Journal of
APPLIED PACKAGING RESEARCH

RESEARCH ARTICLE

Application of Active and Edible Films of Sodium Caseinate to Extend the Shelf-life of Argentine Pategrás Cheese

Brenda Álvarez
Mar del Plata University,
CONICET

Natalia Damiani
Mar del Plata University,
CONICET

Marina Czerner
Mar del Plata University,
CONICET

Josefa Martucci*
Mar del Plata University,
CONICET

Liesel Gende*
Mar del Plata University,
CONICET

ABSTRACT

Argentine Pategrás cheese is one of the most popular types of cheese in Argentina. The main spoilage causes in these products are microbiological contamination and lipid rancidity. Semi-hard cheeses are usually covered with a paraffin wax that favors its preservation. When pieces are stored, cutting faces must be protected to prevent desiccation and contamination. Active biodegradable films are an alternative to the traditional materials. In the present work, active films of sodium caseinate with oregano (Origanum vulgare) and laurel (Laurus nobilis) essential oils were produced. Aseptical portions of Pategrás cheese were packaged by heat sealing, the samples were stored under shelf conditions (12 °C and 75% Relative Humidity) for 56 days. Portions were periodically removed and microbiological count of mesophilic microorganisms, food pathogens like S. aureus, as well as fungi and yeasts determination were made on samples surfaces. The results showed that the incorporation of 10000 µg / ml of essential oil confers antimicrobial properties to sodium caseinate films and oregano essential oil was the most effective. Sensory analysis of packaged cheeses was made; those involved with active films with essential oils, were the freshest at the end of shelf life and showed the highest acceptability by the sensory panel members throughout the study period.

***Josefa Martucci**
Corresponding Author
jmartucci@fi.mdp.edu.ar
***Liesel Gende**
Corresponding Author
lieselgende@gmail.com

INTRODUCTION

Argentine Pategrás cheese is one of the most popular types of cheese in Argentina. This kind of cheese making technology in our country was incorporated by European immigrants and is an adaptation from the one used in their origin countries (France and Italy). However, the introduced changes over time, have given it typical Argentine characteristics, with different sensorial properties [1]. One of the main losses during this type of cheese commercialization happens during storage, where the contamination of cheese by bacteria, moulds and yeasts is common, and therefore the development of offflavours can happen, decreasing the quality of the cheese, mainly when stored without package [2]. Also, the high moisture loss in the cheese can be a problem increasing their hardness and leading to undesired sensorial properties [3].

Different packaging systems have been suggested to solve these problems, including vacuum and modified atmosphere packaging, in all cases materials such as polyethylene, polyamide, and polypropylene are normally used [3], [4]. Nowadays with the environmental and sustainability issues as well as legislation restrictions, the use of active edible film is being investigated, proving to be a very promising possibility since, such as conventional materials, allows spoilage prevention, shelf-life extension and reduction of water loss [3].

Edible films can act as carriers of antimicrobial agents to incorporate functional properties to the packaging material [5]. Several essential oils have been incorporated into protein based films for cheese preservation. The advantage of applying such essential oils through the use of films, is that edible films control the release of the incorporated antimicrobial thus maintaining effective doses of the additive along the storage period [6]. In addition, it allows attaining the desired goal with lower oil concentrations, thus limiting unwanted flavors and odors to the food [7]. Ramos [5] evaluated whey protein isolate (WPI)-based coatings with different combinations of antimicrobial compounds to extend the shelf-life of a semi-hard cheese. Coated cheese showed a reduction of 10% in moisture loss and variation in hardness and color during storage compared with uncoated samples. In the case of WPI–based coatings with chitooligosacharides and lactic acid, the antimicrobial effect is higher against bacteria than yeasts and moulds when compared with a commercial coating [5]. Kavas [8] showed that WPI-based coatings in combination with mint essential oil in traditional whey cheese of Turkey decrease the growth of microorganisms after 15 days of storage at 4 °C. Bactericidal effect was observed on all microorganisms in 4% (v/v) concentration of essential oil.

Between biopolymers films, sodium caseinate-based films are attractive for cheese preservation due to their high nutritional quality (milk protein), excellent sensory properties, and high resistance to chemical denaturation and coagulation (stable over a wide range of pH, temperature and concentration of salt), which increase its potential to adequately protect food products from the surrounding environment [9]. Caseinate edible films incorporated with essential oil were analyzed as a potential active packaging system [10], [11], [12], [13], [14]. To our benefit there is little information about the application of active caseinate films for cheese preservation [15], [16].

The aim of this work was to develop an active biodegradable film based on sodium caseinate and oregano or laurel essential oils for conservation of semihard Pategrás cheese in normal storage conditions (12±2 °C, 75±5 % RH). Microbiological changes were monitored with time to evaluate the deterioration of available cheese. Functional properties and antioxidant activity of the developed films was determined, and sensory evaluation was performed to assess the acceptability of the product

after storage time, wrapped with an active film.

MATERIALS

Sodium caseinate, isoelectric point 5.5, was purchased from Sigma Aldrich (USA). Anhydrous glycerol (Gly, 99.5 %, Ciccarelli, Argentina), Propylene glycol (PG, Química Bolivar, Argentina), 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma Aldrich, USA), potassium hexacyanoferrate III, trichloroacetic acid and methanol (Ciccarelli, Argentina) were analytical grade and used as received. Meat peptone, malt extract, bacteriological glucose, sodium chloride, Mueller Hinton agar, agar medium for fungi and yeasts, Baird Parker agar base (Britania, Argentina), potassium tellurite and yolk were used as components of culture media.

Source of bacterial strains

Bacterial strains of Gram-negative *Escherichia coli* ATCC 8539 (ATCC, American Type Culture Collection) and Gram-positive *Staphylococcus aureus* ATCC 25923 were purchased on Tryptic Soy Agar. The vegetative cells of each microorganism previously cultivated on agar for 24 h at 37±0.5 °C were suspended in double distilled sterile water, and the suspension was standardized to 0.5 of Mac Farland scale ($1.5x10^8$ UFC/mL) before testing antimicrobial activity with microdilution method and the agar diffusion test.

METHODS

Extraction of laurel and oregano essential oils

Essential oils were obtained by hydrodistillation of 100 g of dried leaves using a Clevenger-type apparatus as described by Gende [17]. The yield was 0.22±0.03 % for laurel and 0.75±0.02 % for oregano. Successive distillations were conducted for each species in order to obtain sufficient volumes for the various tests. The produced essential oils were dried over anhydrous sodium sulphate and stored in screw-capped dark glass vials at 5-8 °C until further testing.

Antimicrobial activity of essential oils against *E. coli* and *S. aureus*

A broth microdilution method was used to determine minimal inhibitory concentration (MIC) [18]. Appropriate amounts of essential oil were mixed in sterile water and emulsified with 8 mL propylenglycol/100 mL solution, thus obtaining a mother solution (MS). For broth microdilution, 100 µL of Mueller Hilton medium was placed in each of the 96-well microtiter plates and then diluted with MS to obtain serial dilutions. Microbial biomass suspension of *E. coli* and *S. aureus* was individually added to each essential oil serial dilution, yielding final value concentrations ranging from 12.5 to 2000 µg/mL. All microtiter plates (with positive and negative controls) were incubated at 35±0.5 ºC for 24 h in order to determine MIC values. Experiments were conducted in triplicates.

