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Antimicrobial, mechanical and barrier properties of triticale protein films incorporated with oregano essential oil

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ABSTRACT

Antimicrobial triticale protein films were prepared by incorporating oregano essential oil (OEO). The effect of different concentrations of OEO on antimicrobial activity and on mechanical and barrier properties of films plasticized with glycerol was evaluated and compared with control films (without antimicrobial agent). The addition of OEO did not affect the water vapor permeability of films, increased water solubility and the percent elongation of the films, and reduced tensile strength and Young's modulus. Films with OEO showed higher antimicrobial activity against the Gram positive bacterium *Staphylococcus aureus*, and lower for Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*). The obtained results proved the permanence of OEO in the polymer matrix after processing, making them able to be used as active additives in film formulation.

1. Introduction

Due to increased health and environmental concerns, extensive studies are conducted to develop edible and environmentally friendly biodegradable packaging materials from natural polymers (Arcan & Yemenicioglu, 2011). Protein, lipids and polysaccharides are used for the preparation of bio-based packaging (Ahmad, Benjakul, Prodpran & Agustini, 2012; Paschoalick, García, Sobral & Habitante, 2003). Packaging is used for food quality, extending the shelf life of perishable items, especially those susceptible to microbiological deterioration. Antimicrobial film preparation is increasing the attention from food and packaging industries due to the consumer demands for minimally processed and preservative-free products (López, Sánchez, Batlle & Nerín, 2007). Food-packaging limits possible undesirable flavors caused by the direct addition of additives into food (López et al., 2007; Suppakul, Miltz, Sonneveld & Bigger, 2003). Also, since microbial contamination on the food surface exists during the steps of the process, in order to guarantee food safety, active food packaging could be more interesting than applying antibacterial technologies directly to the food (Ouattara, Simard, Piette, Begin & Holley, 2000).

The use of natural phenolic compounds in food packaging is particularly encouraged since they improve food oxidative and microbial status. The molecular weight and structure of phenolic compounds can show a great variation and they may contain different numbers of hydroxyl groups capable of forming H-bonding with peptide carbonyl groups of proteins (Damodaran, 1996). Phenolic compounds are among the most

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potent and abundant bioactive compounds which can easily be obtained from different plant materials, agro-industrial wastes, and byproducts (Arcan & Yemenicioglu, 2011). Essential plant oils rich in phenolic compounds have been reported to have a wide spectrum of antimicrobial activity. Among these, oregano has been found to be one of the most effective. Its antimicrobial properties have been demonstrated in numerous studies (Emiroglu, Yemis, Coskun & Candogan, 2010). Carvacrol, thymol, c-therpinene and p-cymene are the principal constituents of oregano essential oil (Burt, 2004). Its antimicrobial properties have been demonstrated in numerous studies (Emiroglu et al., 2010; Zivanovic, Chi & Draughon, 2005; Zinoviadou, Koutsoumanis & Biliaderis, 2009).

The incorporation of EOs in films to avoid microbial food spoilage is considered an attractive option by manufacturers and demanding consumers. Essential oils, categorized as GRAS (generally recognized as safe), can be considered as the potential alternatives to synthetic additives (Ahmad et al., 2012). Essential oils extracted from plants are rich sources of biological active compounds (Burt, 2004). Studies on the bacterial activity of several EOs have shown that oregano is one of the most effective antibacterial agent (Emiroglu et al., 2010; Seydim & Sarikus, 2006; Zinoviadou et al., 2009). Therefore, there is an increasing interest in the evaluation and possible application of these compounds for minimizing the superficial contamination of foods, decreasing the microbial growth rate of microorganisms responsible for food degradation (Emiroglu et al., 2010; Sanchez-Garcia, Ocio, Gimenez & Lagaron, 2008).

