Improving the shelf life of low-fat cut cheese using nanoemulsion-based edible coatings containing oregano essential oil and mandarin fiber

Authors:

Artiga-Artigas, María; Acevedo-Fani, Alejandra & Martín-Belloso, Olga*.

Affiliations

Department of Food Technology

University of Lleida – Agrotecnio Center

Av. Alcalde Rovira Roure 191,

25198. Lleida, Spain

***Phone number and email of the corresponding author**:

Fax +34 973702596; Tel +34 973702593; email: omartin@tecal.udl.cat

Abstract

Nanoemulsion-based edible coatings containing oregano essential oil (OEO) as antimicrobial were applied onto low-fat cut cheese to extend its shelf life. Nanoemulsions formulation was 2.0% (w/w) sodium alginate, 0.5% (w/w) mandarin fiber, 2.5% (w/w) Tween 80 and 1.5%, 2.0% or 2.5% (w/w) of OEO. Particle size, ζ-potential, apparent viscosity and whiteness index of nanoemulsions were assessed. Water vapor resistance of coatings was evaluated as well as their antimicrobial efficiency against inoculated *Staphylococcus aureus* and native microbiota growth during refrigerated storage. Headspace gases were measured as an indicator of bacterial activity and sensory alterations such as color and texture of cheese pieces were studied. Coatings with at least 2.0% (w/w) OEO decreased *Staphylococcus aureus* population from 6.0 to 4.6 log CFU/g after 15 days. Coated-cheese pieces containing 2.5% (w/w) OEO inhibited psychrophilic bacteria or molds and yeasts growth during 6 or 24 days of storage, respectively. Consequently, the atmosphere into the sealed tracks was stabilized and the outward appearance of cheese pieces was preserved. Thus, the present work evidences the feasibility of using mandarin fiber with high nutritional properties and sodium alginate acting as texturizing agents, to form OEO-loaded coatings onto low-fat cut cheese in order to extend its shelf life.

Keywords: Nanoemulsions; mandarin fiber; cheese; edible coatings; *Staphylococcus aureus*; molds; oregano essential oil.

1. Introduction

Low-fat cheese is characterized by containing low quantities of calories and salt. The shelf life of this kind of cheeses is limited due to the uncontrolled and extensive fungal and bacterial development on its surface reducing their quality, especially if they are cut. The need of bacterial cultures to obtain the suitable form, taste and texture of cheese introduces a potential risk of infection from cheese-borne species like *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella enterica* and *Escherichia coli* (Kuorwel, Cran, Sonneveld, Miltz, &Bigger, 2011). In this regard, consumers' demand for safe and high-quality foods has motivated the scientific community and food industry in finding new strategies that allow increasing the shelf life of highly perishable foods, but with slight effect on the organoleptic properties of the product.

Over the past few years, it has been an increasing interest in using natural antimicrobials for food preservation, due to the general consumer rejection of synthetic additives such as sulfites, benzoic acid or its derived salts, commonly used to control the microbial growth in foods. Essential oils (EOs) are secondary metabolites produced by aromatic plants that have shown potent antimicrobial effect against several pathogenic and spoilage microorganisms. EOs contain a complex mixture of different constituents (non-volatile and volatile), whose composition is highly variable (Adorjan & Buchbauer, 2010; Bonilla, Atarés, Vargas, & Chiralt, 2012; Salvia-Trujillo et al., 2014). In particular, oregano essential oil (OEO) has been previously utilized to control the microbial growth in foods (Raybaudi-Massilia, Mosqueda-Melgar, & Martín-Belloso, 2006; Tajkarimi, Ibrahim, & Cliver, 2010). Its active compound carvacrol presents strong antifungal capacity and high inhibitory effect against *Listeria monocytogenes*, *Salmonella*, *Escherichia coli* and *Staphylococcus aureus* (Burt, 2004; Rojas-Graü et al., 2009; Tajkarimi et al., 2010). Nonetheless, despite these remarkable properties, EOs have poor water solubility, intense aroma, high volatility and may be toxic at high concentrations, which mainly jeopardize their application as natural preservatives (Svoboda, Brooker, & Zrustova, 2006).

The great challenge of incorporating EOs into food matrices could be overcome if they are incorporated into nanoemulsions. These oil-in-water systems have been described as colloidal dispersions with an extremely small droplet size (< 200 nm) (Li, Zheng, Xiao, & McClements, 2012), which can contain lipophilic ingredients in the oil phase (McClements, 2011; Solans et al., 2005). Nanoemulsions can be directly added to food matrices in liquid state or instead, they can be applied as edible coatings onto food surfaces (solid state) if a biopolymer is incorporated in the aqueous phase of nanoemulsions. Moreover, the combination of different biopolymers (for instance, alginate-pectin or alginate-chitosan), can be used to enhance the physicochemical properties of emulsions (George & Abraham, 2006). In this regard, this combination could be even more interesting if one of these biopolymers is also able to provide added-value to the food product, as in the case of dietary fibers (González-Molina, Domínguez-Perles, Moreno, & García-Viguera, 2010). Specifically, mandarin fiber has been used as functional food additive due to its prebiotic properties (Moreira et al., 2015). It has been reported that the intake of mandarin fiber significantly reduce the risk of developing coronary heart disease, stroke, hypertension, diabetes, obesity, and certain gastrointestinal diseases (Grigelmo-Miguel & Martín-Belloso, 1999; T. Wang, Sun, Zhou, & Chen, 2012).

