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Characterization of antioxidant–antibacterial quince seed mucilage films containing thyme essential oil

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ABSTRACT

In this study thyme essential oil (TEO) concentrations ranging from 0% to 2.0%, incorporated in quince seed mucilage (QSM) film were used. Antibacterial activity, physical, mechanical, barrier and antioxidant properties of QSM films were evaluated. The antimicrobial activity of the QSM films incorporated with thyme essential oil was screened against 11 important food-related bacterial strains by agar disc-diffusion assay. Films containing 1% of thyme essential oil were effective against all test microorganisms and exhibited a strong inhibitory effect on the growth of *Shewanella putrefaciens*, *Listeria monocytogenes* and *Staphylococcus aureus*. QSM films exhibited some antioxidant activity, which was significantly improved by the addition of the essential oil. A reduction of the glass transition temperature, as determined by differential scanning calorimetry (DSC), was caused by addition of thyme essential oil into the QSM films. Scanning electron microscopy was carried out to explain structure–property relationships. Incorporating thyme essential oil into edible QSM films provides a novel way to improve the safety and shelf life of ready-to-eat foods.

1. Introduction

In recent years, research on packaging has given greater attention to biodegradable films, produced from natural biopolymers. This makes sense for the environment because these materials are fully compatibles, from renewable sources, highly recyclable, compostable and biodegradable. On the other hand, due to constant concern on preventing chemical and especially microbiological deterioration in food (Chen, Wang, & Weng, 2010), the interest in active packaging has increased. Edible films can improve shelf life and food quality by serving as selective barriers to moisture transfer, oxygen uptake, lipid oxidation, and losses of volatile aromas and flavors (Kester & Fennema, 1986). Biodegradable film can be used to carry active ingredients, such as antioxidant and antimicrobial agents that provide an extra stress factor against foods' oxidative and microbial deterioration (Sánchez-González, González-Martínez, Chiralt, & Cháfer, 2010; Ojagh, Rezaei, Razavi, & Hosseini, 2010).

Interest in antimicrobial films has risen recently due to increased consumption of fresh-cut produce. In order to control undesirable microorganisms on foods, antimicrobial substances can be incorporated in edible films (Labuza & Breene, 1989; Chen, Yeh, & Chiang, 1996; Padget, Han, & Dawson, 2000; Chung, Papadakis, & Yam, 2001). Among the most effective essential oils, thyme and oregano essential oils have been pointed out to posses better antimicrobial potential for meat applications, which could be ascribed to the presence of phenolic compounds, particularly thymol and carvacrol (Paster, Juven, & Shaaya, 1990; Hammer, Carson, & Riley, 1999; Burt, 2004; Kintzios, 2004; Oussalah, Caillet, Salmieri, Saucier, & Lacroix, 2004; Burt, Vlielander, Haagsman, & Veldhuizen, 2005; López, Sánchez, Batlle, & Nerín, 2005; Oussalah, Caillet, Salmieri, Saucier, & Lacroix, 2006; López, Sánchez, Batlle, & Nerín, 2007; Solomakos, Govaris, Koidis, & Botsoglou, 2008).

Antimicrobial activity of thyme or oregano essential oil incorporated edible films have been evaluated by a number of researchers, however, limited data exist on the application of antimicrobial edible films incorporated with essential oils in real food systems (Seydim & Sarikus, 2006; Chi, Zivanovic, & Penfield 2006; Oussalah et al., 2006; Du, Olsen, Avena-Bustillos, Mchugh, Levin, & Friedman, 2008). Among *Lamiaceae* species, oregano (*Origanum vulgare* L.), thyme (*Thymus vulgaris* L.) and wild thyme (*Thymus serpyllum* L.) have been studied widely for their antioxidant activity, due to the high content of phenolic compounds (Vichi, Zitterl-Eglseer, Jugl, & Fraz, 2001; Zandi & Ahmadi, 2000; Takacsova, Pribela, & Faktorova, 1995).

Quince (*Cydonia oblonga* Miller, Rosaceae family) is one of the most important underutilized fruit species which gives fruits with high nutrient value and positive influence on human health. In Iran, which supplies about 75% of the world production (Bergman, Afifi, & Heidgerip, 1996), the plant has been used in Iranian folk medicine for treatment of variety of disease. Leaves have been used as a sedative and the fruits were used for the treatment of

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dysentery, Diarrhea, stomach ulcers (Saric-Kundalic, Dobes, Klatte-Asselmeyer, & Saukel, 2011; Khoubnasabjafari & Jouyban, 2011). In addition, the seeds of the plant are used as a pharyngeal demulcent and emulsifying agent in the preparation of hair-fixing lotions (Bergman et al., 1996; Elandsen & Magney, 1992). As was suggested by Schmidt (1844) and more definitely shown by Kirchner and Tollens (1875), quince seed mucilage is a complex of a cellulosic fraction with a more readily hydrolyzed polysaccharide.

Arabinose, a mixture of methylated and unmethylated aldobionic acids and a cellulosic fraction were liberated in the hydrolysis of quince seed gum. Xylose was identified in the further hydrolysis of the aldobionic acids (Renfrew & Cretcher, 1932). This polysaccharide is of interest as a potential film or coating component considering the low production cost (in comparison with most biopolymers) and easy extraction. The aims of the present study were (1) to investigate the possibility of producing a novel biodegradable edible film from quince seed mucilage incorporated with thyme essential oil and (2) to determine the physical, optical, mechanical, thermal, barrier and antioxidant properties of QSM films and to evaluate their antimicrobial activity against 11 bacterial species that may cause food poisoning and spoilage.

2. Methods and materials

2.1. Materials

Quince seeds used in this study were purchased from the local market at Tehran in Iran. Thyme Essential oil supplied by Zardnand Company, (Tehran, Iran). Glycerol and Tween 80 (Fluka, Sigma–Aldrich, MO, USA), were used to prepare film-forming dispersions (FFD). Brain Heart Infusion (BHI) (broth and agar) and MRS Broth were bought from Merck Co (Darmstadt, Germany). Folin–Ciocalteu reagent, sodium carbonate, standard gallic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co (St. Louis, MO). Other materials including NaOH, calcium nitrate, sodium and calcium chloride were purchased from Merck Corporation (Whitehouse Station, NJ 08889-0100 USA).