In recent years, food packaging research has focused on biodegradable and/or edible films made from natural polymers. Such polymers may be proteins of agricultural origin and their chemical nature determines the physical properties of the resulting films (Atarés, De Jesús, Talens & Chiralt, 2010; Salgado, López-Caballero, Gómez-Guillén, Mauri & Montero, 2012). A particular interest has been focused on the use of triticale protein to prepare films. Triticale ($\times$ Triticosecale Wittmack) is a hybrid grain produced by crossing wheat and rye. The first area of interest for triticale is for use as a feed grain for livestock because it has proven to be a good source of protein, amino acids and B vitamins. A second area of interest is the use of straws for biofuel production. Triticale outperforms wheat as a biofuel feedstock because it uses less nitrogen to achieve similar yields. The versatility that triticale offers as a grain, a forage and as a biofuel feedstock adds to the economic viability that sustains the interest in the crop. Although triticale is a suitable grain for human diet, the overall food market for triticale is very small. Current triticale varieties do not possess the milling and baking characteristics to be competitive with wheat for use in bread and pasta products. Triticale flour proteins showed suitable film-forming capacity for the formulation of biodegradable films with properties comparable to that of other edible films and could be used as a component of new biopolymeric films (Aguirre, Borneo & León, 2011).

The aim of this work is to analyze the effect of oregano essential oil (OEO) incorporation on antimicrobial activity, solubility, mechanical (tensile strength, percent elongation at break, Young's module and puncture force) and barrier (water vapor permeability) properties of triticale protein films in order to make a contribution to develop novel active film, discussing the potential contributions of interactions between OEO's compounds and proteins.

2. Materials and methods

2.1. Materials

Triticale (variety Buck TK 205) flour (moisture content, 13.25 $\pm$ 0.02 g/100 g, protein content, 8.88 $\pm$ 0.02 g/100 g, ash content, 0.61 $\pm$ 0.02 g/100 g, particle size: pass through a US Standard Sieve no. 100) was donated by Campeloni Semillas S.A. (Córdoba, Argentina). All chemical reagents used in this research were purchased from Sigma-Aldrich Chemie Gmbh (Munich, Germany) and were of analytical grade. *Staphylococcus aureus* (ATCC29737), *Escherichia coli* (ATCC25923) and *Pseudomonas aeruginosa* (ATCC27853) were obtained from the culture Collection of the Microbiology Laboratory of Ceprocor, Córdoba, Argentina.

2.2. Oregano essential oil extraction and analyses.

Samples of at least 200 g of dried leaves of oregano were hydrodistilled in triplicate for 1 h using a Clevenger-type apparatus. The essential oil (EO) content was gravimetrically quantified. Each sample was analyzed three times, and the average content of EO was used for further evaluation. The EOs were dried over anhydrous sodium sulfate and stored at 4–6 °C until further analysis. For the quantification of individual components, the EO was analyzed using a Perkin-Elmer Clarus 500 gas chromatograph equipped with a flame ionization detector (GCFID). A capillary column DB-5 (30 m–0.25 mm i.d. and 0.25 m coating thickness) was used for the separation of individual components of the EO. Helium was employed as the carrier gas with a flow rate of 0.9 ml/min. The temperature program was 60 °C for 5 min, from 60 to 250 at 5 °C/min, with a final hold time of 10 min. The injector and detector were maintained at 260 and 280 °C, respectively. The sample, 0.2 μl, was injected with a 1:100 split ratio. For the determination of the composition, EO samples were diluted with hexane. The injection volume was 1 μl. The identification of the components of the EO was realized by GC–MS. A Perkin-Elmer Q 700 GC–MS coupled with an ion trap mass detector was employed for the identification. A capillary column DB-5 (30 m–0.25 mm i.d. and 0.25 m coating thickness) was used for the separation of the components. Helium was used as carrier gas with a flow rate of 0.9 ml/min. The temperature program for the oven and injector was the same as that for the GC-FID. Ionization was realized by electron impact at 70 eV. Mass spectral data were acquired in the scan mode in the *m*/*z* range 35–450. Retention indices (RI) of the sample components were determined on the basis of homologous n-alkane hydrocarbons under the same conditions. The compounds were identified by comparing their retention indices and mass spectra with published data (Adams, 1995). The main components were further identified by coinjection of authentic standards (SIGMA, USA). Fenchone was used as internal standard at a concentration of 0.1 mg/ml dichloromethane. The quantitative composition was obtained by

peak area normalization, and the response factor for each component was considered to equal 1.

2.3. Proteins-rich film-forming solution preparation

Protein fractions were extracted from triticale flour according to the method described by Aguirre et al. (2011). Albumins and globulins were extracted from 50 g of flour using 0.25 l of NaCl solution (5 g/l). Flour was stirred (20 °C) for 1 h and centrifuged for 15 min. Supernatant was discarded. The precipitate was dispersed in 0.25 l of an ethanol solution (70 ml/100 ml), stirred 1 h (20 °C), and centrifuged for 15 min (20 °C). The supernatant containing proteins was collected.