Antioxidant properties of essential oils

DPPH radical scavenging activity (RSA%) – The DPPH radical scavenging activity was determined by the method of Yen and Hsieh [18] with slight modifications. A volume of 400 µL of each essential oil solution (0-10000 µg/mL) was mixed with 2 mL of a solution of 0.06 mmol/L DPPH in methanol. The mixtures were shaken vigorously and allowed to stand in the dark for 30 min at room temperature. The reduction of the DPPH radical was measured at 517 nm using a UV-Visible (Agilent 8453, China) spectrophotometer. The control is carried out in the same manner, but methanol was used instead of the sample. The percentage of DPPH radical scavenging activity (RSA %) was calculated as follows:

$$RSA(\%) = (1 - \frac{A_{517sample}}{A_{517control}}) * 100 \qquad (1)$$

Where $A_{517sample}$ and $A_{517control}$ are the absorbance at 517 nm of the sample and control respectively.

The control is carried out in the same manner, except that distilled water was used instead of the sample. Assays were performed in triplicate. Then the IC50 was calculated, that is the necessary concentration of oil to produce a 50% decrease in free radicals.

Ferric reducing antioxidant power (FRAP) assay – FRAP assay was carried out according to the method described by Oyaizu [19]. Basically, 1 mL of the essential oils (2000-10000 µg/mL) was mixed with phosphate buffer (2.5 mL, 0.2 mol/L, pH 6.6) and 2.5 mL of potassium hexacyanoferrate III ($K_3Fe(CN)_6$, 1g/mL). The mixture was incubated at 50 °C for 20 min and rapidly cooled. Then 2.5 mL of a trichloroacetic acid (10 g/mL) was added to the mixture, which was then centrifuged at 5000 rpm for 10 min (Sartorius type 4-15, Germany). A 2.5 mL aliquot of the upper layer was mixed with 2.5 mL of deionized water and 0.5 mL of a 0.1 g/mL FeCl3 solution. Absorbance was measured at 700 nm in a UV-visible spectrophotometer (Agilent 8453, China). The increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid (AA) was used as reference material and the final results were expressed as µg/mL of ascorbic acid equivalents (AAE). All tests were performed in triplicate and the graph was plotted with the average of the three determinations.

Preparation of active films based on sodium caseinate

Film-forming solution (FFS) was prepared by dissolving sodium caseinate powder (Cas) in distilled water (pH 6.5) at a ratio of 3 g/mL under mechanical stirring (500 rpm 30±2 °C). After dissolution, glycerol (30 g dry protein basis/100 g solution) was added as plasticizer. Laurel essential oil (LEO) or oregano essential oil (OEO) was incorporated to obtain final concentrations between 2000 and 10000 µg/mL. Control formulation was prepared in the same way, replacing EOs by distilled water. Afterwards, mixtures were homogenized at 12000 rpm for 5 min and 20000 rpm for 5 min by using a homogenizer (UltraturaxT25 basic, IKA-Werke GMBH & Co. KG Staufen, Germany). Films were obtained by casting and dried at 30 ºC in a forced-air oven (Memmert UFE550, Germany) for 20 h until constant weight. Three different types of films were then obtained: Control sodium caseinate films (Cas), laurel essential oil-added Cas film (Cas-xLEO) and oregano essential oil-added Cas film, (Cas-xOEO), where x is the amount (µg/mL or ppm) of EO incorporated. Before performing determinations, the dried films were conditioned in a humidity chamber at 65±2 % RH and 25±2 °C.

Characterization of sodium caseinate films

Film thickness – Film thickness was measured using a 0–25 mm manual micrometer (543-690, Mitutoyo, Japan), with a resolution of 0.01mm. The reported values are the average of five readings taken randomly on each film sample.

Optical properties – Color and opacity of films were determined using a Lovibond colorimeter RT500 (Neu-Isenberg, Germany) with a measuring diameter of 8 mm. The color parameters for colour change were quantified by CIE L* (whiteness/darkness), a* (redness/greenness) and b* (yellowness/blueness) system. Opacity (%) was calculated from the reflectance measurements of each sample with a black backing and each sample with a white backing according to:

$$Opacity(\%) = 100 * \frac{Y^*_{black}}{Y^*_{white}} \qquad (2)$$

where Y is a three stimulus value. All reported values are the average of four replicates.

Water vapor permeability – WVP was determined according to the ASTM E96-95 desiccant method (ASTM E96-95). The films were sealed on polymethylmetacrylate permeation cups containing dry $CaCl_2$ (0% RH) with an exposed area of 28 cm^2. The cups were placed in an environmental chamber maintained at ambient temperature (25±2 °C) and 65±2 % RH. The weight gain was accurately recorded (±0.0001 g) hourly during 24 h period and the water vapor transmission rate (WVTR) was calculated from the initial slope of the weight changes vs. time divided by the effective film area ($kg/s.m^2$). WVP was calculated using the following equation:

$$WVP(kg/Pa.m.s) = \frac{WVTR * e}{\Delta P} \quad (3)$$

where e is the film thickness (m) and ΔP is the vapor pressure difference across the film (Pa), calculated based on the chamber temperature and the relative humidity inside and outside the cup. WVP results were the average of three replicates.

Antimicrobial activity of the films – Antibacterial activity of the films was assessed using the agar diffusion method [20]. Freshly bacterial suspensions (*E. coli* and *S. aureus*) were spread on the surface of solidified Mueller Hinton agar in Petri dish using a sterile cotton swab. Subsequently, edible film discs (diameter: 1.0 cm) were placed on the agar surface. Petri dishes were incubated at 35±0.5 ºC for 24 h. The diameter of the growth inhibition zone surrounding the film discs was accurately measured with a manual caliper from the of the film (Mitutoyo, Japan). Each assay was performed in triplicates.

Antioxidant activity – For RSA % and FRAP assays, the films were immersed in liquid nitrogen and subsequently crushed and grounded with a pestle. A precise amount of crushed film (0.4 g) was mixed with 4 mL of methanol in a caped tube, stirred vigorously and allowed to stand overnight (about 12 h). Afterwards, tubes were centrifuged at 5000 rpm for 10 min (Sartorius type 4-15, Germany) and the supernatant was recovered and reserved for RSA% and FRAP as outlined above for EOs. In the case of reducing power, final results were expressed per g of film.

Wrapping Pategrás cheese into sodium caseinate active films – Commercial Argentine Pategrás semi-hard cheese was obtained from a local dairy manufacturer (Sancor, Argentina). Cylindrical samples (diameter: 1cm, length 1cm) were cut with a sterile puncher to a depth of 8 cm from the cheese bark. Pouches (3x6 cm^2) were made with Cas, Cas-10000OEO and Cas-10000LEO films and a portion of cheese was packed in them and thermo-sealed. The packets were then stored at 12±2 °C and 75±5 % RH and the cheese portions were periodically (0, 14, 28, 42 and 56 days) evaluated by microbiological determinations.

Cheese samples analysis during storage time

Microbiological analysis

To evaluate the microbial development on the surface of each cheese sample, a sterile cotton swab contained in a test tube was used with 9 mL of peptone water (protease peptone, NaCl). The swab was performed on the inner surface of the cheese and then was transferred back to the tube and stirred for 1 minute in vortex. Subsequently, 1 mL aliquots were taken from each tube and necessary decimal dilutions were made, which allowed obtaining between 30 and 300 colony forming units per plate.

Total viable bacteria – A bacterial suspension of 1 mL was placed in sterile plates and molten agar (45 °C) was added by the pour plate method using PCA agar (yeast extract, tryptone, glucose and agar). Plates were incubated at 32-37 °C for 24-48 hours. Analyzes were performed in triplicates.

