = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2}$$

where $\Delta L^* = L^* - L_0^*$, $\Delta a^* = a^* - a_0^*$, $\Delta b^* = b^* - b_0^*$ in which L_0^*, a_0^*, and b_0^* are the color values of the standards and L^*, a^*, and b^* are the film color values.

Morphological Properties

The morphological properties of the films containing the oregano and thyme essential oils were studied using scanning electron microscopy (SEM). The images were obtained using a scanning electron microscope JSM 5800 LV, JEOL (Tokyo, Japan), connected to a secondary electron detector for energy dispersive X-ray spectroscopy (EDS). The films were cut and pasted onto double-sided conducting tape on an aluminum support and coated with a thin film of platinum using a Balted SCD 050 Sputter Coater (Scotia, NY). The micrographs were obtained with a magnification of 550× at an accelerating voltage of 5 kV. Several images were taken from different points on the surface of the film to ensure that the final image was representative of the entire sample.

Thermal Stability

The thermal stability of the films was studied using thermogravimetric analysis. This was performed under argon flow on a Shimadzu Instrument, model TGA-502, with a heating rate of 10°C min^{-1} from room temperature up to 600°C.

Antimicrobial Activity

Antimicrobial activity tests of the films was performed using the agar diffusion method.[20] Strains of *Escherichia coli* (ATCC 25972) and *Staphylococcus aureus* (ATCC 1901) were inoculated in Brain Heart Infusion (BHI, HiMedia, India) and incubated at 37°C for 12 h. The films were cut into a disc form of 10 mm diameter and were placed on *Trypticase Soy Agar* (*TSA*, HiMedia, India) plates that were previously seeded with 0.1 mL of inoculum containing indicator microorganisms in the range of 10^6 CFU mL^{-1}. The plates were incubated at 37°C for 24 h. The diameter of the inhibition zone surrounding the film discs with the agar surface were measured, with digital micrometer (DIGIMESS Precision: 0.001 mm, resolution: 0–25 mm, Brazil), and the total area was calculated. The entire zone area was calculated by subtracting from the film disk area, and this difference was reported as the zone of inhibition in mm^2. We also observed the antimicrobial activity by contact inhibition, i.e., even without the presence of a clear zone inhibition, if the film inhibited microbial growth on the surface contact of film disk with the agar.

Statistical Analysis

The results were evaluated using analysis of variance (ANOVA) and Tukey's test at a significance level of 0.05 using the Statistica 12.0 software (STATSOFT Inc., São Paulo, Brazil).

RESULTS AND DISCUSSION

Proximate Analysis of Quinoa Flour

The quinoa flour obtained in this work had a moisture content of 11 ± 0.1%, and the proximate analysis for the dry basis found 13.0 ± 0.1% of protein, 6.0 ± 1.5% of total lipids, 3 ± 0.1% of ash, 6.1 ± 0.3% of total fiber and 71 ± 1% of carbohydrates. These values were similar to other studies in the literature reporting the proximate analysis of grain quinoa.[18,30–32]

Selection of Essential Oils

In Figure 1, the zone of inhibition from the preliminary tests of antimicrobial activity of the EOs is observable. The oils that showed visually larger inhibition zones to the growth of *S. aureus* (ATCC 1901) included oregano (*Origanum vulgare* L.) and thyme (*Thymus vulgaris* L.), these added in the formulations. Oregano and thyme EOs are known for their antimicrobial activity *in vitro* due to the presence of carvacrol (traces to 80% in oregano and 2–11% in thyme) and thymol (traces to 64% in oregano and 10–64% in thyme).[33] Several studies have shown that certain essential oils have strong antimicrobial properties; therefore, they could be used in food production as a possible alternative to synthetic preservative additives to limit the growth of food pathogens and increasing the shelf life of some food.[33–35]

Mechanical Properties

Regarding the thickness, independent of the type of essential oil and concentration used, we did not observe a statistically significant difference ($P > 0.05$), with thickness ranging from 181 ± 16 μm to 209 ± 10 μm. The mechanical properties of the tensile strength, TS (MPa), elongation at break, EB (%) and Young's modulus, YM (MPa), of the quinoa flour films containing the EOs are provided in Table I.

The tensile strength (TS) is a measurement of the maximum strength of the film under an applied tensile stress. Our analysis of the films revealed that the addition of the EOs, including increasing their concentration, produced a significant reduction ($P < 0.05$) in the TS compared with the film produced only with quinoa flour (0% EO). The values reduced from 3.5 ± 0.2 MPa to 1.2 ± 0.2 MPa for the film with the EO of oregano and to 1.8 ± 0.1 MPa for the film with the EO of thyme. The reverse effect was observed for the elongation at break (EB), which increased significantly ($P < 0.05$) with an increase in EO concentration from 14.3 ± 0.9% for the film formulated only with quinoa flour to 37.3 ± 3.1% and 20.1 ± 0.3% for the films with