2.4. Film preparation

Films were obtained by a casting process for a final 7.5 g of protein/100 ml film-forming solution. Glycerol was added at 20 g/100 g protein as plasticizer, and stirred for 20 min. This proportion was chosen for the incorporation of OEO because this formulation had the best mechanical properties results in a previous study (Aguirre et al., 2011). The antimicrobial essential oil was added at concentrations of 0, 1 and 2% (w/v) in relation to the total basic formulation of the film forming solution. The solution was stirred again for 15 min.

Measured volumes (20 ml) of the film forming solution were poured onto a horizontal flat silicon tray (12 cm diameter) to allow water and ethanol to evaporate. Films were dried at 40 °C in an oven with air circulation. Dry films were peeled off the casting surface and preconditioned in an environmental chamber at 25 °C and 32% relative humidity (RH) that was obtained using saturated salt solutions of $MgCl_2$ for at least 72 h prior to testing. Film thickness was determined using a manual micrometer at five random positions on the film to obtain an average value.

2.5. Moisture content and film solubility in water

Moisture content of films was determined gravimetrically by drying small pieces in a ventilated oven at 105 °C for 24 h in triplicate. Film solubility was defined by the content of dry matter that was lost after 24 h immersion in water. The initial dry matter in each film was determined at 105 °C for 24 h. Discs of film were cut, weighed and immersed in 50 ml of distilled water for 24 h at room temperature. The pieces of film were taken out and dried (105 °C for 24 h) to determine the weight of dry matter that was dispersed in water.

2.6. Water vapor permeability (WVP)

WVP was measured gravimetrically according to the method described by Aguirre et al. (2011). Each film sample was sealed over a circular permeation cup containing silica gel (desiccant at RH 0%). The permeability cups were 3.5 cm in diameter. The air gaps between the silica surface and the films were less than 4 mm. The cups were then placed in a pre-equilibrated cabinet. The environment within the cabinet was held at constant RH and controlled temperature. The RH inside the cell was always lower than the outside, and water vapor transport was determined from the weight gain of the permeation cell. Cups were periodically weighted and water vapor transfer rates (WVTR, $g\,m^{-2}\,s^{-1}$) of films were determined from the slope of weight gain versus time plots using:

$$WVTR = (\Delta m/A \times \Delta t),$$

where Δm is weight gain of permeation cell (g), A is the exposed area and Δt is time. Water vapor permeability (WVP, $gm^{-1}\,s^{-1}\,Pa^{-1}$) were calculated using the following equation:

$$WVP = WVTR \times X/\Delta P$$

where X is film thickness and ΔP is vapor partial pressure difference (Pa) across the film.

2.7. Mechanical properties

Tensile strength (TS), percent of elongation at break (%E) and Young's modulus (EM) were determined using a texture analyzer TA.XT2i (SMS, Surrey, England) in accordance with ASTM D-882-91 method (1996), directly from the stress–strain curves using the software Texture Expert V.1.22 (SMS). Films were cut into 20 mm wide and 50 mm long strips, and mounted between the grips of the texture analyzer. The initial grip separation was set at 30 mm and the crosshead speed at 1.0 mm/s. All determinations are the means of at least five measurements.

2.8. Puncture test

The force at the breaking point of the triticale films were determined by a puncture test using a texture analyzer TA.XT2i (SMS, Surrey, England). The films were fixed on a still flat surface with a 10 mm diameter hole and perforated with a P/2 N probe (needle probe), moving at 1 mm/s until the film broke. The puncture force (PF) at break was determined with the software Texture Expert V.1.15 (SMS) directly from the force × displacement curves. All determinations were made five times. Mean and standard deviations were calculated.