2.2. Chemical composition of thyme essential oil

For the quantification of individual components, the *thyme essential oil* was analyzed using a Hewlett-Packard 6890 series gas chromatograph (Perkin Elmer (PE) Auto System XL, USA), connected to a Hewlett-Packard, model 5973, mass spectrometric detector (Agilent Technologies, Wilmington, DE, USA). A capillary column DB-5MS (60 m × 0.320 mm i.d. and 1 μm, film thickness) was used for the separation of individual components of the EO. Chemical composition of essential oils was determined according to the method described by Karabagias, Badeka, and Kontominas (2011). The oven temperature was programmed at 100 °C for 5 min, increased by 10 °C/min to 130 °C, increased by 7 °C/min to 270 and held to 270 °C for 3 min. Helium was used as the carrier gas at a flow rate 1.5 ml/min. 0.1% solution of EO was prepared in hexane and 1 μl of this solution was injected. The injector was operated in split mode (20:1 split ratio) at a temperature of 270 °C. The mass spectrometer was operated under the following conditions: scan range 30–330, source temperature: 230 °C, quadruple temperature: 150 °C, electron impact (EI) ionization at 70 eV. Identification of compounds was achieved by comparing the mass spectra of the recorded chromatographic peaks with the Wiley 275 MS database.

2.3. Bacterial strains

Escherichia coli (ATCC 25922), *Yersinia enterocolitica* (ATCC 9610), *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (PTCC 1154), *Pseudomonas aeruginosa* (ATCC 27853), *Lactobacillus plantarum* (ATCC 8014), *Salmonella typhimurium* (ATCC 14028), *E. coli* O157:H7 (ATCC 25922), *Listeria monocytogenes* (ATCC 1915), *Vibrio cholera* (ATCC 14033) and *Shewanella putrefaciens* (CECT 5346) were provided by Iranian Research Organization for Science and Technology (Tehran, Iran).

2.4. Emulsion preparation

Sequential processes employed to extract mucilage from quince seed. About 10 g quince seeds sieved and washed with its triple weight of ethanol (96% w/v) for 5 min under constant stirring. Then ethanol removed and the seeds dried in an oven at 45 °C. Aqueous quince seed mucilage was extracted from whole seeds using distilled water (water to seed ratio of 30:1). Then, the swelled seeds were stirred with a rod paddle blender (Rondo-2500, KA702, France) at 1100 rpm, at 45 °C for 15 min to scrape the mucilage layer off the seed surface. The solutions were then filtered with cheese cloth and the insoluble residue if any was filtered out leaving a concentration of 13.9% (w/w). Film solution was prepared by slowly dissolving 1% mucilage and glycerol as a plasticizer in 35% (w/w) based on QSM weight were prepared under constant stirring (750 rpm) at 45 ± 2 °C for 15 min. Tween 80 at level of 0.2% (v/v) of EO was added in film forming solutions to assist essential oil dissolution, and then thyme oil was incorporated into the film solution at various concentrations of 0% (as control), 1%, 1.5% and 2% (v/v) of edible film solution. The solution was homogenized (IKA T25 basic, Staufen, Germany) at 12,000 rpm for 3 min to obtain an emulsion. Finally, the emulsion was placed into a centrifuge (6000 rpm for 5 min) to remove air bubbles. To avoid any rheological changes, like creaming phenomenon in the film forming emulsions, centrifugation was done in minimum required time to remove air bubbles.

2.5. Preparation of QSM films

The film solution (50 ± 0.1 g) was casted onto the sterile plastic petri dishes (130 mm diameter) and dried at room temperature (25 ± 2 °C) and room relative humidity (37 ± 2%) for 24 h. Finally, the obtained QSM films were peeled from plates and conditioned at 25 ± 2 °C in desiccators containing saturated solutions of $Ca(NO_3)_2$, $6H_2O$ (50 ± 2% relative humidity, RH) for at least 48 h prior to tests.

2.6. Determination of physical properties of films

2.6.1. Film thickness

Film thicknesses were measured with a manual digital micrometer (Mitutoyo No. 293-766, Tokyo, Japan) to the nearest 0.001 mm. Measurements were taken at twelve random locations of the films. The mean value was used to calculate water vapor permeability (WVP), oxygen permeability (O_2P), and tensile strength.

2.6.2. Moisture content

Moisture content of film pieces ($3 \times 3\,cm^2$) was determined measuring weight loss of films, upon drying in an oven at 110 °C until constant weight (dry sample weight). Moisture content (%) was calculated as follows (Eq. (1)):

$$M_t = \left(\frac{w_i - w_d}{w_i}\right) \times 100 \quad (1)$$

where M_t is the moisture content of the sample (%); W_i is the initial sample weight (g) and W_d is the dry sample weight (g).

2.6.3. Water solubility

The solubility in water of the different QSM-films was measured from immersion assays under constant agitation in of distilled

water for 6 h according to method of Jouki, Khazaei, Ghasemlou, and HadiNezhad (2013).The initial dry matter of the films was determined by drying 2 cm diameter films in a vacuum oven, at 110 °C, for 24 h. Other disks were cut, weighed and immersed in 50 ml of distilled water, with periodic stirring, for 6 h at 25 °C. The solubility was reported as the difference between the initial and final dry matter with respect to the initial dry matter. The tests were conducted in triplicate.

2.7. Water vapor permeability (WVP)

WVP of the film samples was determined at 25 °C and 75% RH gradient according to the ASTM E96 gravimetric method (ASTM, 1995a,b). The circular test cups containing anhydrous calcium chloride (0% RH, assay cup) were sealed by the test films (0.00263 m^2 film area). The cells were placed in a desiccators maintained at 75% RH with a saturated solution of sodium chloride. Then the cells were weighed at 6 h intervals over a 48 h period. The difference in RH corresponds to a driving force of 1753.55 Pa, expressed as water vapor partial pressure. The slope of the weight gain vs. time plot was divided by exposed film area to obtain the water vapor transmission rate. This was multiplied by the thickness of the film and divided by the pressure difference between the inner outer surfaces to obtain the WVP. Water vapor permeability of each film was measured as the mean and standard deviations of three replications.

2.8. Oxygen permeability (O_2P) of films

Oxygen permeability (O_2P) was determined based on the ASTM D3985-95 (ASTM, 1995a,b) method. O_2P was measured at 23 ± 2 °C and 50 ± 1% RH using a GDP-C-Gas Permeability Tester (D-80335 Munchen, Brugger, Germany). Briefly, each film was placed on a stainless steel mask with an open testing area of 0.0144 m^2. Masked films were placed into the test cell and exposed to oxygen O_2 flow. Units for O_2P were cm^3 μm/m^2 d kPa. The analysis was performed in duplicate and the results were averaged.

2.9. Tensile properties

Tests were performed using a Testometric Machine M350-10CT (Testometric Co. Ltd., Rochdale, Lancashire, England) according to the American Society for Testing and Material method D882-91 (ASTM, 1996). Tensile strength, elastic modulus, and percent elongation were determined according to the method described by Hosseini, Razavi, and Mousavi (2009).