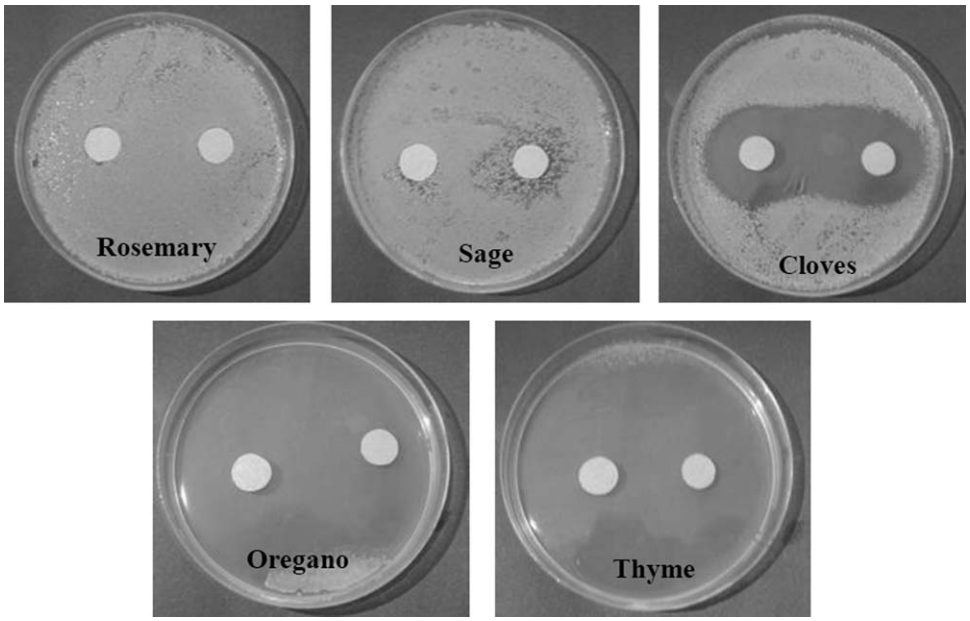

Figure 1. Images of zones of inhibition of essential oils of rosemary (*rosmarinus officinalis*), cloves (*Eugenia caryophyllata*), sage (*Salvia officinalis*), oregano (*Origanum vulgare* L.) and thyme (*Thymus vulgaris* L.) against *Staphylococcus aureus*.

2% of oregano and thyme EOs, respectively. For the Young's modulus (YM), we observed that the addition of the EO led to a significant reduction ($P < 0.05$) in the values. The results indicated that the addition of the EOs in the films led to a significant decrease in strength and rigidity of the films; however, the EOs significantly increased the elasticity.

According to Sanchez-Gonzalez *et al.*[36] the oil incorporation in the film results in discontinuities in the polymer matrix of the dried film, which results in a decrease in the mechanical properties of the TS and EB. The same authors observed that the addition of tea tree essential oil (TTO) in films based on hydroxypropylmethylcellulose (HPMC) results in a significant decrease in the TS and elastic modulus of the films. A similar effect was observed by Pranoto *et al.*,[24] in which the incorporation of garlic oil in chitosan edible film significantly reduced the TS from 37.03 ± 1.29 MPa (control) to 28.97 ± 1.92 (400 µl/g of chitosan).

Another group,[37] developed gelatin-based edible films that were incorporated with olive oil and observed an increase in the tensile strength (TS) from 6.10 (control) to 6.7 MPa with a final value of 10.9 MPa (emulsified films). According to the authors, the inclusion of oil favored protein–protein interactions, and as a consequence, the obtained films exhibited higher TS values in comparison with those films without the oil. Jouki *et al.*,[20] developed films of quince seed mucilage (QSM) containing oregano essential oil (OEO), observed that the TS and YM of the films decreased significantly ($P < 0.05$) after addition of the EO from 21.14 to 13.28 MPa and 79.29 to 66.13 MPa for the TS and YM, respectively. This was accompanied by significant increase ($P < 0.05$) in the EB from 29.02% to 34.67% with an increase in the oil concentration of up to 2.0% (w/w). The EO is liquid at room temperature, and it is present in the film in the form of oil droplets that can easily be deformed, enhancing the film's extensibility. Thus, the OE can act as a plasticizer to reduce the TS and increasing the EB of the films.

Solubility and Water Vapor Permeability (WVP)

The moisture content (MC) and water solubility of the quinoa flour films are shown in Table II. Biodegradable packaging should maintain moisture levels within the packaged product. Therefore, knowledge of the solubility and MC of the film is

Table I. Properties Mechanical of Tensile Strength (TS), Elongation at Break (EB), and Modulo de Young (YM), of Films Prepared Quinoa Flour with and without Essential Oils (EOs)

	TS [MPa]		EB [%]		YM [MPa]	
	Oregano	Thyme	Oregano	Thyme	Oregano	Thyme
0%	3.5 ± 0.2^{a}	3.5 ± 0.2^{a}	14.3 ± 0.9^{c}	14.3 ± 0.9^{c}	128.3 ± 10.3^{a}	128.3 ± 10.3^{a}
0.5%	3.0 ± 0.2^{b}	2.8 ± 0.1^{b}	19.2 ± 1.8^{b}	16.6 ± 1.3^{b}	115.2 ± 6.9^{b}	101.3 ± 3.5^{b}
1%	2.7 ± 0.3^{b}	2.8 ± 0.2^{b}	19.7 ± 0.9^{b}	19.2 ± 1.2^{a}	82.3 ± 7.6^{c}	88.4 ± 7.0^{b}
2%	1.2 ± 0.2^{c}	1.8 ± 0.1^{c}	27.3 ± 3.1^{a}	20.1 ± 0.3^{a}	51.9 ± 4.2^{d}	54.2 ± 3.6^{c}

The results are represented as the means ± standard deviation of the ten repetitions. Values with the same lowercase letter in the column show significant similarity ($P > 0.05$) according to Tukey's test, for films containing essential oils of oregano or thyme in different concentrations.