2.9. Antimicrobial activity of the films

The antimicrobial activity of the protein films were determined by the agar disk diffusion method against *S. aureus* (ATCC29737), *E. coli* (ATCC25923), *P. aeruginosa* (ATCC27853) by measuring the inhibition by digital image analysis. The strains were selected because of its importance in human health for being responsible for food spoilage. We measured the diameter of inhibition zone against test microorganisms. The culture medium used was Trypticase Soy Agar (TSA) and Brain Heart Infusion broth (BHI), autoclaved at 121 °C × 15 min. We conducted confirmatory biochemical tests and microorganisms before each use. Microorganisms were reac tivated in BHI. Swab was distributed evenly over the agar surface, an inoculum of a tube-like turbidity no. 0.5 (1 × 108 CFU/ml) of the scale and subsequently deposited MacFarland disks on the plate. There were two replicates for each microorganism. Then, the (Petri dishes) plates were incu bated at 37 ± 1 °C for 24 h. After the incubation period, the antimicrobial activity of each material was evaluated by

observing the formation of zones of inhibition and measuring the diameter in mm with Image J (http://rsbweb.nih.gov/ij/).

2.10. Statistical analyses

Statistical data was analyzed using Microsoft Excel 2003. ANOVA was carried out to test mean differences. Student's t-test was applied to compare averages of properties with a level of 95% confidence interval.

3. Results and discussion

3.1. Oregano essential oil analyses

The oregano essential oil (OEO) content of dry leaves was $3.90 \pm 0.25\ mg/g^{-1}$ dry matter. The major components (only those components with concentrations greater than 1.5%) in oregano oil are listed in Table 1. The principal components were monoterpene *trans*-sabinene hydrate (34.30%) and thymol (17.93%). Carvacrol, thymol, γ-therpinene and p-cymene are the principal constituents of OEO (Burt, 2004; Lambert, Skandamis, Coote & Nychas, 2001). The activity of the essential oils is related to the respective composition, the structural configuration of the constituent components and their functional groups and possible synergistic interactions between components (Dorman & Deans, 2000). The components with phenolic structures, such as carvacrol, eugenol and thymol, were very active against microorganisms (Gutierrez, Barry-Ryan & Bourke, 2008). The importance of the hydroxyl group in the phenolic structure was confirmed by Dorman and Deans (2000). Also important is the presence of phenolics together, since the work of Lambert et al. (2001) showed that the combination of carvacrol and thymol were additive against *S. aureus* and *P. aeruginosa*. The compound β caryophyllene, also present in the essential oil used, has antimicrobial activity against Gram-positive bacteria, according to the study published by Longaray Delamar, Moschen-Pistorello, Artico, Tai-Serafini and Echeverrigaray (2005).

3.2. Film properties

Thickness of control triticale protein films and those incorporated with oregano essentials oil at various concentrations was $200 \pm 50\ \mu m$. No significant differences in thickness was observed between films incorporated with essential oil. 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 star anise.

3.3. Moisture content hand water solubility of films

Incorporation of OEO into triticale films did not significantly affect the moisture content relative to plain triticale films (control films), except in 2% OEO films, from 11.83% to 15.15% (Table 2). These findings are in agreement with the data reported by Zinoviadou et al. (2009), who also showed that oregano oil addition at 0.5, 1 and 1.5% did not markedly affect the water content of whey protein isolate films.

The film water solubility is directly related with the structural properties of proteins and the presence of other non-proteinaceous components in the films (like phenolic compounds). The incorporation of OEO increased water