Mould and yeast – A bacterial suspension of 1

mL was planted in depth on plates with YGC agar (yeast extract, glucose, chloramphenicol and agar). Plates were incubated at 22-25 °C for 3-5 days. Analyzes were performed in triplicates.

Lactose-fermenting coliform microorganisms – A bacterial suspension of 1 mL was planted in depth in plates with VRB agar (yeast extract, peptone, lactose, mixture of bile salts, sodium chloride, neutral red, crystal violet and agar). The plates were incubated under anaerobic conditions at 32-37 °C for 24 hours. Analyzes were performed in triplicates.

***Staphylococcus spp.* –** Pieces of 10 g of cheese was placed in 90 mL of peptone solution and then stirred. Serial dilutions were used for microbial enumeration, 0.1 mL of the appropriate dilutions were spread on Baird Parker agar surface (meat peptone, meat extract, yeast extract, lithium chloride, glycerin, sodium pyruvate and emulsion agar of yolk and potassium tellurite solution). They were incubated at 32-37 °C for 24-48 hours. Microbiological analyzes were carried out in triplicates.

In all cases, to determine the bacterial or fungal species adequate staining and microscopic observations were made.

Sensory evaluation

Sensory evaluation was performed by 8 female panelists of different ages and occupations in a conditioned room at 21°C (optimal temperature to taste the flavor of cheese [21]). Judges were trained in several sessions, using the terminology adapted from Candioti [22]. Cheese portions were aseptically cut, packed in the different films as described above and stored at 12±2 °C and 75±5 % RH for 42 days. Portions of newly purchased fresh cheese were added to the samples in each evaluation session. Samples of cheese, coded with three-digit random numbers, were given to the panelists asked to rate the global acceptance of the different batches by judging the freshness, global flavor intensity, oils flavor and global acceptability [23]. Each descriptor was quantified on a 10 points unstructured scale, anchored at each extreme, except for acceptability parameter, which was scored from 1 to 9. For freshness 0 corresponding to very fresh and 10 to no fresh at all, for global cheese flavor intensity and laurel/oregano essential oil flavor parameters 0 corresponding to imperceptible and 10 to very intense, and finally for acceptability 1 corresponding to "I dislike it a lot" and 9 to "I like it a lot". Coffee and water were used to neutralize aroma and flavor, respectively, between each sample evaluated.

Statistical analysis

Experiment data were statistically analyzed by one-way ANOVA. Differences between means pairs were assessed on the basis of confidence intervals using the Tukey's test. The significance level was $p < 0.05$.

RESULTS AND DISCUSSION

Extraction and characterization of the oils

Essential oils of oregano and laurel were obtained by the hydrodistillation technique. Gas chromatography-mass spectrophotometry analyses revealed that carvacrol (26.70 %), p-cymene (15.20 %), γ- terpinene (15.10 %) and terpinene (7.50 %) were the predominant constituents in OEO [24]. The prevailing compounds of OEO were phenolic monoterpenes in concordance with other authors [4], [13]. In LEO, the main constituents were 1,8-cineole (41.40 %), linalool (15.30 %) and methyl eugenol (5.10 %) [25].

The antimicrobial activity of the oils were quantified by determining the minimum inhibitory concentration (MIC) against *E. coli* (Gram (-)) and *S. aureus* (Gram (+)). The results are shown in the Table 1, both food pathogens presented more sensitivity to oregano essential oil, being lower its MIC

values in comparison to laurel oil.

In the tested concentrations (up to 2000 µg/mL) LEO produced no inhibition of microbial growth. These results suggest that phenolic compounds (carvacrol and methyl eugenol) in OEO are primarily responsible for the antimicrobial activity against pathogens analyzed. Similar results were observed by other authors [26]. Bagamboula, Uyttendaele and Debevere [27] found that phenolics compounds (carvacrol and thymol) showed high antimicrobial activity against *E. coli*, while terpene compounds (estragol and linalool) exhibited limited activity. In addition, Tassou, Koutsoumanis and Nychas [28] observed that the escape of intracellular material from *E. coli* and *S. aureus* was consistent with the phenolic concentration in essential oils composition.

Regarding to the found differences between the microorganisms analyzed for oregano oil, the MIC obtained for *S. aureus* is considerably lower than that for *E. coli*. This indicates a higher sensitivity of the Gram (+) bacteria, in line with other studies [23], [29], [30]. The Gram-negative ones possess an outer membrane surrounding the cell wall which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering while Gram-positive bacteria do not have rigid cell walls for being protected by their thick peptidoglycan layer [30].

The antioxidant capacity of EOs was revealed through their FRAP assay and RSA% efficiency, respectively. The results are summarized in Figure 1.

Both EOs exhibited a strong dose-dependent reducing power (Figure 1-solid lines) until reaching a saturation level, where no change in the activity with the concentration. The OEO showed higher reducing capacity than laurel oil in agreement with EOs chemical composition [29], [26] because carvacrol, the main component of OEO, was an electronic donors. The phenolic compounds are free radical acceptors that delay or inhibit the autoxidation initiation step or interrupt the autoxidation propagation [31], [32]. In the case of LEO, chemical composition did not show phenol structures but this EO had antioxidant capacity also. LEO presents molecules with reducing capacity that, probably, are responsible for this activity [33].

The scavenging ability of the essential oils on DPPH free radical is shown in Figure 1 (dashed

	Minimum inhibitory concentration (MIC) ppm	
	E. coli	*S. aureus*
Oregano oil (OEO)	1600-1800	800-900
Laurel oil (LEO)	>2000	>2000

Table 1. MIC values expressed in ppm (µg/mL) for each oil against isolates of E. coli and S. aureus. (n = 3)

lines). The EOs showed a dose dependent radical scavenging activity (RSA%), being OEO the most efficient antioxidant. OEO showed five times higher activity than LEO ($IC50_{LEO}/IC50_{OEO}$= 5.12), in accordance with the five times higher content of phenolic compounds in its composition (carvacrol: 26.70 % and methyl eugenol: 5.10 %, respectively). The IC50 values of EOs were higher than ascorbic acid (IC50=4.28 µg/mL), indicating that they have lower RSA % than this reference compound.

Properties of EO-added sodium caseinate films

Sodium caseinate active films with LEO and OEO were obtained in a range of concentration from 1000 to 10000 µg/mL of the active ingredient. Antioxidant and antimicrobial activity of films were evaluated to obtain an optimum formulation of film for food packaging. RSA% and FRAP results are shown in Figure 2. The addition of EOs significantly increased ($p< 0.05$) antioxidant activity of sodium caseinate films. The films showed higher IC50 values (Figure 2) than the respective oil (1600 vs 787 µg/mL to oregano and 4740 vs 4030 µg/mL to laurel). This indicates that the amount of active components extracted from the film is less than the amount incorporated in the FFS. Similar results were reported by other authors in different biopolymer films [24], [34], [35]. The effect is more pronounced for the oregano oil, possibly due to the less hydrophobicity of carvacrol versus methyl eugenol [13]. In the same way, Hosseini [35] showed that the hydrophobic nature of eugenol limited the retention of the active component from chitosan matrix. The same behavior is observed in the reducing power capacity where saturation is achieved at higher concentrations (4000 µg/mL for oregano oil and 6000 µg/mL for the film) (Figure 2).