Table II. Moisture Content (MC) and Solubility (S) of the Films of Quinoa Flour Incorporated with Essential Oils (EOs)

	MC (%)		S (%)	
	Oregano	Thyme	Oregano	Thyme
0%	18.70 ± 0.9^{a}	18.70 ± 0.9^{a}	43.1 ± 1.5^{a}	43.1 ± 1.5^{a}
0.5%	16.3 ± 0.8^{b}	16.1 ± 0.4^{b}	41.2 ± 0.4^{b}	42.1 ± 0.6^{ab}
1%	15.8 ± 0.6^{b}	15.5 ± 0.2^{b}	38.4 ± 0.1^{bc}	39.2 ± 0.2^{bc}
2%	14.7 ± 0.2^{b}	14.5 ± 0.4^{b}	36.5 ± 0.3^{c}	37.0 ± 0.4^{c}

The results are represented as the means ± standard deviation of three repetitions. Values with the same lowercase letter in the column show significant similarity ($P > 0.05$) according to Tukey's test, for films containing essential oils of oregano or thyme in different concentrations.

highly important for food packaging applications. The MC values of the films significantly decreased ($P < 0.05$) with the presence of the EO; however, the increase in the concentration of the OE was not significant ($P > 0.05$) for MC.

The solubility of a film is an important property for edible films, and the water insolubility or resistance is usually required for a potentially commercial film.[37] The solubility of the quinoa flour film ($43.1 \pm 1.5\%$) showed a significant reduction ($P < 0.05$) when compared with films incorporated with the EO of oregano ($36.5 \pm 0.3\%$) and thyme ($37.0 \pm 0.4\%$) at higher concentrations. According to Jiménez *et al.*,[38] this effect is a consequence of the increase in the film's hydrophobicity due to the incorporation of the oil. Ma *et al.*,[37] developed gelatin-based edible films incorporated with olive oil and observed that the solubility significantly decreased ($P < 0.05$) from 44.67% (control) to 27–33% (emulsified films). A similar effect was observed by Tapia-Blacido *et al.*,[4] in their study of the interactions of the lipid fraction with the properties of amaranth flour films. The authors observed that the presence of lipid in the flour films increased their hydrophobicity and decreased their solubility from $42.2 \pm 1.8\%$ (flour) to $39.9 \pm 2.5\%$.

The Figure 2 shows that an increase in EO concentration results in a significant reduction ($P < 0.05$) in the WVP from $1.48 \pm 0.07 \times 10^{-10}$ g m^{-1} s^{-1} Pa^{-1} for the film produced only with quinoa flour (0% OE) to $1.05 \pm 0.04 \times 10^{-10}$ g m^{-1} s^{-1} Pa^{-1} and $1.11 \pm 0.08 \times 10^{-10}$ g m^{-1} s^{-1} Pa^{-1} for films with 2% oregano or thyme EOs, respectively. Ma *et al.*,[37] developed gelatin-based edible films that were incorporated with olive oil and observed a significant reduction in the WVP with an increased concentration of olive oil from $5.610 \pm 0.068 \times 10^{-10}$ (g m^{-1} s^{-1} Pa^{-1}) to $4.194 \pm 0.044 \times 10^{-10}$ (g m^{-1} s^{-1} Pa^{-1}) (emulsified films). According to these authors, the olive oil incorporation in the gelatin matrix improved the water barrier ability of the films for any given oil to protein ratio. Pereda *et al.* (2012)[39] reported that the WVP is a crucial property for films intended for use as edible food coatings because most natural biopolymers are very susceptible to water absorption. The same authors investigated the effect of the addition of olive oil in edible chitosan films, and they found that the WVP through the films decreased as the oil concentration increased with values on the order of 10^{-9} g m^{-1} s^{-1} Pa^{-1}, which may be quite high for preventing migration of moisture from the fruit, e.g., to the environment during freezing and thawing.

Because of the hydrophobic nature of the oils, their incorporation in the quinoa flour films improved the solubility and water barrier ability. A primary function of food packaging is to avoid or to decrease moisture transfer between the food and the atmosphere or between two components in a heterogeneous food product.