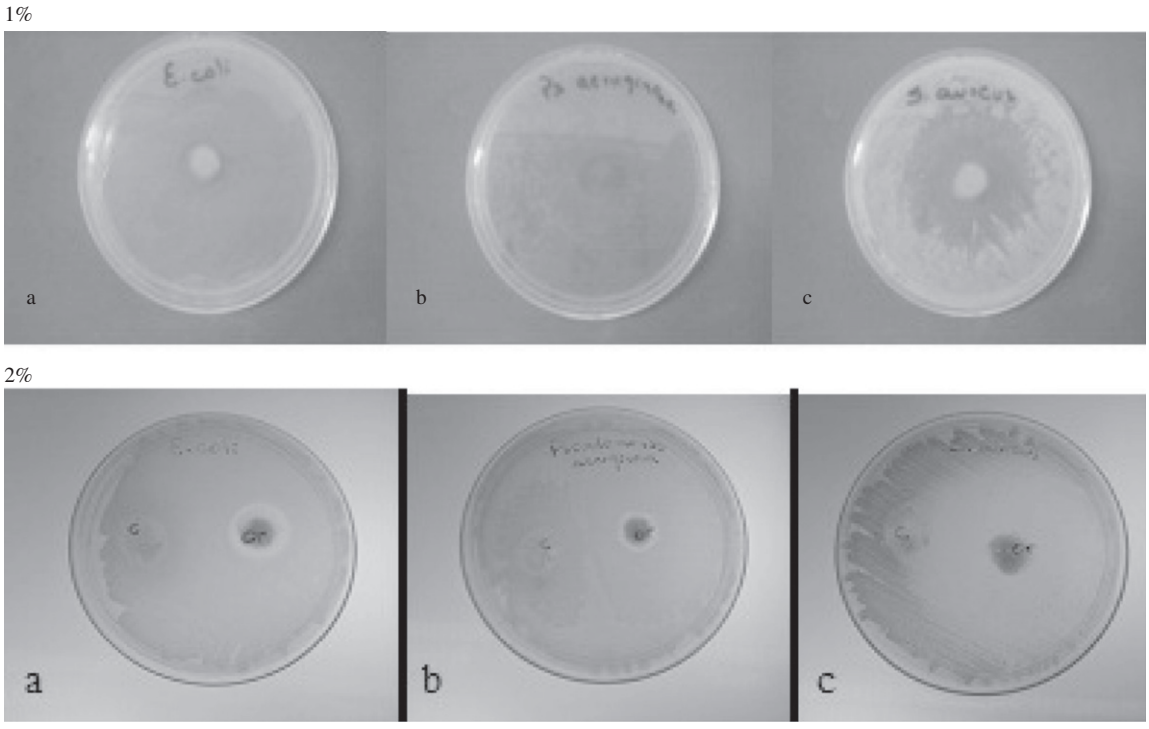

Fig. 1 – Inhibition zones for triticale proteína films incorporated with 1 and 2% OEO against the microorganisms (a) *E. coli*, (b) *P. aeruginosa* and (c) *S. aureus*.

solubility of triticale films (Table 2) with respect to control, but there was no significant differences between the two concentration of OEO added. This increase in soluble matter could be due to a lower density of interaction of the polymeric network by the presence of OEO, because of the similarities in the structure of glycerol and polyphenols (e.g. OH groups).

3.4. Mechanical properties and puncture test

The tensile strength (TS), Young's modulus (EM) and elongation at break (%*E*) are parameters representing the mechanical properties of the films, which depend on their microstructural characteristics. TS, EM and %*E* of triticale films are summarized in Table 3. According to it, a 71% reduction in the TS values (2.9–0.85 MPa) was obtained when adding 2% of OEO to the triticale films. Consequently, the %*E* increased significantly (Table 3), ranging from 9.67% (control) to 84.56% for films containing 2% of OEO ($p<0.05$). This trend is consistent with those obtained by Benavidez, Villalobos-Carvajal and Reyes (2012), Pelissari, Grossman, Yamashita and Pineda (2009), Zinoviadou et al. (2009) and Zivanovic et al. (2005). The incorporation of essential oils usually reduce the TS as a result of the development of a heterogeneous film structure featuring discontinuities. Because OEO 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).

The presence of OEO in the film-forming solution may have interfered with interactions between the triticale proteins, reducing the intermolecular forces along polymer chains, improving the flexibility and chain mobility. Thus, OEO can act as a plasticizer, reducing TS and increasing %*E* of the films.

The Young's modulus (EM) indicates the rigidity of the film; a larger EM indicates a more rigid material. Table 3 shows that the OEO led to a significant reduction of the Young's modulus and, therefore, the formation of less rigid films. The film produced with only triticale protein and glycerol presented a higher EM of 147.59 MPa. The addition of OEO resulted in a film matrix that was less dense, which facilitated the movement of the polymer chains and improved the film flexibility. The addition of OEO also decreases the EM of whey protein isolate films (Zinoviadou et al., 2009).

The films produced in this work presented puncture force values (Table 3) equivalent to amaranth flour films (Tapia-Blacido, do Amaral Sobral & Menegalli, 2011). The incorporation of OEO into triticale films did not significantly affect the puncture force relative to control films.

Table 1 – Relative percentage of the constituent of oregano leaves, according to their elution order in GC analysis.