The antimicrobial activity of the obtained films was evaluated using the inhibition zone technique. Sodium caseinate control films showed no antimicrobial activity against tested microorganisms (Inhibition halo against *E.coli* and *S. aureus* 0.0±0.0 mm), in accordance with other authors [10], [12]. In the case of active films, inhibition of bacterial growth was observed only in the highest concentrations of the active ingredient (10000 µg/mL), being more effective Cas-OEO (Inhibition halo against *E.coli* 8.2±0.2 vs. 5.0±0.0 mm, for Cas-10000OEO and Cas-10000LEO respectively). As discussed above, this antibacterial effect has been mainly attributed to phenolic components of the essential oil; these compounds form channels through the bacterial membrane by pushing apart the fatty acid chains of the phospholipids, allowing ions to leave the cytoplasm [36]. In our study, no significant differences between the antimicrobial activity of oregano and laurel against *S. aureus* and *E. coli* for both films were observed (Inhibition halo against *S. aureus*: 8.0±0.7 vs. 5.0±0.0 mm, for Cas-10000OEO and Cas-10000LEO respectively). Antimicrobial activity of incorporated oils in casein films was lower than that of free oils due to interactions between the components of films and essential oils [24], [34], [35].

In our results, only the films with 10000 µg/mL of essential oil showed antimicrobial and antioxidant activity, with potential for food packaging applications. This formulation was selected to wrap a Pategrás cheese.

Some films properties are relevant for food packaging. In particular, optical properties play a major role in the appearance of the coated product. In order to compare the differences between control films and those prepared with the essential oils, color parameters of the obtained films were reported (Table 2).

Visually, Cas films were quite transparent, though they turned yellowish when the essential oil was added (photographs were shows in Table 2). This is according to color parameters and measured opacity, which did not reveal statistical differences among films, may be due to the little amount of EOs

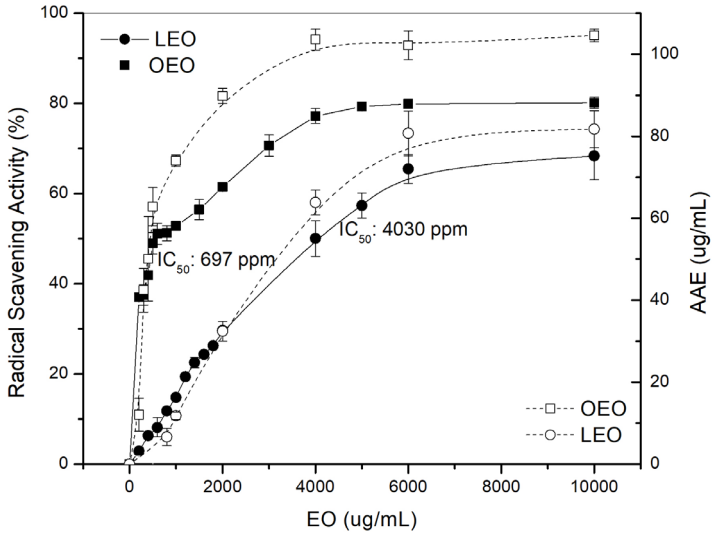


Figure 1. Antioxidant capacity of essential oils (EO) measured as: radical scavenging activity (RSA %) (solid lines) and ferric reducing power (µg/mL of ascorbic acid equivalents (AAE)) (dashed lines) (n = 3, p <0.05).

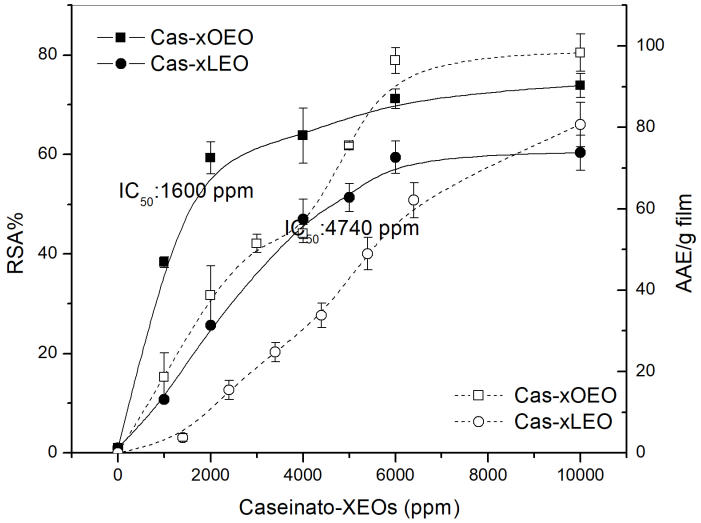
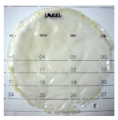


Figure 2. Antioxidant capacity of sodium caseinate based films with EOs, measured as: radical scavenging activity (RSA %) (solid lines) and ferric reducing power (ascorbic acid equivalents/g) (dashed lines) (n = 3, p <0.05).

Film	Colour parameters			Opacity	WVP*10^{13} (Kg/Pa.m.s) 25 °C, 65% RH
	L*	a*	b*	%	
Cas 	93.97 ±0.36 a	1.41 ±0.07 a	0.92 ±0.34 a	10.11 ±0.03 a	1.21 ±0.16 a
Cas-10000OEO 	89.97 ±6.52 a	1.34 ±0.16 a	1.56± 0.18 b	10.15 ±0.92 a	1.16±0.17 a
Cas-10000LEO 	89.27 ±5.76 a	1.54 ±0.17 a	3.55 ±0.26 c	10.31 ±0.18 a	0.96±0.09 a

The same letters in the same column indicate no significant difference between the results. Tukey test, 95% confidence; n = 4.

Table 2. Colour parameters, opacity and water vapor permeability (WVP) of control and active films.

incorporated. Other authors observed a marked increase in the opacity of caseinate films doped with tung oil (10 mL/100 mL solution) associated with the presence of large oil droplets dispersed in the matrix [37]. Regarding the color parameters, there is no significant differences ($p>0.05$) in L* and a* parameters. Moreover, the incorporation of oils produces an increase in the b* parameter ($p<0.05$), indicating an increase in yellowish coloration, more pronounced in LEO. The incorporation of essential oil had an influence on film color, though changes depended on the type of essential oil [37], [24], [38]. Pereda [37] showed values of 8.11 in b* parameters in sodium caseinate films plasticized with glycerol and modified with tung oil.

The low moisture resistance of the protein-based material is perhaps the biggest limitation that they present for it use in technical applications. Water is a ubiquitous plasticizer that can form hydrogen bonds with the protein´s polar groups and in this manner inflate it, causing the films to lose dimensional stability and making them more susceptible to hydrolytic degradation and to microbial attack [39], [40]. To evaluate the oils addition´s effects on the moisture resistance of the materials, studies about water absorption and water vapor permeability (WVP) at 65% RH and 25 °C were performed. Incorporation of oil produced a reduction in the absorption capacity of the material due to the presence of a hydrophobic phase dispersed in the matrix (Figure 3). Similar results were obtained for chitosan films doped with garlic oil [41], for caseinate films doped

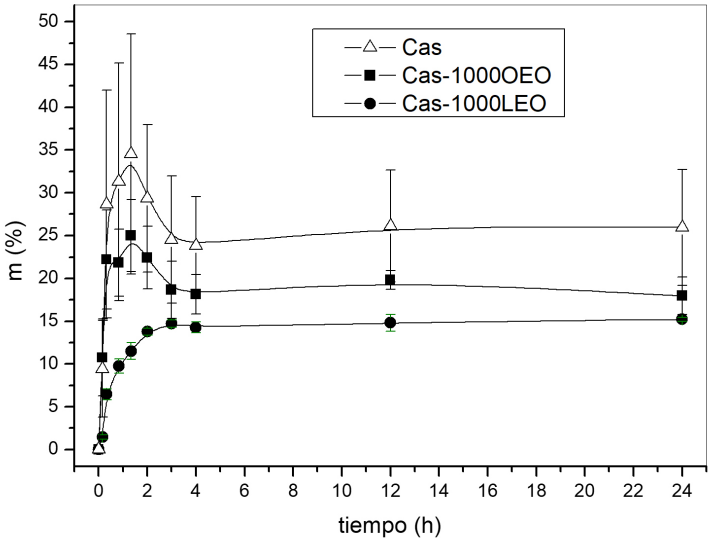


Figure 3. Water absorption curves of films, evaluated at 65 % RH and 25 ° C (n = 3, p <0.05).

with tung oil [37] and for chitosan films doped with cinnamon oil [40].