Optical Properties (Color and Opacity)

The optical properties of the films are relevant properties because they have a direct impact on the film appearance and application.[36]

Table III shows the values for the color parameters (a^*, b^*, and L) of the quinoa flour containing the EO. The color parameter, L, provides a measure of the lightness, and the color values

Table III. Color Parameters of Films of Quinoa Flour Incorporated with Essential Oils

	L^*	a^*	b^*	ΔE^*
Oregano				
0%	89.3 ± 1.1^{a}	-0.3 ± 0.0^{a}	22.6 ± 0.2^{a}	1398.8 ± 41.1^{a}
0.5%	89.4 ± 1.1^{a}	-0.3 ± 0.1^{a}	22.8 ± 0.3^{a}	1351.2 ± 86.7^{a}
1%	89.8 ± 0.1^{a}	-0.3 ± 0.1^{a}	22.5 ± 0.1^{a}	1499.2 ± 52.4^{a}
2%	89.6 ± 0.4^{a}	-0.4 ± 0.0^{a}	23.0 ± 0.2^{a}	1606.8 ± 98.2^{a}
Thyme				
0%	89.3 ± 1.1^{a}	-0.3 ± 0.0^{a}	22.7 ± 0.2^{a}	$1448.1 \pm 41,4^{a}$
0.5%	89.8 ± 0.2^{a}	-0.3 ± 0.1^{a}	22.8 ± 0.1^{a}	1499.2 ± 90.5^{a}
1%	88.1 ± 1.3^{a}	-0.3 ± 0.1^{a}	22.8 ± 0.1^{a}	1499.2 ± 51.4^{a}
2%	89.8 ± 0.4^{a}	-0.3 ± 0.0^{a}	22.9 ± 0.1^{a}	1559.0 ± 52.8^{a}

The data are presented as the means plus or minus the standard deviations of three repetitions. Means in the same column followed by different letters were significantly different ($P < 0.05$) according to Tukey's test for films containing essential oils of oregano or thyme in different concentrations.

Table IV. Opacity (A mm^{-1}) of Quinoa Flour Films and Films Containing Essential Oils at Different Wavelengths (nm)

	280 nm	350 nm	500 nm	600 nm	700 nm
Oregano					
0%	23.8 ± 1.2^{a}	15.1 ± 1.2^{a}	4.6 ± 0.3^{b}	3.8 ± 0.2^{b}	3.4 ± 0.3^{b}
0.5%	18.6 ± 0.5^{a}	15.0 ± 0.6^{a}	4.7 ± 0.1^{b}	4.1 ± 0.1^{b}	3.8 ± 0.1^{b}
1%	22.3 ± 1.8^{a}	15.8 ± 0.8^{a}	5.1 ± 0.4^{b}	4.3 ± 0.4^{b}	3.9 ± 0.4^{b}
2%	22.5 ± 1.9^{a}	17.6 ± 1.5^{a}	6.8 ± 0.3^{a}	6.1 ± 0.3^{a}	5.8 ± 0.3^{a}
Thyme					
0%	23.3 ± 1.2^{a}	15.1 ± 1.2^{a}	4.6 ± 0.3^{b}	3.8 ± 0.2^{b}	3.4 ± 0.4^{b}
0.5%	20.3 ± 1.6^{a}	15.2 ± 0.4^{a}	4.7 ± 0.4^{ab}	3.9 ± 0.3^{b}	3.4 ± 0.2^{b}
1%	19.5 ± 0.9^{a}	15.2 ± 0.5^{a}	5.0 ± 0.2^{ab}	$4.2 \pm 0.3a^{b}$	3.8 ± 0.3^{ab}
2%	21.6 ± 1.1^{a}	16.5 ± 0.6^{a}	5.6 ± 0.2^{a}	4.8 ± 0.2^{a}	4.5 ± 0.2^{a}

The data are presented as the means (in triplicate) plus or minus the standard deviations. Means in the same column followed by different letters were significantly different ($P < 0.05$) according to Tukey's test.

range from 0 to 100 with 0 designating a perfect black and 100 designating pure white. With regard to the color difference of the films (ΔE), higher values of DE indicate films with a more significant color intensity.

The addition of the EO into the films did not modify significantly ($P > 0.05$) the ΔE, demonstrating that for the tested concentrations the oregano and thyme oils did not significantly change the color parameters in our quinoa flour films. The parameter, CIE Lab b^*, indicates that the quinoa flour films exhibit a strong tendency to a yellowish color, and the parameter, a^*, is negative for both films, indicating a tendency toward the characteristic tones of the color green. Flour films exhibit a tendency toward a yellowish color,[1] this result indicated that the yellowish color of the flour films would be related to the presence of proteins in their composition.[4]

The analysis of the opacity (Amm^{-1}) at different wavelengths (nm) for the quinoa flour films and films containing the EOs related to the absorption of UV and visible light in the range of 280–700 nm are presented in Table IV. High absorbance values indicate films with less transparency or a higher degree of opacity. In the UV region, independent of the concentration, the addition of an EO did not significantly ($P > 0.05$) change the opacity. The films exhibited excellent barrier properties to light in the UV range (280–350 nm). These results indicate that the films had a protective ability against UV radiation because of their UV-barrier capabilities, which suggests their potential preventive effect on product oxidation due to UV light.