2.10. Color measurements

The changes in color of the QSM films incorporated with thyme essential oil (TEO) were evaluated by measuring the L^*, a^* and b^* parameters using a portable colorimeter (Minolta CR360 Series, Minolta Camera Co. Ltd., Osaka, Japan). The measured color parameters were used to calculate changes in total color (ΔE) and whiteness index (WI), according to the following equations: (Eqs. (2) and (3)).

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

$$WI = 100 - \sqrt{(100 - L)^2 + a^2 + b^2} \quad (3)$$

where L^*, a^* and b^* are the color parameter values of the white standard backgrounds (L^* = 93.49, a^* = −0.25 and b^* = −0.09) and L, a and b are the color parameter values of the sample. The colorimeter was calibrated using a white standard plate. For each treatment five samples were measured and on each film three readings were made.

2.11. Scanning electron microscopy of QSM-based films

Microstructure of the cross-sections of dried films was observed by scanning electron microscopy (EM-3200, KYKY, China). The QSM films were mounted on the specimen holder with aluminum tape and then sputtered with gold in BAL-TEC SCD 005 sputter coater (BAL-TEC AG, Balzers, Liechtenstein). Samples were photographed at tilt angles of 60–90° to the electron beam for the views in the cross section.

2.12. Differential scanning calorimetry (DSC)

The thermal properties of films were determined by differential scanning calorimetry (DSC) (Model F3 200 DSC Netzsch, Germany). Samples were scanned at a heating rate of 10 °C/min between temperature ranges of −100 and 200 °C. *Tg* was identified as the midpoint temperature of the shift in the baseline due to the change in heat capacity upon glass transition. Ghanbarzadeh and Oromiehi (2009) indicated that the glass transition temperature (*Tg*) is the temperature at which the material undergoes a structural transition from an amorphous solid (glassy) state to a more viscous rubbery state. Also the melting point (*Tm*) of the films was determined. The *Tg* and *Tm* of the each film were determined in duplicate and the results were averaged.

2.13. Total phenol measurement

Total phenol (TP) content of films were estimated according to the method described by Siripatrawan and Harte (2010) using Folin–Ciocalteu colorimetrically method. Briefly, 25 mg of each film sample was dissolved in 5 ml of distilled water, then extract solution (0.1 ml), distilled water (7 ml), and Folin–Ciocalteu reagent (0.5 ml) were mixed and kept at room temperature for 8 min, after which 1.5 ml sodium carbonate (2%, w/v) and water were added to obtain a final volume of 10 ml. The mixture was stirred thoroughly and allowed to stand for 2 h at room temperature prior to an absorbance reading at 765 nm in a spectrophotometer (Shimadzu UV-VIS, Japan). The results were expressed as mg gallic acid equivalents (GAE) per gram of dried film according to the following equation:

$$T = \frac{C \times V}{M} \quad (4)$$

where T is total content of phenolics compound (milligram per gram dried film, in GAE), C is the concentration of gallic acid obtained from the calibration curve (milligram per milliliter), V is the volume of film extract (milliliter) and M is the weight of dried film (gram).

2.14. Determination of antioxidant activity

The radical scavenging activity of QSM-based films was measured using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to Brand-Williams, Cuvelier and Berset (1995) on basis of bleaching of the bluish-red or purple-colored methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a reagent. Briefly, 25 mg of each film sample was dissolved in 5 ml of distilled water, and then a 0.1 ml of film extract solution were added to 3.9 ml of the DPPH solution (0.1 mM methanol solution) followed by 60 min incubation in the dark at ambient temperature. The absorbance was read against pure methanol at 517 nm by using a Perkin-Elmer spectrophotometer and the percentage of DPPH radical-scavenging activity was calculated using following equation:

$$\text{Radical scavenging activity}(\%) = 100 \times (1 - A_{\text{sample}}/A_{\text{DPPH}}) \quad (5)$$

Table 1
Chemical composition and their relative abundance of thyme oil[a].

No.	Compounds	Thyme (%)[a]
1	Thymol	46.42
2	*p*-Cymene	22.31
3	γ-Terpinene	7.50
4	Linalool	5.37
5	Carvacrol	12.42
6	β-Myrcene	2.27
7	Caryophyllene	0.69

[a] Expressed as percentage of the total peak area of the chromatograms.

where A_{sample} represents the absorbance of the sample solution and $A_{control}$ represents the absorbance of DPPH solution without the addition of the film. All experiments were carried out in triplicate.

2.15. Evaluation of antimicrobial activity of films

Overnight culture of *L. plantarum* was grown in MRS Broth at 28 °C. *L. monocytogenes*, *S. typhimurium*, *B. cereus*, *Y. enterocolitica*, *P. aeruginosa*, *S. aureus*, *E. coli*, *E. coli* O157:H7, *S. putrefaciens*, and *V. cholera* were grown in BHI broth (Merck, Germany) at 37 °C for 24 h with agitation. Antibacterial activity testing was carried out using agar diffusion method. Disks (6 mm diameter) cut from the films were placed on BHI agar (Merck, Germany) plates, previously surface spreaded with the inoculum containing indicator microorganisms in the range of 10^8 CFU/mL. The plates were then incubated at 37 °C for 24 h (Seydim & Sarikus, 2006). Antimicrobial activity was evaluated by measuring the diameter of inhibitory zone (mm) surrounding film disks against the test organisms. Each assay was repeated three times.

2.16. Statistical analysis

Data for each test were statistically analyzed. The analysis of variance (ANOVA) was used to evaluate the significance in the difference between factors and levels. Comparison of the means was done by the Duncan's multiple range test. The statistical analysis of the data was performed using SPSS statistical software version 18 (SPSS Inc., Chicago, IL).

3. Results and discussion

3.1. Identification of essential oil components

The major components (only those components with concentrations greater than 0.5%) in thyme essential oil are listed in Table 1. Thymol, Carvacrol and γ-therpinene are the principal constituents of thyme essential oil (Safaei-Ghomi, Ebrahimabadi, Djafari-Bidgoli, & Batooli, 2009; Hudaib, Speroni, Di Pietra, & Cavrini, 2002). GC/MS results for thyme are reported in the literature. Some variances between our results and others were seen, as Chamanara, Shabanpour, Gorgin, and Khomeiri, (2012) reports 21.89% and 3.63% for carvacrol and thymol, respectively. Schubring and Oehlenschlager (1997) and Chamanara et al. (2012) mentioned these variances could be because of difference of herbal species and their ecotypes, and other environmental parameters. At least, seven chemotypes of *T. vulgaris* exist. With regard to EOs, these findings may be related to the high concentrations primarily of thymol and carvacrol in thyme essential oil (Table 1), compounds of documented antimicrobial activity (Burt, 2004).