The results of water vapor permeability of films are shown in Table 2. Unlike synthetic polymeric materials, the WVP of biopolymers is dependent on the material thickness. In the present study, there is no significant difference in the thickness of the films ($p>0.05$), which was maintained between 90 and 110 µm. Water vapor permeability of control films was similar to values obtained in previous studies with protein plasticized films [37], [24]. The incorporation of oil did not produce a significant change in WVP ($p>0.05$); some authors observed the same behavior in protein systems added with similar amounts of oil [11], [37], [40]. This is associated with the fact that the water molecules can permeate through the non lipidic phase. In other studies, the incorporation of oils produces a considerable improvement in the WVP, but the amount of dispersed phase incorporated is higher [38]. In comparison with synthetic polymers, sodium caseinate films showed higher WVP than high and low density polyethylene (HPDE: 2.4 10^{-16} kg/m.s.Pa; LDPE: 3.6-9.7 10^{-16} kg/m.s. Pa) [40].

Analysis of cheese samples wrapped with active films during storage time

Microbiological analysis

Counts of total mesophilic microorganisms on cheese surface in relation with the storage time in a different kind of packaging are shown in Figure 4.

The pattern of microbial growth of total mesophilic microorganisms distribution was similar for all samples, with slight differences in the microbial count values, an increase in the microbial load from day 14 to 28, and then decrease up to day 42 (Figure 4). The death rate of some microorganisms in the cheese during ripening can be influenced by the different lactic cultures used, which inhibit them, due to acid production and the consequent low pH, a parameter that can help control the growth of the

microorganisms [42]. In our work, the results of the microbiological counts were expressed as CFU/cm^2, due to the samples were taken by swabbing those cheeses surfaces in contact with the wrapping films and, in this way, evaluate the antimicrobial activity from them.

Cas films reached a maximum value of $1210x10^3 \pm 543.06$ $x10^3$ CFU/cm^2 at day 28, this value is lower than those registered by Cogan [43] for matured cheeses, with counts of around 10^8 CFU/cm^2. Similarly, Brooks [44] and Aygun [45] obtained a similar trend for cheeses made with raw milk, but expressed as CFU/g. For those samples wrapped in films with essential oils (Cas-10000LEO, Cas-10000OEO) the distribution was alike, observing the lowest microbial count of all the samples at same storage day, in cheeses wrapped with a film of laurel essential oil, with an average value of $738x10^3 \pm 325.27x10^3$ CFU/cm^2. At day 56, the end of the storage time, the samples wrapped with Cas film showed a decrease of the total mesophilic microorganisms count until reaching values of $17.125x10^3 \pm 7.72$ $x10^3$ CFU/cm^2, this value being a logarithmic unit lower than the result obtained for the unwrapped cheese ($165.35x10^3 \pm 64.17x10^3$ CFU/cm^2, $p<0.05$). On the other hand, at same storage time, the samples wrapped with Cas-10000OEO film showed the lowest value of $4775x10^3 \pm 590.9x10^3$ CFU/cm^2, with two logarithmic units lower than the unwrapped samples and a smaller unit that those wrapped with Cas and Cas-10000LEO ($p < 0.05$). The distribution in shelf time of aerobic mesophilic microorganisms could be due to the increased in lactic flora at first, which then generates a reduction in the pH resulting in a decrease in the total microbial load [46].

Cheeses have rinds mostly to protect the anaerobic environment from the inside, but these are generally not dry enough to prevent fungal growth. Likewise, cheese acidity does not impede the development of molds and storage temperatures are not low enough to stop them [47]. Cheese susceptibility to alterations increases with the water content of the food product, so the microorganisms that develop on the surface become important. For this purpose, the cheese can be coated; coating used during ripening serves to regulate the moisture content while protecting the cheese against microorganisms (CODEX) [48]. From the results of our assays in relation to fungi and yeasts, in the microscopic observation, after growth on Petri dishes it could be seen mainly fungi of the genus *Penicillium spp.* and *Aspergillus spp.* The use of films in cheese packaging could contribute to maintain the food water content and thus favor fungal development, in the case of wrapped samples with oregano film, the counts decreased from 5 CFU/cm^2 on day 14 to 0 CFU/cm^2 on day 56. For all samples, the counts were lower than those reported by Cogan [43] for ripened cheeses, with counts in order of 10^6 CFU/cm^2, Aygun [45] in the order of 10^7 and Brooks [43] in the order of 10^4 CFU/g of analyzed product.

The Argentine Food Code (CAA) [49] its acronym in Spanish, does not provide specifications in relation to the maximum number of aerobic mesophilic bacteria or maximum fungi and yeast count, only indicates the reference values for indicators microorganisms such as coliforms, *Salmonella spp.*, *Listeria monocytogenes* and *Staphylococcus aureus*.

Results from microbiological counts in Baird Parker medium on cheese depending on the storage time are shown in Table 3.

According to CAA 605 (SPRyRS and SAGPyA, Joint Resolution No. 33/2006 and No. 563/2006), for medium moisture cheeses, the acceptance criteria for *S. aureus* are m = 100 (maximum level of microorganisms, for acceptable quality) and M = 1000 (maximum microorganisms in the food, for a provisionally acceptable quality). After 24 hours of incubation, the observed colonies were black and bright and had a circular morphology. Cheese wrapped

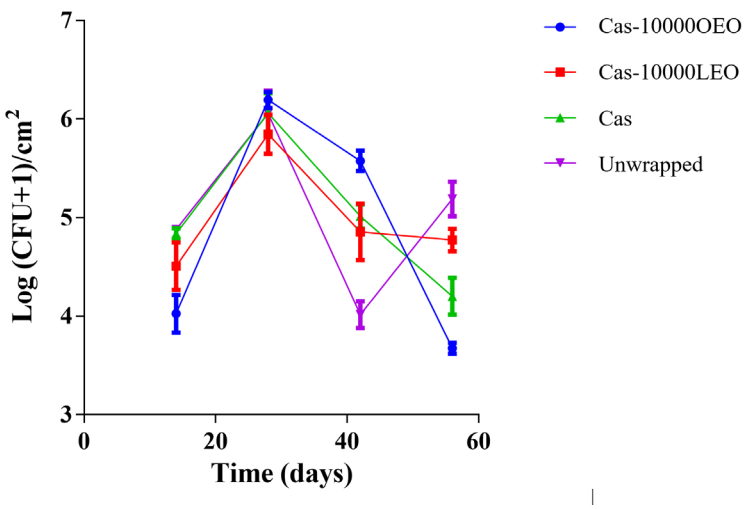


Figure 4. Microbiological counts expressed as Log (CFU+1)/cm2 of total mesophilic microorganisms in unwrapped Pategrás cheese and wrapped with sodium caseinate films with or without essential oils. Values are expressed as Mean±sd. a, b, c, d expressed significant differences between the treatments by time ($p<0.05$).

with Cas-10000OEO at day 56 showed one logarithmic unit less than the rest, due to the addition of the antimicrobial agent. However, no observed colony showed the distinctive characteristics of *S. aureus* in Braid Parker medium, in difference with Brooks [44] who obtained counts in the order of 10^1 CFU/g and Aygun [45] in the order of 10^3 CFU/g. It was verified the absence of this microorganism at all evaluated times for all treatments. No presumptive colonies of *E. coli* (CFU/cm^2) were observed in VBRNB medium for all treatments (refrigerated at 10 ± 2 ° C and 75 ± 5% RH).