RI	Component	%
1017	α-terpinene	4.77±0.32
1029	limonene	4.33±2.73
1031	1,8 cineole	3.9±0.0
1060	γ terpinene	6.9±0.95
1070	*cis*-hydrate sabinene	3.40±0.1
1098	*trans*-hydrate sabinene	34.3±5.82
1177	terpinel 4 ol	6.37±1.42
1189	α terpineol	1.9±0.1
1290	thymol	17.93±0.4
1299	carvacrol	3.8±0.36
1419	β caryophylene	1.7±0.6
	Total	97.9±2.2

RI: Retention index. Reported values are means±standard deviation.

3.5. Water vapor permeability

WVTR and WVP of triticale films incorporated with OEO are shown in Table 2. No significant change ($p<0.05$) was observed after the incorporation of OEO. WVP depends on the hydrophilic–hydrophobic ratio of the film constituents (Pellisari et al., 2009), however some studies on the incorporation of essential oils and natural extracts have not shown

Table 2 – Effects of concentration of oregano essentials oil on moisture content, film solubility and permeability of triticale protein films.

Oregano essential oil (%)	Moisture content (%)	Film solubility (%)	WVP × 10^{10} (g m^{-1} s^{-1} Pa^{-1})
0	11.83±1.03[a]	41.22±0.09[a]	0.33±0.04[a]
1	11.43±1.02[a]	45.91±0.36[b]	0.35±0.05[a]
2	15.15±0.82[b]	46.48±0.49[b]	0.40±0.05[a]

Reported values are means±standard deviation. Different letters (a, b) in the same column indicate significant differences ($p<0.05$).

Table 3 – Effects of concentration of oregano essentials oil on mechanical properties and puncture test of protein triticale films.

Oregano essential oil (%)	Tensile strength (MPa)	Young's modulus (MPa)	Elongation at break (%)	Puncture force (N)
0	2.90±0.03[a]	147.59±0.71[a]	9.67±1.25[a]	1.17±0.17[a]
1	2.08±0.19[a]	82.53±11.22[b]	12.10±0.26[b]	1.04±0.43[a]
2	0.85±0.16[b]	21.75±10.58[c]	84.56±5.18[c]	0.98±0.29[a]

Reported values are means±standard deviation. Different letters (a, b, c) in the same column indicate significant differences ($p<0.05$).

improvements in WVP (Atarés et al., 2010; Zinoviadou et al., 2009). OEO is hydrophobic and complex mixture, but also according to Atarés et al. (2010), it cannot be assumed that the WVP of films is reduced simply by adding a hydrophobic component to the formulation.

3.6. Antimicrobial activity

Table 4 shows the results of the antimicrobial tests performed against *E. coli*, *S. aureus* and *P. aeruginosa* by the agar disk diffusion method. 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 triticale protein films without OEO (control) showed no antimicrobial activity against the studied microorganisms. But, the effect of OEO was incorporated and expressed in triticale films (Fig. 1). Films containing 2% of OEO were the most effective. The larger zones of inhibition were observed for the Gram positive bacterium *S. aureus*, while the lower for Gram negative (*E. coli* and *P. aeruginosa*). The OEO concentration of 1% was not effective against *P. aeruginosa*. Numerous studies investigating the action of OEO 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 incorporation of 1% of OEO into whey protein films was effective against *E. coli* O157: H7 and *Pseudomonas* spp. (Oussallah, Caillet, Salmieri, Saucier & Lacroix, 2004). The antimicrobial activity against *L. plantarum*, *S. enteritidis*, *E. coli*, *L. monocytogenes* and *S. aureus* of the essential oil of oregano (2% *w/v* film forming solution) was also expressed in films whey proteins films (Seydim & Sarikus, 2006). Lopez, Sanchez, Batlle and Nerin (2005) also reported high effectiveness of oregano essential oil in Gram-positive bacteria.

The inhibitory efficacy of essential oils is mainly due to the most abundant components (Cosentino et al., 1999) and there is a relationship between the chemical structure of these components in the essential oil, their concentration and the antimicrobial efficacy (Vigil, 2005). Xu, Zhou, Ji, Pei and Xu (2008) have described the antimicrobial effect of thymol and carvacrol against *E. coli*, attributing this effect to its ability to permeate and depolarize the cytoplasmic membrane. The study of the combined activity by carvacrol and thymol in the same film showed that some additive effect between them took place (Ramos, Jiménez, Peltzer & Garrigós, 2012). The mechanism proposed by Zivanovic et al. (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. Thus, the resistance of Gram-negative bacteria to essential oils lies in the protective role of the cell wall lipopolysaccharides or extrinsic membrane proteins, which restricts diffusion of hydrophobic compounds through the cover lipopolysaccharide (Burt, 2004). The lower antibacterial activity against *E. coli* and *P. aeruginosa* could be due to the higher resistance of Gram-negative microorganisms to these compounds.