3.2. Physical properties of QSM films

Effects of incorporating thyme essential oil on the physical properties of QSM films are presented in Table 2. The addition of thyme essential oil to the film-forming emulsion led to an increase in the thickness of the films, which ranged between 0.063 and 0.076 mm (Table 2). However, this effect was only significant at the highest level of TEO used (2%). Altiok, Altiok and Tihminlioglu (2010) reported that the incorporation of thyme oil had no effect on thickness of chitosan films. Hoque, Benkajul and Prodpran (2011) did not find any difference in the thickness of film incorporated with various extract of cinnamon, clove and staranise. Altiok et al. (2010) reported that the incorporation of thyme oil had no effect on thickness of chitosan films. According to Han and Krochta (1999), film thickness is influenced by the solid content of the film forming solution. Thus, thyme essential oil might contribute to the formation of the loose film matrix with the increased thickness.

In general, the moisture content value increased as TEO was incorporated into QSM film, which is attributed to breakup of film network. This phenomenon increased the amount of water molecules which were present between polymer chains by hydrogen bonding. Incorporation of thyme essential oil into QSM films increased moisture content (%) from 18.67% to 18.83% (Table 2). Addition of TEO at a level of 2% v/v decreased the moisture content value significantly ($P < 0.05$), which is attributed to an increase in hydrophobicity of films. Similar results were found by Hosseini et al. (2009) for chitosan films incorporating thyme and clove essential oils. The film water solubility is directly related with the structural properties of film and the presence of components in the films (like phenolic compounds).

The incorporation of thyme essential oil decreased water solubility of QSM films with respect to control. Addition of TEO at a level of 2% v/v decreased the water solubility value. Addition of thyme and clove essential oils at a level of 1.5% v/v decreased the water solubility value of the chitosan-based films (Hosseini et al., 2009). Similar results were found by Shojaee-Aliabadi et al. (2013) for κ-carrageenan films incorporating *Satureja hortensis*. They reported these results could be attributed to a decrease in the hydrophilic nature of the films, as well as interaction between the components of essential oil and the hydroxyl groups of carrageenan film, which would reduce availability of hydroxyl groups for interaction with water molecules, consequently leading to a more water-resistant film. In our study lower moisture content with minimum solubility (17.29% and 43.23%, respectively) was achieved for films formulated with 2% thyme essential oil.

3.3. Water vapor permeability

WVP of QSM films incorporated with TEO at various concentrations is presented in Table 2. WVP of TEO incorporated films were slightly increased from 7.62 to 8.97–14.91 $\times 10^{-11}$ as the

Table 2
Physical and thermal properties of plasticized quince seed mucilage (QSM) films incorporated with various thyme essential oil (TEO) concentrations[a].

TEO concentration (%V/V)	Thickness (mm)	Moisture content (%)	Solubility in water (%)	WVP ($\times 10^{-11}$ g/s m Pa)	O_2P (cm^3 μ/m^2 d kpa)
0 (Control)	$0.063 \pm 0.002_b$	$18.67 \pm 0.25_a$	$47.69 \pm 0.86_a$	$7.62 \pm 1.01_c$	$38.81 \pm 2.32_c$
1	$0.066 \pm 0.003_b$	$18.65 \pm 0.24_a$	$47.49 \pm 1.24_a$	$8.77 \pm 1.14_{bc}$	$40.30 \pm 2.44_c$
1.5	$0.070 \pm 0.002_b$	$18.33 \pm 0.19_a$	$46.39 \pm 1.04_a$	$9.92 \pm 0.78_b$	$46.98 \pm 3.46_b$
2	$0.076 \pm 0.003_a$	$17.29 \pm 0.18_b$	$43.23 \pm 1.02_b$	$14.91 \pm 1.09_a$	$56.02 \pm 4.59_a$

[a] Reported values correspond to the mean ± standard deviation. Different letters in the same column indicate significant differences ($P < 0.05$).

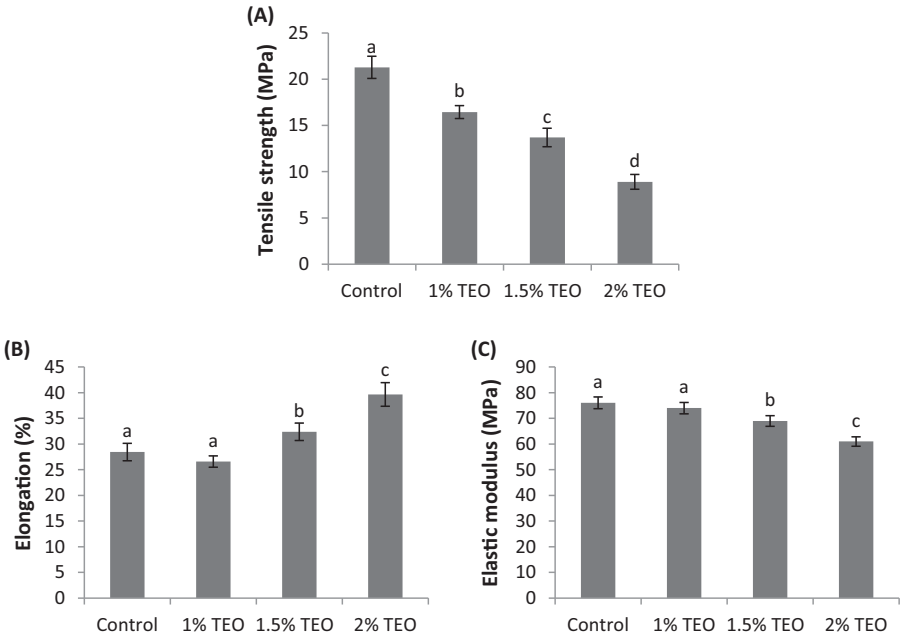

Fig. 1. Tensile strength (A), elongation at break (B), and Elastic modulus (C), of the quince seed mucilage (QSM) films incorporated with various concentrations (0%, 1%, 1.5% and 2%) of thyme essential oil (TEO).

concentrations of TEO ($P < 0.05$). Hernandez (1994) indicated that water vapor transfer generally occurs through the hydrophilic portion of the film and depends on the hydrophilic–hydrophobic ratio of the film components. WVP depends on the hydrophilic–hydrophobic ratio of the film constituents (Pelissari, Grossmann, Yamashita, & Pineda, 2009). However some studies on the incorporation of essential oils and natural extracts have not shown improvements in WVP (Atarés, De Jesús, Talens, & Chiralt 2010; Zinoviadou, Koutsoumanis, & Biliaderis, 2009). This might be due to the differences in the hygroscopic nature of the oils used, which caused the different ability to attract water to the film network. In the other study, Rojas-Grau et al. (2007) found that the WVP of alginate–apple puree edible films were not affected by the incorporation of oregano oil, because EO consist mostly of terpene-like compounds, not lipids. Similar results were found by Bonilla, Atarés, Vargas, and Chiralt (2011) in chitosan based films containing thyme essential oils and Martins, Cerqueira, and Vicente (2012) in chitosan based films containing α-tocopherol. They showed that increase of thyme essential oils and a-tocopherol concentration leads to the increase of WVP values. A great number of factors can affect the WVP of the films as e.g. film thickness, water sensitivity and crystallinity. The obtained