In summary, after 56 days cheeses wrapped with Cas-10000OEO were those that showed the lowest value in the microbial count. In vitro studies have demonstrated antibacterial activity of essential oils (EOs) against several microorganisms, such us *E. coli* and *S. aureus* and different compounds of EO has been identified as effective antibacterials, e.g. carvacrol. It has also been seen that higher concentration is needed of them to achieve the same antimicrobial effect in foods [29].

Sodium caseinate protein films did not exert a definite inhibitory effect on the growth of the evaluated microorganisms. It has been reported that the addition of oregano essential oil to milk protein films reduces the growth of microorganisms in beef muscle [13]. According to Gomez Estaca [50], films that are incorporated with essential oil would ensure a longer shelf-life for fish in cold storage. Yangilar [51] observed a shelf-life extension in Göbek Kashar cheese coated with chitosan films enriched with fish oil.

Although there was an obvious antimicrobial activity of the films with oregano essential oil in the model system (agar disk diffusion), the polyphenol-protein interaction may reduce the activity of the phenolic compounds [46]. Therefore, our results showed that the addition of this essential oil to the sodium caseinate films contributes to the decrease in the mesophilic aerobic count of the cheeses wrapped with these active films at 56 days, although

Time (days)	Unwrapped (CFU/g)	Cas (CFU/g)	Cas-10000LEO (CFU/g)	Cas-10000OEO (CFU/g)
28	1217.5 ±297.4**ac**	4850.0±947.56 **b**	542.5±176.71 **a**	1450.0±825.67 **a**
56	900±318.85 **b**	637.5±228.67 **b**	875±304.25 **b**	60±56.6 **a**

Values are expressed as Mean±sd. a, b, c expressed significant differences between the treatments by time ($p<0.05$).

Table 3.Microbiological counts (CFU/g) of bacteria in Baird Parker medium for unwrapped Pategrás cheese and wrapped and with sodium caseinate films with or without essential oils.

it does not provide a general microbiological stabilization of the Pategrás cheese during storage.

In relation to the standard deviations found for microbiological analyzes, the dispersion could be attributed to the variability of cheese samples, although all parameters were controlled, including differences that may exist may also have contributed to this deviation.

Sensory evaluation of cheese samples during storage time

Once the microbial wrapped cheese safety was verified, a quantitative descriptive sensorial analysis was carried out by a trained sensory panel. The results of the different attributes evaluated are shown in Figure 5.

From the results of our work, it could be seen that a texture parameter like freshness (Figure 5.a) had lower score during all storage time for samples wrapped with essential oils films ($p <0.05$). Similar trend was observed in the global flavor intensity of product (Figure 5.b), reaching the same values for all samples from the 28 days of conservation. It is possible that the use of edible films may increase sensory properties of cheese during ripening [51]. Panelists also detected an atypical oily aroma in samples wrapped with active films. The most pronounced change in these attributes (Figure 5.c), occurred mainly during the first 14 days of packaging and its kept constant during the remaining time of evaluation. The same behavior was observed in Göbek Kashar cheese coated with chitosan enriched fish oil films [51], in meat products by the incorporation of oregano oil [52], in cooked chicken wrapped with tomato-based films with carvacrol [53] and in breaded hake medallions wrapped with fish gelatin films with carvacrol [54]. However, in the mentioned works the incorporation of flavor to the packed product produces a decrease in the acceptability of food by the consumer [51], [53], [54]. In our work, samples packaged with active films showed higher acceptability than the samples wrapped in Cas films ($p<0.05$; Figure 5.d). Part of the essential oil present in films migrates towards the cheese imparting a different flavor to the traditional Pategrás cheese and it is accepted by the sensorial panel. It was reported by Zantar [55] that cheese sample aromatized using *T. vulgaris* got

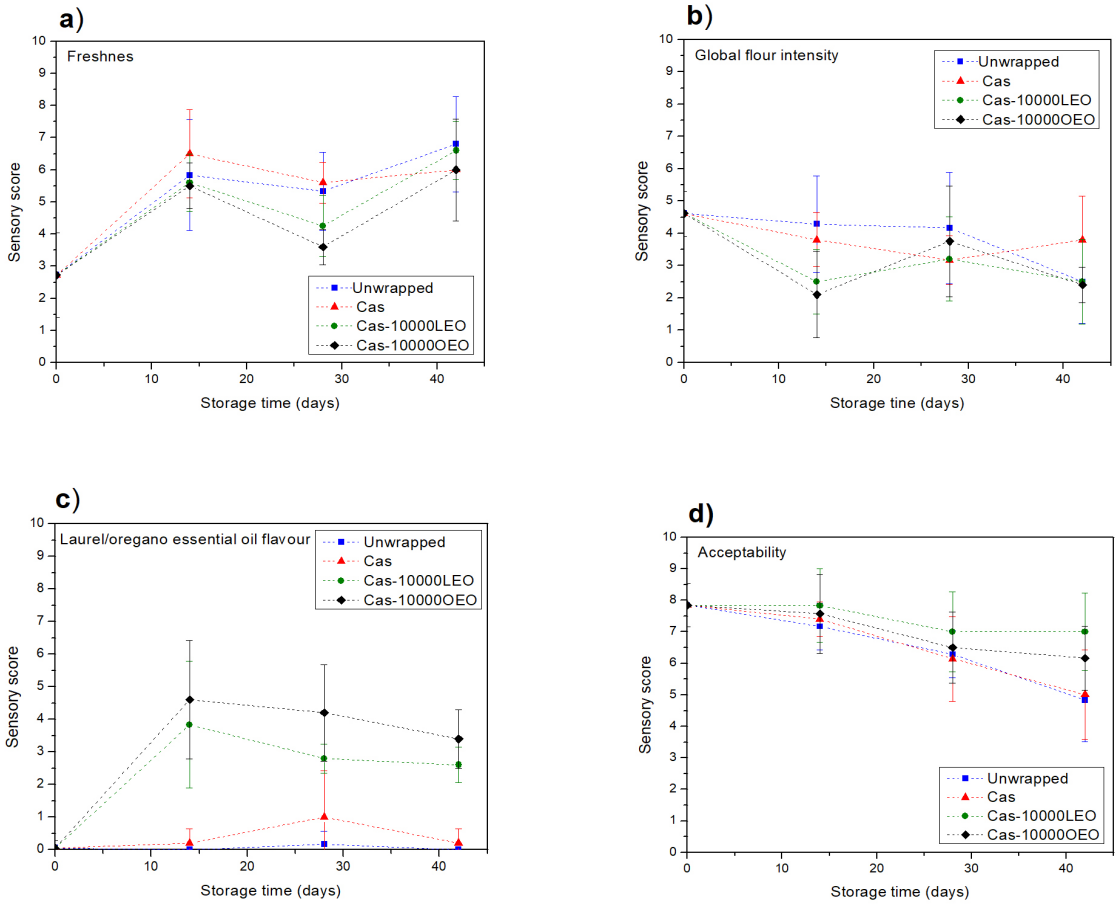


Figure 5. Sensorial attributes evaluated on control and wrapped cheese samples by sensory panel throughout the storage time at 4 ° C. a) Freshness, b) global flavour intensity, c) laurel/oregano essential oil flavor, and d) acceptability (n = 8, p <0.05).

the highest overall acceptability score and it was followed by the control and the sample aromatized using *O. compactum*.