The evaluation of the opacity in the visible region (500–700 nm) revealed that all of the tested films generally showed low absorbance values, indicating their high transparency and low opacity. However, with an increase in essential oil concentration, we observed a significant increase ($P < 0.05$) in the light absorption of the films, consequently leading to a reduction in the transparency of the films. According to Pereda *et al.*,[39] oil droplets that are dispersed in the carbohydrate matrix affect the transparency by preventing light transmission through the resulting film. The same authors added olive oil in chitosan films (5%, 10%, and 15% olive oil/chitosan, wt %), and they observed an increase in the film opacity as the concentration of lipids increased. To evaluate the opacity of gelatin-based edible films incorporated with olive oil, Ma *et al.*,[37] observed that the addition of olive oil (5, 10, 15, and 20% olive oil/protein weight ratios) produced a significant increase ($P < 0.05$) in the opacity of films from 0.58 $A_{600}.mm^{-1}$ for the control film to values between 3.28 $A_{600}.mm^{-1}$ and 6.50 $A_{600}.mm^{-1}$.

Morphological Properties

The scanning electron microscopy (SEM) images for the quinoa flour-based films and quinoa flour-based films containing the EOs are shown in Figure 3. The results show that for the studied magnification (550×) the quinoa flour-based films (without EOs), presented a compact structure without the presence of cracks or blistering, with small irregularities. However, with an increase in the EO concentration, an increase in the irregularities of the film surface was observable that were possibly caused by the increased lipid droplet concentration that was dispersed in the matrix of the film.

For the films with 2% of the EOs of oregano or thyme, we observed a dispersal of the lipid droplets in the emulsion that were continuously distributed throughout the polymer network after the film formed. This dispersal and the higher lipid concentration on the surface of the film may explain the greater

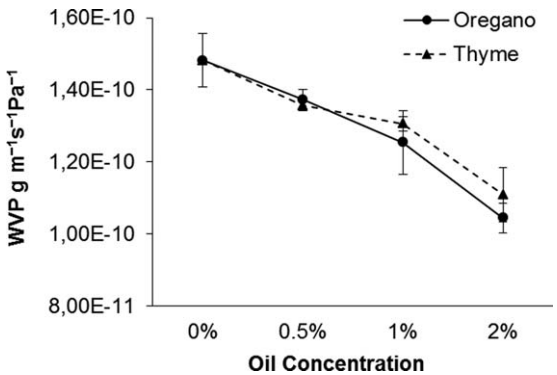

Figure 2. Water vapor permeability (WVP) of the films of quinoa flour incorporated with essential oils (EOs).

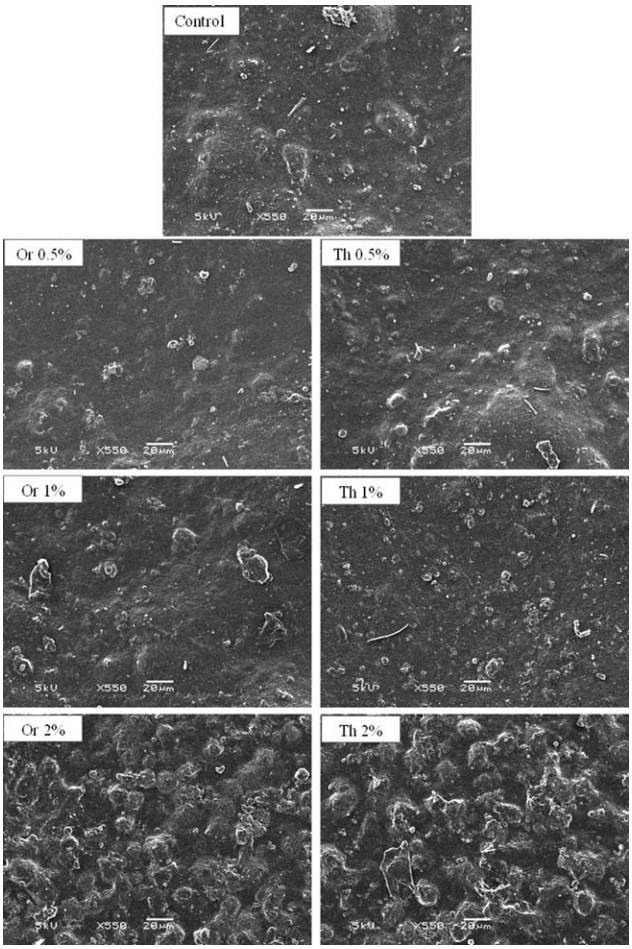

Figure 3. Scanning Electron Microscopy (SEM), images of film surface without essential oil (control) and with essential oil of oregano at concentration of 0.5% (OR 0.5%), 1% (OR 1%), 2% (OR 2%) and oil of thyme at concentration of 0.5 (Th0.5%), 1% (Th 1%), 2% (Th 2%).

opacity of the film with higher levels of EO because, according to Pereda *et al.*,[39] the lipid droplets cause a greater dispersion of light on the surface of the film, leading to an increase in opacity.