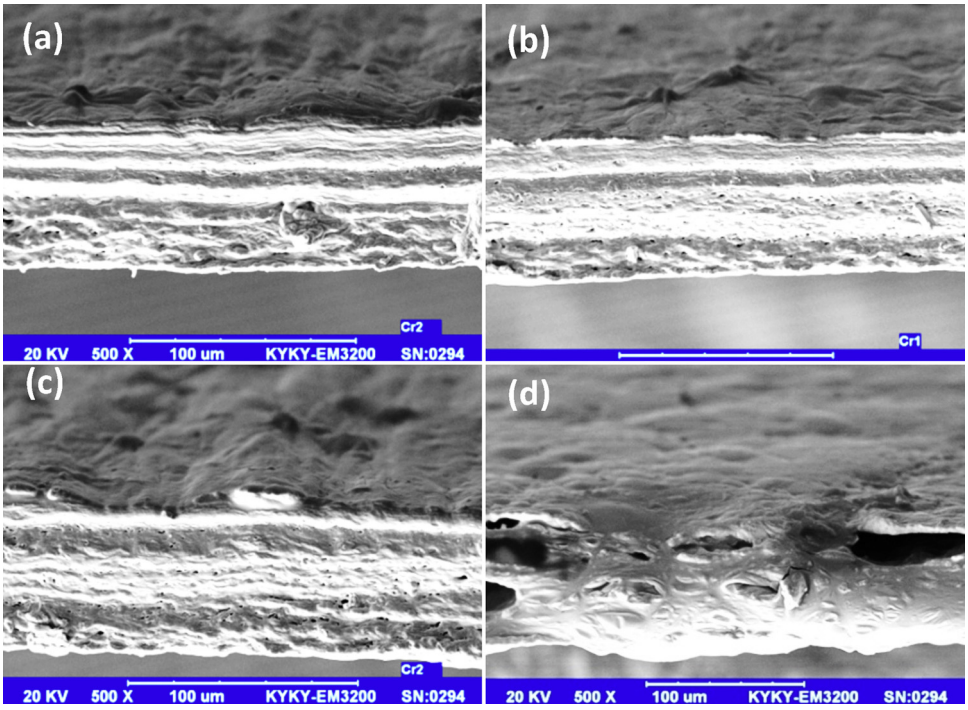

Fig. 2. SEM micrographs of the cross-sections of the films. (a) control (0% TEO), (b) 1% TEO, (c) 1.5% TEO, (d) 2% TEO.

Table 3
Effect of different concentrations of TEO on color values including, *L*, *a*, and *b*, total color difference (ΔE) and whiteness index (WI) of plasticized quince seed mucilage (QSM) films [a].

Thyme essential oil concentration (%V/V film forming solution)	*L*	*a*	*B*	ΔE	*WI*
0 (Control)	$87.91 \pm 0.05_a$	$-0.54 \pm 0.07_a$	$3.93 \pm 0.06_d$	$6.22 \pm 0.06_c$	$87.19 \pm 0.07_a$
1	$85.49 \pm 0.04_b$	$-0.57 \pm 0.08_{ab}$	$7.45 \pm 0.11_c$	$10.38 \pm 0.15_b$	$83.53 \pm 0.07_b$
1.5	$84.11 \pm 0.09_c$	$-0.66 \pm 0.09_b$	$8.45 \pm 0.10_b$	$12.60 \pm 0.17_b$	$81.79 \pm 0.09_c$
2	$83.56 \pm 0.05_d$	$-0.79 \pm 0.06_c$	$10.03 \pm 0.13_a$	$13.81 \pm 0.08_a$	$80.34 \pm 0.06_d$

[a] Reported values correspond to the mean ± standard deviation. Different letters in the same column indicate significant differences ($P < 0.05$).

results from mechanical (Fig. 1) and thermal properties (Fig. 2) also noticed that the presence of TEO originated less crystalline films, leading to an increase of WVP.

This hypothesis was advanced since it is well known that polymers with high crystallinity usually are less permeable due their ordered structure, and that the mass transfer of a gas in a semi-crystalline polymer is primarily a function of the amorphous phase (Miller & Krochta, 1997). Hosseini et al. (2009) stated that, although the hydrophobic nature of essential oils could affect the hydrophilic or hydrophobic property of the film, the physical factors had a dominant influence on the water vapor transmission rate through the film. Ahmad, Benjakul, Prodpran, and Agustini (2012), it cannot be assumed that the WVP of films is reduced simply by adding a hydrophobic component to the formulation. QSM films incorporated with 2% TEO showed a loose texture with sponge-like structure, with pores distributed throughout the QSM matrix (Fig. 2). Essential oil might be evaporated during drying, leading to the formation of micro-pores throughout the films (Ahmad et al., 2012). This enhanced moisture passing through the films and thereby increases the water vapor transmission rate values of the films. These latter structures could explain the observation of higher WVP in QSM films with higher thyme essential oil content.

3.4. Oxygen permeability of the films

Oxygen permeability of the QSM films with and without TEO is summarized in Table 2. The O_2P of the control QSM film was 36.81 ± 2.32 cm^3 μm/m^2 d kPa or 42.60×10^{-14} cm^3/m s Pa indicating that this film is a good oxygen barrier. The results encouraged for the oxygen permeability of QSM films show that they were more permeable than those made from polyvinylidene chloride (PVDC) (5.1 cm^3 μm/m^2 d kPa) (McHugh & Krochta, 1994), and PET films (13 cm^3 μm/m^2 d kpa) (Han & Krochta, 2007), PE films (18 cm^3 μm/m^2 d kPa) (Hong & Krochta, 2006), although the value were lower than those of high density polyethylene films (HDPE) (427 cm^3 μm/m^2 d kPa) (Miller & Krochta, 1997), low density polyethylene films (LDPE) (1870 cm^3 μm/m^2 d kPa) (Miller & Krochta, 1997). Kester and Fennema (1986) have also mentioned that hydrophilic films and coatings (polysaccharide or protein-based) generally provide a good barrier to oxygen transference.