It was also included a question in the sheet that judges must complete: Would you consume the product? In response to this question, all samples were accepted after 14 days of storage, while after 28 days the Cas-wrapped and unwrapped cheese were rejected and until 42 days of storage the samples wrapped with active films continued to be accepted by the panel.

This result, although preliminary, would indicate that possibly the use of films with essential oils would extend the products shelf-life. This aspect is an open line for future work.

CONCLUSIONS

Laurel and oregano essential oils were characterized regarding to their antioxidant capacity and antimicrobial activity against *E. coli* and *S. aureus*. Oregano oil showed higher activity for both

analyzed parameters, which was associated with a higher content of phenolic compounds in their chemical composition. The addition of EOs into sodium caseinate films produce films with antioxidant and antimicrobial properties without changes in opacity and WVP.

The preservation of Pategrás cheese with active films of sodium caseinate with 10000ug/mL of LEO or OEO allowed concluding that the cheese would be suitable for consumption until 42 days of conservation. The presence of the film protects the food against dehydration and the cheese samples wrapped with essential oils films had higher acceptability than those wrapped with caseinate control films or unwrapped ones, being the only accepted by consumers until the end of treatment.

These results suggest that sodium caseinate films have found an important use as a carrier of active compounds, since the use of said active films would prolong the useful life of the products.

REFERENCES

[1] A. Gauna. "Elaboración de quesos de pasta semidura con ojos". Instituto Nacional de Tecnología Industrial, 2005.

[2] P. Walstra and R. Janness. "Química y Física Lactológica". Ed. Acribia, 1984.

[3] M.J. Costa, L.C. Maciel, J.A. Teixeira, A.A. Vicente and M.A. Cerqueira. "Use of edible films and coatings in cheese preservation: Opportunities and challenges". Food Research International, 2018, 107, 84-92.

[4] M.L. Rooney. "Introduction to Food Active Packaging technologies". Innovations in Food Packaging. Han, J.H. Ed., Elsevier, Amsterdam, 2005.

[5] Ó.L. Ramos, J.O. Pereira, S.I. Silva, J.C. Fernandes, M.I. Franco, J.A. Lopes-da-Silva and F.X. Malcata. "Evaluation of antimicrobial edible coatings from a whey protein isolate base to improve the shelf-life of cheese". Journal of dairy science, 2012, 95(11), 6282-6292.

[6] I. Fernández Pan, J.I. Maté, C. Gardrat and V. Coma. "Effect of chitosan molecular weight on the antimicrobial activity and release rate of carvacrol enriched films". Food Hydrocolloids, 2015, 51, 60-68.

[7] J. Quesada, E. Sendra, C. Navarro and E. Sayas Barberá. "Antimicrobial active packaging including chitosan films with Thymus vulgaris L. essential oil for ready to eat meat". Foods, 2016, 5(3), 57.

[8] G. Kavas and N. Kavas. "The effects of mint (Mentha spicata) essential oil fortified edible films on the physical, chemical and microbiological characteristics of lor cheese". Journal of Food, Agriculture & Environment, 2014, 12(3,4), 40-45.

[9] J.L. Audic and B. Chaufer. "Influence of plasticizers and cross linking on the properties of biodegradable films made from sodium caseinate". Eurpean Polymer Journal, 2005, 41, 1934–1942.

[10] E. Kristo, K.P. Koutsoumanis and C.G. Biliaderis. "Thermal, mechanical and water vapor barrier properties of sodium caseinate films containing antimicrobials and their inhibitory action on Listeria monocytogenes". Food Hydrocolloid, 2008, 22, 373-386.

[11] L. Atares, J. Bonilla and A. Chiraldt. “Characterization of sodium caseinate based edible films incorporated with cinnamon or ginger essential oils”. Journal of Food Engineering, 2010, 100, 678–687.

[12] M.P. Arrieta, M.A. Peltzer, J. Lopez, M.C. Garrigos Selva, J.M. Artur and A. Jimenez Migallón. “Functional properties of sodium and calcium caseinate antimicrobialactive films containing carvacrol”. Journal of Food Engineering, 2014, 121, 94–101.

[13] M. Oussalah, S. Caillet, S. Salmiea Ri, L. Saucier and M. Lacroix. “Antimicrobial and antioxidant effects of milk protein based film containing essential oils for the preservation of whole beef muscle”. Journal of Agricultural and Food Chemistry, 2004, 52(18), 5598-5605.

[14] A. Matsakidou, M.Z. Tsimidou and V. Kiosseoglou. “Storage behavior of caseinate-based films incorporating maize germ oil bodies”. Food Research International, 2019, 116, 1031-1040.

[15] M.D.R. Moreira, M. Pereda, N.E. Marcovich and S.I. Roura. “Antimicrobial effectiveness of bioactive packaging materials from edible chitosan and casein polymers: assessment on carrot, cheese, and salami”. Journal of food science, 2011, 76(1), M54-M63.

[16] F. Yangılar and P. Oğuzhan Yıldız. “Casein/natamycin edible films efficiency for controlling mould growth and on microbiological, chemical and sensory properties during the ripening of Kashar cheese”. Journal of the Science of Food and Agriculture, 2016, 96(7), 2328-2336.

[17] L.B. Gende, M. Maggi, C. van Baren, A. di Leo Lira, A. Bandoni, R. Fritz and M. Eguaras. “Antimicrobial and miticide activities of Eucalyptus globulus essential oils obtained from different Argentine regions”. Spanish Journal of Agricultural Research, 2010, 8(3), 642-650.

[18] G.C. Yen and P.P. Hsieh. “Antioxidative activity and scavenging effects on active oxygen of xylose lysine maillard reaction products”. Journal of Science Food and agriculture, 1995, 67(3), 415–420.

[19] M. Oyaizu. “Studies on product of browning reaction prepared from glucose amine”. Japanese Journal of Nutrition, 1986, 44, 307–315.

[20] M. Pereda, A.G. Ponce, N.E. Marcovich, R.A. Ruseckaite and J.F. Martucci. “Chitosan gelatin composites and bi-layer films with potential antimicrobial activity”. Food Hydrocolloid, 2011, 25, 1372-1381.

[21] F. Kosikowski. “Cheese and Fermented Milk Foods”, 2nd Ed., F.V. Kosikowski and Associates, New York , 1982.

[22] M.C. Candioti, E. Hynes, A. Quiberoni, S.B. Palma, N. Sabbag and C.A. Zalazar. “Reggianito Argentino cheese: Influence of Lactobacillus helveticus strains isolated from natural whey cultures on cheese making and ripening processes”. International Dairy Journal, 2002, 12, 923–931.

[23] H.T. Lawless and H. Heymann. “Sensory evaluation of food: principles and practices”. Springer Science & Business Media, 2010.

[24] J.F. Martucci, L.B. Gende, L.M. Neira and R.A. Ruseckaite. “Oregano and lavender essential oils as antioxidant and antimicrobial additives of biogenic gelatin films”. Industrial Crops and Products, 2015, 71, 205–213.