Thermal Stability

TGA thermograms showing thermal degradation behavior of the films with or without EOs are shown in Figure 4. It can be seen that the addition of EOs, does not affect the thermal stability of the films, exhibiting three main stages of weight loss (Δw).

The first weight loss ($\Delta w_1 = 10–15\%$), observed between room temperature and 180°C, is generally due to the evaporation of residual water. The estimated amount of water desorbed was nearly 10–15%, these values are similar to those seen in Table II for moisture content (MC). The weight loss ($\Delta w2 = 18–20\%$) in the second stage, which corresponds to the degradation of lower molecular weight fractions or structurally bound water in the film network, occurred at the range 180–250°C. For the third stage, the major weight loss (Δw_3 of 21–33%) occurred between 250 and 350°C for all of the samples, which is ascribed to the organic phase decomposition. Therefore, all films exhibit high thermal stability at least up to 250°C. It was not possible to observe a larger residual weight with the gradual increase in EOs concentration or between the different oils that were tested.

Antimicrobial Activity

The antibacterial activities (inhibitory zone and contact inhibition) of the quinoa flour films incorporated with oregano and thyme EOs against *E. coli (Gram −)* and S. *aureus (Gram +)* bacteria are shown in Table V. The inhibitory zone is based on the measurement of the clear zone that was caused by growth inhibition produced by a film disk (10 mm) that contained the antimicrobial agent when placed in direct contact with a bacterial culture. When antimicrobial agents are incorporated into food packaging films, these materials diffuse through the agar gel and result in a clear zone around the cut films.[40]

As shown in Table V, an increase of the zone inhibition of the tested microorganisms occurred with an increase in both EOs concentrations. Nonetheless, in lowest concentration tested (0.5%) for both EOs did not produce a zone of inhibition against *E. coli* growth, there was only contact inhibition. However, the same concentration produced a small zone of inhibition against *S. aureus*, as well as contact inhibition.

This behavior was observed for other concentrations, independent of the EO, in which larger zones of inhibition were observed for *S. aureus* (Gram +). These results are in accordance with other authors who reported that Gram-positive microorganisms are more sensitive to essential oils than Gram-negative bacteria. This behavior may be related to the presence of an additional external membrane surrounding the cell wall in Gram-negative bacteria, which may restrict the diffusion of hydrophobic compounds through its lipopolysaccharide covering.[19,33]

In Table V, we show that, with the same concentrations and independent of the microorganisms, the EO of oregano had the

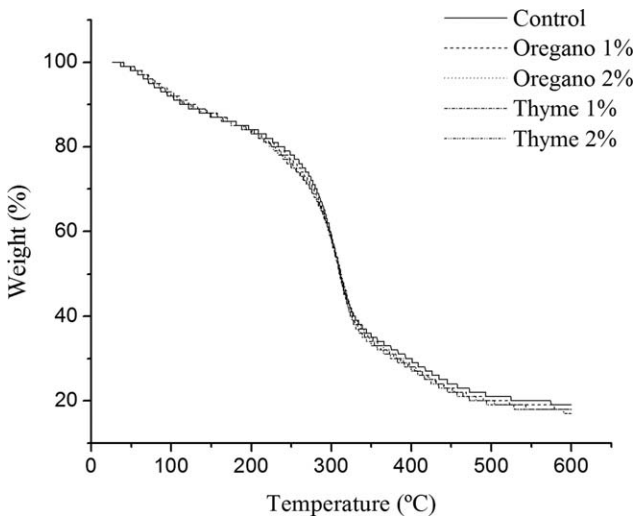

Figure 4. Thermogravimetric Analysis (TGA) analyses of control films and films containing essential oil of de oregano (1% and 2%) and thyme (1% and 2%).

Table V. Zone of Inhibition of Edible Films Prepared with Quinoa Flour and Films Containing Essential Oils Against *E. coli* ATCC 25972 and *S. aureus* ATCC 1901

	E. coli (Gram −)		*S. aureus (Gram +)*	
Antimicrobial Agents	Inhibitory (mm^2)	Contact	Inhibitory (mm^2)	Contact
Control	0	−	0	−
Oregano				
0.5%	0	+	16.5 ± 0.9	+
1%	23.5 ± 0.8	+	50.1 ± 0.9	+
2%	56.2 ± 1.0	+	122.5 ± 1.2	+
Thyme				
0.5%	0	+	13.1 ± 0.8	+
1%	25.3 ± 0.8	+	36.4 ± 0.9	+
2%	36.4 ± 0.9	+	98.1 ± 1.1	+

highest inhibition zones in comparison with thyme. These results are probably related to the composition of the EOs used. Thymol and carvacrol are the main antimicrobial constituents of thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare*). They represent the monoterpenes with the highest bactericidal power, which are present in the composition of many EOs, due to their phenolic nature.[41] As previously mentioned, these are compounds found in higher quantities in the EOs of oregano when compared with thyme. Pesavento *et al.*,[34] analyzed the chemical composition (%) of OE's and found concentrations for carvacrol and thymol that are approximately 71.8% and 1.6% (vol/vol), respectively, for oregano (*Origanum vulgare*) and concentrations of ~0.40% and 43.1% (vol/vol), respectively, for thyme (*Thymus vulgaris*).