Addition of 1% thyme essential oil to the QSM film did not affect this property significantly ($P > 0.05$). Similar study on O_2P of alginate–apple puree edible films containing 0.1% oregano essential oil also showed no significant differences when any of the essential oil was incorporated in the films (Rojas-Grau et al., 2007). The oxygen permeability values of this film increased as higher amounts of TEO were incorporated. McHugh and Krochta (1994) indicated that films containing EOs exhibit relatively poor oxygen barrier properties. This can be explained by the greater oxygen solubility in the non-polar oil phase which contributes to increase the transfer rate of the oxygen molecules into the plasticized polymer matrix. Similar results were found by Atarés, Pérez-Masiá, and Chiralt (2011) in hydroxy-propyl-methylcellulose and Fabra, Talens, Gavara, and Chiralt (2012) in sodium caseinate films. As SEM images show QSM films incorporated with TEO showed a loose texture with sponge-like structure, with pores distributed throughout the QSM matrix. This could explain the higher O_2 permeability of films containing TEO compared to control films.

3.5. Mechanical properties

TS, EM and %EB of QSM-based films are summarized in Fig. 1.Tensile strength expresses the maximum stress developed in a film during tensile testing (Gennadios, Brandenburg, Park, Weller, & Testin, 1994). Film without thyme essential oil had a tensile strength of 21.29 MPa. Tensile strengths were weaker for films containing TEO than for the control film, significantly ($P < 0.05$) decreasing as oil concentration increased. These studies concluded that the incorporation of essential oils usually reduce the TS as a result of the development of a heterogeneous film structure featuring discontinuities (Benavides, Villalobos-Carvajal, & Reyes, 2012; Shojaee-Aliabadi et al., 2013). Conversely, EB of QSM films increased significantly ($P < 0.05$) from 28.45% to 39.67%. Elongation at break is a measure of the film's stretch ability prior to breakage (Krochta & DeMulder-Johnston, 1997). Because EO is liquid at room temperature, it will be present in the film in the form of oil droplets that can easily be deformed, enhancing the film's extensibility (Fabra, Talens, & Chiralt, 2008).

Thus, TEO can act as a plasticizer, reducing TS and increasing %E of the films. Also this effect could be explained by the partial replacement of stronger polymer-polymer interactions by weaker polymer-oil interactions in the film network in the presence of the essential oil, which may weaken the network structure, and hence the tensile strength of the emulsified films (Sánchez-González et al., 2010). Similar results were obtained by Rojas-Grau et al. (2007), who observed an increment in the elongation percentage in films of alginate and apple puree added with oregano, cinnamon and lemongrass EOs. The Elastic modulus (EM) of control films (76.07 ± 1.09 MPa) was significantly greater than most of the films containing thyme essential oil (Fig. 1). The addition of thyme essential oil resulted in a film matrix that was less dense, which facilitated the movement of the polymer chains and improved the film flexibility.

3.6. Surface color

The effects of TEO concentration on color values, total color difference (ΔE) and whiteness index (WI) of films are shown in Table 3. Films without TEO were lighter (higher *L* value). *L* values of the films was decreased from 87.91 to 83.56 but *a* was decreased from −0.54 to −0.79 and *b* values was increased from 3.93 to 10.03, as the TEO concentrations were increased from 0% to 2%. Control film had ΔE value of 6.22. Incorporating thyme essential oil revealed ΔE values that were significantly higher than that of the control.

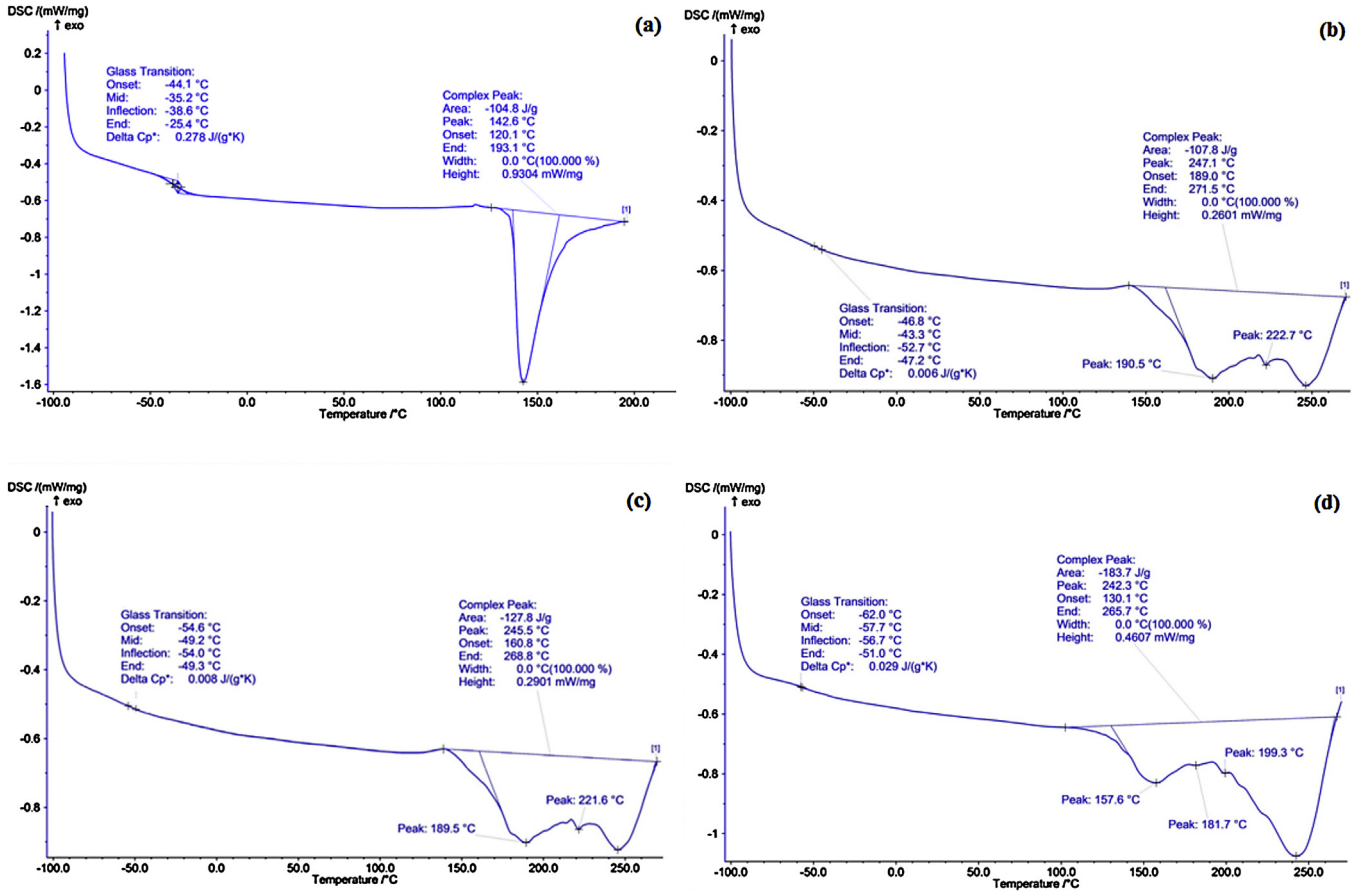

Fig. 3. Representative examples of DSC curves of pure QSM + 0%TEO, (a) QSM + 1%TEO, (b) QSM + 1.5%TEO (c) and QSM + 2%TEO, (d) films.