Table 4 – Antimicrobial activity, expressed as inhibition zone (mm), of triticale protein films incorporated with oregano essential oil (OEO) against test microorganisms.

OEO	Test microorganism		
(%)	*E. coli*	*S. aureus*	*P. aeruginosa*
0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
1	10.81 ± 1.59^{b}	166.90 ± 4.07^{b}	0.00 ± 0.00^{a}
2	21.53 ± 2.05^{c}	342.36 ± 10.63^{c}	9.70 ± 1.07^{b}

Reported values are means ± standard deviation ($n=3$). Different letters (a, b, c) in the same column indicate significant differences ($p<0.05$).

Dadalioglu and Evrendilek (2004) attribute OEO antimicrobial activity of two components: carvacrol (phenolic compound) and p-cymene (monoterpene). Table 1 shows no concentration of p-cymene since it was only 0.93%, but it has been described synergism between it and carvacrol (Burt, 2004; Gutierrez et al., 2008). The p-cymene component itself is a weak antibacterial, but produces swelling of the bacterial cell membrane and thus carvacrol probably may easily enter the bacterial cell (Ultee, Kets, Alberda, Hoekstra & Smid, 2000). Burt (2004) describes the action of carvacrol as disintegration of the outer membrane of Gram-negative bacteria followed by the release of lipopolysaccharides, resulting in an increase in the ATP permeability of the cytoplasmic membrane.

Among the Gram-negative bacteria, *P. aeruginosa* is less sensitive to the action of essential oils (Cosentino et al., 1999; Dorman & Deans, 2000; Gutierrez et al., 2008; Wilkinson, Hipwell, Ryan & Cavanagh, 2003). This resistance is due to the intrinsic barrier of the outer membrane of the bacterium (Mann, Cox & Markham, 2000). The present results show that this tendency occurs also when the essential oil is incorporated into films based on triticale proteins.

In some cases the essential oil antimicrobial activity decreases considerably in a complex system (Gutierrez et al., 2008). Therefore, an important aspect of the application of plant essential oils is the evaluation of their efficacy in the system used. Baranauskien, Venskutonis, Dewettinck and Verhe (2006) showed that proteins usually have a high ability to bind to aromatic volatile compounds. Other studies have shown that milk proteins are limiting factors in the antimicrobial activity (Devlieghere, Vermeleulen & Debevere, 2004). Our study showed that the antimicrobial activity of oregano essential oil can be expressed in protein triticale films.

4. Conclusions

We investigated the physicochemical and antimicrobial properties of protein triticale films with oregano essential oil (OEO). We studied the effect of the OEO on the moisture content, water solubility, water vapor permeability, mechanical properties and antimicrobial activity of triticale films. The antimicrobial effects of films against *S. aureus* (ATCC29737), *E. coli* (ATCC25923), *P. aeruginosa* (ATCC27853) were deter mined by the zone of inhibition method. The addition of OEO did not significantly affect the water vapor permeability

of films, but increased water solubility and modified the mechanical properties. OEO significantly reduced Tensile strength and Young's modulus, while increasing the percent elongation of the films, which suggest the existence of different characteristics between the matrix protein and films without OEO. As the films were prepared with the same concentration of protein and plasticizer, differences are probably related to the way in which proteins interact in the film matrix. The addition of OEO to films produced a triticale protein matrix less dense and less rigid, the typical behavior of small molecules that cause plasticization. The antimicrobial effect of OEO was incorporated and expressed in triticale films. The zones of inhibition increased signifi cantly with increasing concentration of OEO. The larger zones of inhibition observed for the Gram positive bacterium *S. aureus*, while lower for Gram negative (*E. coli and P. aeruginosa*). The OEO concentration of 1% was not effective against *P. aeruginosa*. Therefore, it could be concluded that the addition of OEO to protein triticale films shows some poten tial in food packaging applications. As concerns active packa ging, edible films enriched with essential oils offer many possibilities in the field of food preservation. Nevertheless, further research is necessary to improve their mechanical and barrier properties.

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