The inhibition zone values obtained in this work are similar to those obtained in the literature for the same oils that are incorporated in films with different matrices. Seydim and Sarikus[22] found a zone of inhibition against *S. aureus* (ATCC 43300) that was 33.65 mm^2 for whey protein isolate films containing 2% oregano EO, and they did not observe a zone of inhibition for the same films with 1% oregano EO. Other authors[40] reported that in chitosan-based films that contained thyme EO, large inhibition zones were present against *S. aureus* (PTCC 143) that were 109.03 mm^2 (films containing 1% EO) and 61.43 mm^2 (films containing 0.5% EO). Jouki *et al.*[42] observed inhibition zones against *S. aureus* (ATCC 25923) that were 74.56 mm^2 for quince seed mucilage film that was incorporated with 1% thyme EO. Another group of researchers[19] developed a soy protein-based edible film that was incorporated with oregano and thyme essential oil (2%) for use against *S. aureus*. They observed inhibition zones that were 42 mm^2 and 41 mm^2, respectively. Pelissari *et al.*[1] reported inhibition zones against *E. coli* (ATCC 25922) that were 23.73 mm^2 for starch-chitosan films incorporated with 1% oregano EO.

Several mechanisms are involved in the inhibition of microorganisms in edible films that depend on the source and concentration of the active compounds in the plant extracts and the composition of the film material.[22] In this work, the film with 2% oregano EO showed the best results with respect to antimicrobial activity primarily against the Gram positive bacteria, *S. aureus*, demonstrating its potential use and application in food packaging.

CONCLUSIONS

Our results indicate that quinoa flour containing the essential oil (EO) of oregano or thyme is a potentially useful material for the formulation of active coatings and edible films. The addition or the increase in EOs concentration both significantly changed the physical and mechanical properties. The incorporation of the EOs, despite the reduction in the resistance of the films, led to an increase in their elasticity, and they became more malleable. It also led to a reduction in the WVP and solubility, which is a positive attribute for their application in food. The films exhibited good light absorption while maintaining the protective barrier against UV and visible light, which are important characteristics for their application in food with high lipid contents to minimize the oxidative effects of light. Even for the low concentrations, all of the films incorporated with the EOs of oregano and thyme demonstrated greater antimicrobial activities against *S. aureus* and *E. coli*. However, larger zones of inhibition were observed for films with the oregano EO compared to films with the thyme EO. Therefore, our results are encouraging because they demonstrate that the use of EOs, in addition to improving the mechanical and barrier properties of films, may offer an alternative method for limiting pathogens and aerobic spoilage in food.

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REFERENCES

1. Pelissari, F. M.; Andrade-Mahecha, M. M.; Sobral, P. J. dA.; Menegalli, F. C. *Food Hydrocolloids* **2013**, *30*, 681.
2. Guerrero, P.; Stefani, P. M.; Ruseckaite, R. A.; de la Caba, K. *J. Food Eng.* **2011**, *105*, 65.