However, emulsified films had a slightly yellow appearance, as indicated by a significant ($P < 0.05$) increase in the b value and ΔE but a decrease in the L, a, and WI values as a function of TEO concentration. This phenomenon is probably due to the phenolic compounds of TEO, which might have light absorption at low wavelengths. QSM-based films incorporated with a concentration greater than 1% of thyme essential oil showed a total color difference (ΔE) significantly greater ($P < 0.05$) than the control film (Table 3). This behavior is attributed to the decrease in brightness (L^*) and the increase observed in the colorimetric coordinate b^*. Similar results have been reported by Benavides et al. (2012), who studied the incorporation of oregano essential oil into alginate films.

3.7. Microstructural characteristics

Scanning electron micrographs of cross-sections of QSM films incorporated with TEO are shown in Fig. 2.Scanning electron microscopy (SEM) can provide a better understanding of the relationships of water vapor transmission, mechanical and optical properties with the films' structural characteristics. Films incorporated with thyme essential oil had relatively smoother surfaces, however micro-pores and cavities were found in the films. Essential oil might be evaporated during drying, leading to the formation of micro-pores throughout the films (Ahmad et al., 2012). The film images showed that adding essential oil concentrations of 1 and 1.5% caused a heterogeneous structure in which oil droplets were entrapped in the continuous polysaccharide network (Fig. 2b and c). QSM films incorporated with thyme essential oil concentrations of 2% showed a loose texture with sponge-like structure, with pores distributed throughout the QSM matrix (Fig. 2d). These latter structures could explain the observation of higher WVP in QSM films with higher oil content.

3.8. Thermal properties

DSC studies of QSM-based films containing different proportions of thyme essential oil were performed to further understand the structure and interaction between polymers and thyme essential oil. Tg and Tm can be associated with the crystallinity of the film samples (Peesan, Supaphol, & Rujiravanit, 2005). Below Tg, films are rigid and brittle, whereas above it films become flexible and pliable (Ghasemlou, Khodaiyan, Oromiehie, & Yarmand (2011)). The glass transition temperatures (Tg) and melting temperatures (Tm) of the QSM-based films with different OEO concentrations are shown in Fig. 3. Pure QSM film had Tg of −35.2 °C, which is higher than TEO-incorporated QSM films (Tg, −43 to −57 °C). All Tg values were lower than kefiran films (−18 to −21) (Ghasemlou et al., 2011), but higher than the Tg of high-density polyethylene (−125 °C) (Robertson, 1993) and pure glycerol liquid (−93 °C) (Cherian, Gennadios, Weller, & Chinachoti, 1995). The films incorporated with TEO had slightly higher Tm than the films containing glycerol; this could be attributed to the larger molecular weight and more hydrophobic nature of TEO compared to glycerol.

This decrease may be explained by the molecular structure of TEO, which has an effect on the overall chain mobility in the QSM film. This trend is consistent with those obtained by Yang and Paulson (2000), Ghasemlou et al. (2011). They showed the lower Tm values of plasticized carbohydrate films could be attributed to their inherent structural characteristics (high chain mobility) and to their relatively high hydrophilicity, which lets them absorb more water molecules than other films; this causes a marked decrease in the

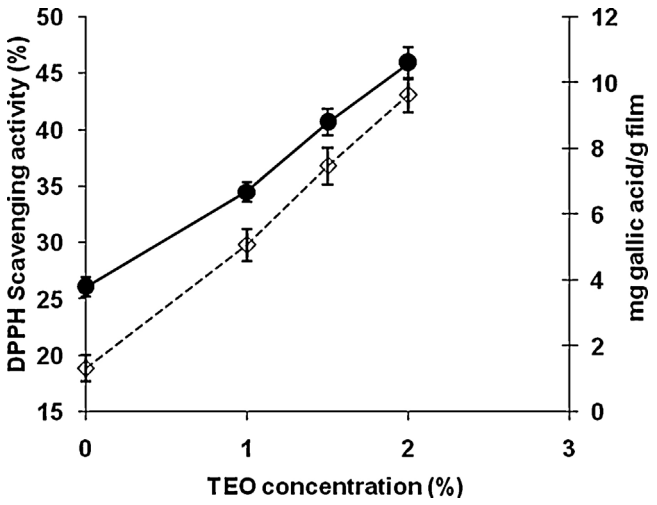

Fig. 4. DPPH radical-scavenging activity and Total phenolic content of QSM films incorporated with different concentrations (0%, 1%, 1.5% and 2%) of thyme essential oil (TEO).

Tm due to the plasticizing effect of the absorbed water molecules. The DSC curve of film containing 2%TEO showed two *T*m peaks at 157 and 242 °C. This result coincides closely with those from the SEM studies, where a partial separation of the two phases may have occurred.

3.9. Antioxidant activity and Total phenolic content of QSM films

Fig. 4 shows the antioxidant activity of different TEO-formulated QSM films. The radical scavenging activity of QSM films with and without incorporated thyme essential oil was determined and is shown in Fig. 4. QSM films showed radical scavenging activity of 18.39% on DPPH. Films with TEO exhibited a higher level of radical scavenging activity with values of 30.11%, 37.29% and 43.14% for 1%, 1.5% and 2% of TEO containing films, respectively. The results showed that DPPH scavenging activity of the QSM films significantly was increased ($P < 0.05$) with increasing TEO concentration as shown in Fig. 4. Similar results were found for hake proteins film formulated with thyme extracts (Pires et al., 2011), κ-carrageenan films film incorporated with *S. hortensis* essential oil (Shojaee-Aliabadi et al., 2013) and chitosan film incorporated with *Zataria multiflora* essential oil (Moradi et al., 2012).

The antioxidant power of essential oils, caused mainly by their phenolic compounds, has been reviewed by Dimitrios (2006). Among *Lamiaceae* species, thyme (*T. vulgaris* L.) has been studied widely for its antioxidant activity, due to the high content of phenolic compounds (Zandi & Ahmadi, 2000; Takacsova et al., 1995). Adding the thyme essential oil raised the phenol contents of the films. The phenol content was higher in the films containing thyme than in the pure QSM films (control). TP content for pure QSM films (3.98 mg gallic acid/g film) was measured. The results showed that total phenolic content in the QSM films significantly was increased ($P < 0.05$) with increasing thyme essential oil concentration (Fig. 4). As by Shojaee-Aliabadi et al. (2013) and Shan, Cai, Sun, and Corke (2005) mentioned, in our study all the EO concentrations studied, there was a linear correlation between TP content and antioxidant activity.