[25] P. Di Leo Lira, D. Retta, E. Tkacik, J. Ringuelet, J.D. Coussio, C. Van Baren and A.L. Bandoni. “Essential oil and by-products of distillation of bay leaves (Laurus nobilis) from Argentina”. Industrial Crops and Products, 2009, 30(2), 259–264.

[26] M.T. Baratta, D. Dorman, S.G. Deans, D.M. Biondi and G. Ruberto. “Chemical composition, antimicrobial and antioxidative activity of laurel, sage, rosemary, oregano and coriander essential oils”. Journal of Essential Oil Research, 1998, 10 (6), 618-627.

[27] C.F. Bagamboula, M. Uyttendaele and J. Debevere. “Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards Shigella sonnei and S. flexneri”. Food Microbiology, 2004, 21(1), 33–42.

[28] C. Tassou, K. Koutsoumanis and G.J.E. Nychas. “Inhibition of Salmmonella enteritidis and Staphylococcus aureus in nutrient broth by mint essential oil”. Food Research International, 2000, 33, 273–280.

[29] S. Burt. “Essential oils: their antibacterial properties and potential applications in foods e a review”. International Journal of Food Microbiology, 2004, 94: 223-253.

[30] S. Inouye, T. Takizawa and H. Yamaguchi. “Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact”. Journal of Antimicrobian Chemotherapy, 2001, 47, 565-573.

[31] M. Kačániová, N. Vukovič, L. Hleba, A. Bobková, A. Pavelková, K. Rovná and H. Arpášová. “Antimicrobial and antiradicals activity of Origanum vulgare L. and Thymus vulgaris essential oils”. Journal of Microbiology and Biotechnology Food Science, 2012, 2(1), 263–271.

[32] K.S. Eça, T. Sartori and F.C. Menegalli. “Films and edible coatings containing antioxidants – A review”. Brazilian Journal of Food Technology, 2014, ISSN 1981-6723 volume 17 (2) Campinas.

[33] R.H. Olmedo, C.M. Asensio and N.R. Grosso. “Thermal stability and antioxidant activity of essential oils from aromatic plants farmed in Argentina”. Industrial Crops and Products, 2015, 69, 21–28.

[34] J. Gómez Estaca, L. Bravo, M.C. Gómez Guillén, A. Alemán and P. Montero. “Antioxidant properties of tuna skin and bovine hide gelatin films induced by the addition of oregano and rosemary extracts”. Food Chemistry, 2009, 112, 18-25.

[35] M.H. Hosseini, S.H. Razavi and M.A. Mousavi. “Antimicrobial, physical and mechanical properties of chitosan based films incorporated with thyme, clove and cinnamon essential oils”. Journal of Food Processing and Preservation, 2009, 33(6), 727-743.

[36] M. Corrales, A. Fernández and J.H. Han. "Antimicrobial Packaging Systems". In: Han J.H. (Ed.), Innovations in Food Packaging. Elsevier Academic Press, San Diego, 2014, pp. 133–170.

[37] M. Pereda, M.I. Aranguren and N.E. Marcovich. "Caseinate films modified with tung oil". Food hydrocolloids, 2010, 24(8), 800-808.

[38] K.G. Zinoviadou, K.P. Koutsoumanis and C.G. Biliaderis. "Physico-chemical properties of whey protein isolate films containing oregano oil and their antimicrobial action against spoilage flora of fresh beef". Meat Science, 2009, 82, 338-345.

[39] J.W. Rhim, K.A. Mohanty, S.P. Singh and P.K.W. Ng. "Preparation and properties of biodegradable multilayer films based on soy protein isolate and poly(lactide)". Industrial and Engineering Chemistry Research, 2006, 45, 3059-3066.

[40] S.M. Ojagh, M. Rezaei, S.H. Razavi and S.M.H. Hosseini. "Development and evaluation of a novel biodegradable film made from chitosan and cinnamon essential oil with low affinity toward water". Food Chemistry, 2010, 122 (1), 161-166.

[41] Y. Pranoto, S.K. Rakshit and V.M. Salokhe. "Enhancing antimicrobial activity of chitosan films by incorporating garlic oil, potassium sorbate and nisin". LWT - Food Science and Technology, 2005, 38 (8), 859–865.

[42] R. Scott. Fabricación de queso. Acribia. Zaragoza. España, 1991.

[43] T.M. Cogan. "Bacteria, beneficial Brevibacterium linens, Brevibacterium aurantiacum and other smear microorganisms". In: Fuquay, J.W., Fox, P.F., McSweeney, P. (Eds.), Encyclopedia of Dairy Sciences, 2011, pp. 395–400.

[44] J.C. Brooks, B. Martinez, J. Stratton, A. Bianchini, R. Krokstrom and R. Hutkins. "Survey of raw milk cheeses for microbiological quality and prevalence of foodborne pathogens". Food Microbiology, 2012, 31(2), 154-158.

[45] O. Aygun, O. Aslantas and S. Oner. "A survey on the microbiological quality of Carra, a traditional Turkish cheese". Journal of Food Engineering, 2005, 66(3), 401-404.

[46] P.R. Salgado, M.E. López Caballero, M.C. Gómez Guillén, A.N. Mauri and M.P. Montero. "Sunflower protein films incorporated with clove essential oil have potential application for the preservation of fish patties". Food Hydrocolloids, 2013, 33(1), 74-84.

[47] W.C. Frazier and D.C. Westhoff. "Microbiología de los alimentos". Ed. Acribia. Zaragoza, España, 1993.

[48] Codex Alimentarius. "Codex General Standard for Cheese". CODEX STAN, 2003, vol. 283-1978, pp. 1-5.

[49] Código Alimentario Argentino. Capitulo VIII, Artículo 605 – 2006, (Resolución Conjunta SPRyRS y SAGPyA N° 33/2006 y N° 563/2006).

[50] J. Gómez Estaca, A.L. De Lacey, M.E. López Caballero, M.C. Gómez Guillén and P. Montero. "Biodegradable gelatin chitosan films incorporated with essential oils as antimicrobial agents for fish preservation". Food Microbiology, 2010, 27(7), 889-896.

[51] F. Yangilar. "Effect of the fish oil fortified chitosan edible film on microbiological, chemical composition and sensory properties of Göbek Kashar Cheese during ripening time". Korean journal for food science of animal resources, 2016, 36(3), 377.

[52] S.M. Scramlin, M.C. Newman, R.B. Cox, H.A. Sepe, A.L. Alderton, J. O'Leary and W.B. Mikel. "Effects of oregano oil brine enhancement on quality attributes of beef longissimus dorsi and semimembranosus muscles from various age animals". Journal of food science, 2010, 75(2), S89-S94.

[53] W.X. Du, R.J. Avena-Bustillos, R. Woods, A.P. Breksa, T.H. McHugh, M. Friedman, M., C.E. Levin and R. Mandrell. "Sensory evaluation of baked chicken wrapped with antimicrobial apple and tomato edible films formulated with cinnamaldehyde and carvacrol". Journal of agricultural and food chemistry, 2012, 60(32), 7799-7804.

[54] L.M. Neira, S.P. Agustinelli, R.A. Ruseckaite and J.F. Martucci. "Shelf life extension of refrigerated breaded hake medallions packed into active edible fish gelatin films". Packaging Technology and Science, 2019.

[55] S. Zantar, F. Yedri, R. Mrabet, A. Laglaoui, M. Bakkali and M.H. Zerrouk. "Effect of Thymus vulgaris and Origanum compactum essential oils on the shelf life of fresh goat cheese". Journal of Essential Oil Research, 2014, 26(2), 76-84.