3. Andrade-Mahecha, M. M.; Tapia-Blacido, D. R.; Menegalli, F. C. *Carbohydr. Polym.* **2012**, *88*, 449.
4. Tapia-Blacido, D.; Mauri, A. N.; Menegalli, F. C.; Sobral, P. J. A.; Anon, M. C. *J. Food Sci.* **2007**, *72*, E293.
5. Tapia-Blacido, D.; Sobral, P. J.; Menegalli, F. C. *J. Food Eng.* **2005**, *67*, 215.
6. Colla, E.; do Amaral Sobral, P. J.; Menegalli, F. C. *J. Agricultural Food Chem.* **2006**, *54*, 6645.
7. Dias, A. B.; Muller, C. M. O.; Larotonda, F. D. S.; Laurindo, J. B. *Lwt-Food Sci. Technol.* **2011**, *44*, 535.
8. Tapia-Blacido, D. R.; Sobral, P. J. D.; Menegalli, F. C. *Lwt-Food Sci. Technol.* **2013**, *50*, 392.
9. Valenzuela, C.; Abugoch, L.; Tapia, C.; Gamboa, A. *Int. J. Food Sci. Technol.* **2013**, *48*, 843.
10. Tapia-Blacido, D. R.; Sobral, P. J. D.; Menegalli, F. C. *Lwt-Food Sci. Technol.* **2011**, *44*, 1731.
11. Tapia-Blacido, D.; Sobral, P. J.; Menegalli, F. C. *Brazilian J. Chem. Eng.* **2005**, *22*, 249.
12. Mariniello, L.; Di Pierro, P.; Esposito, C.; Sorrentino, A.; Masi, P.; Porta, R. *J. Biotechnol.* **2003**, *102*, 191.
13. Bonilla, J.; Talón, E.; Atarés, L.; Vargas, M.; Chiralt, A. *J. Food Eng.* **2013**, *118*, 271.
14. Tanada-Palmu, P. S.; Grosso, C. R. F. *Postharvest Biol. Technol.* **2005**, *36*, 199.
15. Dias, A. B.; Muller, C. M. O.; Larotonda, F. D. S.; Laurindo, J. B. *J. Cereal Sci.* **2010**, *51*, 213.
16. Araujo-Farro, P. C.; Podadera, G.; Sobral, P. J. A.; Menegalli, F. C. *Carbohydr. Polym.* **2010**, *81*, 839.
17. Ahamed, N. T.; Singhal, R. S.; Kulkarni, P. R.; Kale, D. D.; Pal, M. *Carbohydr. Polym.* **1996**, *31*, 157.
18. Abugoch, L. E.; Tapia, C.; Villamán, M. C.; Yazdani-Pedram, M.; Díaz-Dosque, M. *Food Hydrocolloids* **2011**, *25*, 879.
19. Emiroğlu, Z. K.; Yemiş, G. P.; Coşkun, B. K.; Candoğan, K. *Meat Sci.* **2010**, *86*, 283.
20. Jouki, M.; Yazdi, F. T.; Mortazavi, S. A.; Koocheki, A. *Food Hydrocolloids* **2014**, *36*, 9.
21. Campos, C.; Gerschenson, L.; Flores, S. *Food Bioprocess Technol.* **2011**, *4*, 849.
22. Seydim, A. C.; Sarikus, G. *Food Res. Int.* **2007**, *40*, 949.
23. Zivanovic, S.; Chi, S.; Draughon, A. F. *J. Food Sci.* **2005**, *70*, M45.
24. Pranoto, Y.; Rakshit, S. K.; Salokhe, V. M. *Lwt-Food Sci. Technol.* **2005**, *38*, 859.
25. Gómez-Estaca, J.; López de Lacey, A.; López-Caballero, M. E.; Gómez-Guillén, M. C.; Montero, P. *Food Microbiol.* **2010**, *27*, 889.
26. Silva-Weiss, A.; Ihl, M.; Sobral, P. J. A.; Gomez-Guillen, M. C.; Bifani, V. *Food Eng. Rev.* **2013**, *5*, 200.
27. Pagno, C. H.; Costa, T. M. H.; de Menezes, E. W.; Benvenutti, E. V.; Hertz, P. F.; Matte, C. R.; Tosati, J. V.; Monteiro, A. R.; Rios, A. O.; Flores, S. H. *Food Chem.* **2015**, *173*, 755.
28. Wang, L.; Dong, Y.; Men, H.; Tong, J.; Zhou, J. *Food Hydrocolloids* **2013**, *32*, 35.
29. Dick, M.; Henrique Pagno, C.; Haas Costa, T. M.; Gomaa, A.; Subirade, M.; de Oliveira Rios, A.; Hickmann Flôres, S. *J. Appl. Polym. Sci.* **2015**, DOI: 10.1002/app.43311.
30. Abugoch, L. E.; Romero, N.; Tapia, C. nA.; Silva, J.; Rivera, M. *J. Agri. Food Chem.* **2008**, *56*, 4745.
31. Abugoch James, L. E. In Advances in Food and Nutrition Research; Steve, L. T., Ed.; Elsevier: New York, 2009; Vol. *58*, Chapter 1, pp 1–31, **2009**.
32. Repo-Carrasco, R.; Espinoza, C.; Jacobsen, S. E. *Food Rev. Int.* **2003**, *19*, 179.
33. Burt, S. *Int. J. Food Microbiol.* **2004**, *94*, 223.
34. Pesavento, G.; Calonico, C.; Bilia, A. R.; Barnabei, M.; Calesini, F.; Addona, R.; Mencarelli, L.; Carmagnini, L.; Di Martino, M. C.; Lo Nostro, A. *Food Control* **2015**, *54*, 188.
35. Calo, J. R.; Crandall, P. G.; O'Bryan, C. A.; Ricke, S. C. *Food Control* **2015**, *54*, 111.
36. Sanchez-Gonzalez, L.; Vargas, M.; Gonzalez-Martinez, C.; Chiralt, A.; Chafer, M. *Food Hydrocolloids* **2009**, *23*, 2102.
37. Ma, W.; Tang, C. H.; Yin, S. W.; Yang, X. Q.; Wang, Q.; Liu, F.; Wei, Z. H. *Food Res. Int.* **2012**, *49*, 572.
38. Jiménez, A.; Fabra, M. J.; Talens, P.; Chiralt, A. *Carbohydr. Polym.* **2010**, *82*, 585.
39. Pereda, M.; Amica, G.; Marcovich, N. E. *Carbohydr. Polym.* **2012**, *87*, 1318.
40. Hosseini, M. H.; Razavi, S. H.; Mousavi, M. A. *J. Food Processing Preserv.* **2009**, *33*, 727.
41. Yanishlieva, N. V.; Marinova, E.; Pokorný, J. *Eur. J. Lipid Sci. Technol.* **2006**, *108*, 776.
42. Jouki, M.; Mortazavi, S. A.; Yazdi, F. T.; Koocheki, A. *Carbohydr. Polym.* **2014**, *99*, 537.