3.10. Antibacterial activity

The antibacterial activities of QSM films incorporated with thyme essential oil against Gram-negative and Gram-positive bacteria are shown in Table 4. This method is based on the measurement of the clear zone caused by growth inhibition produced by a film disk containing the antimicrobial agent when putting in direct contact with a bacterial culture (Weerakkody, Caffin, Turner, & Dykes, 2010). The results show that the control films did not inhibit the growth of the ten pathogenic bacteria. QSM films showed antibacterial effect against all bacteria studied after the incorporation of 1.0% thyme essential oil. As Hosseini et al. (2009) mentioned films containing thyme essential oil in general were very hydrophilic, thus, they absorbed water quickly and resulted in swelling. Therefore, the active components in these films migrated very fast.

Films containing 1% of TEO were effective against all test microorganisms and exhibited a strong inhibitory effect on the growth of *S. putrefaciens*, *L. monocytogenes* and *S. aureus* as evidenced by minimal growth around the film cuts. Similar antimicrobial activities of essential oils from oregano against *S. aureus* were also determined by Emiroğlu, Yemiş Coşkun and Candoğan (2010), and Nedorostova, Kloucek, Kokoska, Stolcova and Pulkrabek (2009). Inhibition was increased with increasing concentration of essential oil. As expected, the films containing the highest oil

Table 4
Inhibition zone diameters yielded by QSM film disks with various concentrations (0, 1, 1.5 and 2%) of thyme essential oil (TEO)[a].

Test organisms	Inhibition zone diameters(mm^2)			
	TEO concentration (%)			
	0	1	1.5	2
Staphylococcus aureus	0.0_d	$74.56 \pm 5.45_c$	$159.65 \pm 11.65_b$	$255.12 \pm 21.43_a$
Pseudomonas aeruginosa	0.0_d	$57.90 \pm 4.39_c$	$120.12 \pm 10.78_b$	$196.38 \pm 18.69_a$
Listeria monocytogenes	0.0_d	$77.70 \pm 6.94_c$	$150.89 \pm 14.91_b$	$290.60 \pm 16.06_a$
Escherichia coli	0.0_d	$63.69 \pm 9.13_c$	$143.08 \pm 15.05_b$	$269.55 \pm 20.17_a$
Escherichia coli O157:H7	0.0_d	$61.29 \pm 8.95_c$	$139.08 \pm 17.40_b$	$237.06 \pm 22.19_a$
Salmonella Typhimurium	0.0_d	$53.24 \pm 9.23_c$	$110.60 \pm 10.82_b$	$188.33 \pm 18.54_a$
Lactobacillus plantarum	0.0_d	$51.77 \pm 3.45_c$	$83.98 \pm 9.24_b$	$125.16 \pm 19.46_a$
Bacillus cereus	0.0_d	$61.80 \pm 6.03_c$	$119.49 \pm 12.11_b$	$170.72 \pm 18.22_a$
Yersinia enterocolitica	0.0_d	$89.41 \pm 7.79_c$	$184.95 \pm 16.42_b$	$226.89 \pm 21.09_a$
Vibrio cholera	0.0_d	$75.59 \pm 8.41_c$	$144.17 \pm 16.15_b$	$221.50 \pm 20.29_a$
Shewanella putrefaciens	0.0_d	$88.40 \pm 6.16_c$	$184.09 \pm 17.89_b$	$313.61 \pm 22.70_a$

[a] Reported values correspond to the mean ± standard deviation. Different letters in the same column indicate significant differences ($P < 0.05$).

content (2%) presented the greatest zone of inhibition ($P < 0.05$). However, inhibition of film towards some microorganisms reached the maximum at some particular concentration. Among the bacteria examined, *P. aeruginosa* showed the highest resistance, while *S. putrefaciens* was the most sensitive to TEO-containing films, with an inhibition zone of 313.61 mm^2.

The antimicrobial activity of thyme and oregano has been attributed to their essential oils, which contain the terpenes: carvacrol (2-methyl-5-[1-methylethyl] phenol) and thymol (5-methyl-2-[1- methylethyl) phenol]), respectively. According to Burt (2004), carvacrol and thymol disintegrate the outer membrane of Gram-negative bacteria, releasing lipopolysaccharides and increasing the permeability of the cytoplasmic membrane to adenosine tri-phosphate. Carvacrol dissolves in the phospholipid bilayer of *B. cereus* (Gram-positive) and causes expansion and destabilization of the membrane leading to cell death (Ultee, Kets, & Smid, 1999). QSM films with TEO were significantly more effective against Gram-positive bacteria (*S. aureus* and *L. monocytogenes*) than against Gram-negative bacteria (*E. coli*, *P. aeruginosa* and *S. typhimurium*).

The lower antibacterial activity against *S. enteritidis* and *P. aeroginosa* could be due to the higher resistance of Gram-negative microorganisms to these compounds. Numerous studies investigating the action of essential oil against pathogenic microorganisms agree that essential oils are most effective against Gram-positive bacteria than against Gram-negative (Burt, 2004; Pelissari et al., 2009). The mechanism proposed by Zivanovic, Chi and Draughon, (2005) for the antimicrobial activity of phenolic compounds from essential oils is their attack to phospholipids of the cellular membrane, causing an increase in permeability and loss of cytoplasm, or interactions with enzymes localized in the cell wall. Burt (2004) suggests that this behavior may be related to the presence of an additional external membrane surrounding the cell wall in Gram-negative bacteria, which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering.

4. Conclusion

The properties of QSM-based films were affected by the addition of thyme essential oil. The present work shows that thyme essential oil can be added to a QSM-based film matrix, imparting a good antioxidant activity. QSM films exhibited some antioxidant activity, which was significantly improved by the addition of the essential oil. The QSM films prepared with thyme essential oil exhibited highest inhibition against *S. putrefaciens*, *L. monocytogenes* and *E. coli*. Addition of 1% oregano essential oil did not affect the oxygen and water vapor permeability of the films. Films incorporated with thyme essential oil exhibited significant antibacterial activity against Gram-positive bacteria (*S. aureus* and *L. monocytogenes*) than Gram-negative bacteria (*P. aeruginosa* and *S. typhimurium*). Thyme essential oil significantly reduced Tensile strength and Young's modulus, while increasing the percent elongation of the films. Scanning electron microscopy showed that the microstructure of biopolymer-based films has a critical effect on their physical and mechanical properties that is important in food packaging applications. The results of the present study suggest that the QSM films incorporated with TEO as a natural antioxidant-antibacterial agent can potentially be used in packaging a wide range of food products, particularly those that are highly oxidative and microbial sensitive.

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