Furthermore, mandarin fiber, which contains a high percentage of soluble fiber (mainly pectin), has been shown to have high water holding capacity and apparent viscosity in combination with sodium alginate, which may lead to the formation of nanoemulsion-based edible coatings (Lundberg, Pan, White, Chau, & Hotchkiss, 2014). Edible coatings are defined as thin layers of eatable material, which are applied in liquid form on the food surface, usually by immersing the product in a solution formed by the structural matrix (carbohydrate, protein, lipid or multicomponent mixture) (Rojas-Graü, M.A. et al., 2009). some of its functions are to protect the product from mechanical damage and chemical reactions acting as moisture barriers (Miller & Krochta, 1997). Otherwise, if the coatings contain antimicrobial agents, they are able to protect high perishable food products, such as

low-fat cheese, from the microbial growth extending their shelf life (Raybaudi-Massilia, Mosqueda-Melgar, & Martín-Belloso, 2008; Rojas-Grau et al., 2007).

In this regard, nanoemulsions containing EOs could be used to form antimicrobial coatings on the cheese surface, as a way to limit the negative changes that occur during the time. Thus, the aim of the current work was to assess the antimicrobial effectiveness of nanoemulsions-based edible coatings containing OEO and enriched with mandarin fiber against inoculated *Staphylococcus aureus*, and their capability to improve the shelf life of a highly perishable low-fat cut cheese.

2. Materials and methods

2.1. Materials

Low-fat cheese (CADICOOP light) was kindly donated by CADÍ® (Lleida, Spain). Oregano essential oil was supplied by Essential arôms (Lleida, Spain). Tween 80 was purchased from Panreac (Barcelona, Spain). Sodium alginate (MANUCOL®DH) was obtained from FMC Biopolymer Ltd (Scotland, U.K.). Information provided by the manufacturer indicates that viscosity and pH of a solution 1% is 40-90 mPa·s and 5.0-7.5, respectively. Mandarin fiber containing 231.30 g/kg of soluble fiber (mainly pectin), 202.38 g/kg of insoluble fiber, 81.25 g/kg of proteins, 7,74 g/kg of lipids, 29.61 g/kg of ashes and 423.76 g/kg of carbohydrates was kindly donated by Indulleida (Lleida, Spain). Ultrapure water obtained from a Milli-Q filtration system was used to the preparation of all solutions.

2.2. Methods

2.2.1. Nanoemulsions preparation

Formulation of oil-in-water nanoemulsions contained OEO (1.5 – 2.5% w/w), Tween 80 (2.5% w/w), sodium alginate (2.0% w/w) and mandarin fiber (0.5% w/w).

92 The aqueous phase was prepared by solving sodium alginate in ultrapure water at 70ºC for 3 h.
93 After reaching room temperature, mandarin fiber was added to alginate solution and mixed using a
94 laboratory high-shear homogenizer (T25 digital Ultra-Turrax, IKA, Staufen, Germany) at 9,600 rpm
95 for 3 min. Ultimately, the aqueous phase was filtered in order to remove the fiber in excess. An
96 accurate amount of the lipid phase consisted of the mixture of OEO and Tween 80 at room
97 temperature was added to the aqueous phase, and blended with the high-shear homogenizer at
98 11,000 rpm for 2 min, leading to coarse emulsions. Lastly, nanoemulsions were formed passing the
99 respective coarse emulsion through a microfluidizer (M110P, Microfluidics, Massachusetts, USA)
100 at 150 MPa for 5 cycles.

101 ### 2.2.2. Physicochemical characterization of emulsions and nanoemulsions.

102 #### *2.2.2.1. Droplet size, size distribution and ζ-potential*

103 The particle size distribution and mean droplet diameter (nm) of emulsions and nanoemulsions were
104 measured by a Zetasizer Nano-ZS laser diffractometer (Malvern Instruments Ltd, Worcestershire,
105 UK) working at 633 nm and 25 ºC, equipped with a backscatter detector (173º) (Salvia-Trujillo,
106 Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2015).

107 The ζ-potential (mV), was measured by phase-analysis light scattering (PALS) with a Zetasizer
108 Nano-ZS laser diffractometer (Malvern Instruments Ltd, Worcestershire, UK). It determines the
109 electrical charge at the interface of the droplets dispersed in the aqueous phase.

110 In both types of determinations, samples were prior diluted in ultrapure water using a dilution factor
111 of 1:9 sample-to-solvent.

112 #### *2.2.2.2. Apparent viscosity and whiteness index*

113 Viscosity measurements (mPa·s) were performed by using a vibro-viscometer (SV-10, A&D
114 Company, Tokyo, Japan) vibrating at 30 Hz, with constant amplitude and working at room
115 temperature. Aliquots of 10 mL of each emulsion and nanoemulsion were used for determinations.

A colorimeter (CR-400, Konica Minolta Sensing Inc., Osaka, Japan) set up for illuminant D65 and 10^{o} observer angle was used to measure the CIE L^*, a^* and b^* parameters of emulsions and nanoemulsions at room temperature. The device was calibrated with a standard white plate (Y =94.0; x = 0.3133; y = 0.3194). The whiteness index (WI) was calculated with eq. (1) (Vargas et al., 2008):

$$WI = 100-((100-L^*)^2+(a^{*2}+b^{*2}))^{0.5} \quad \textbf{eq.(1)}$$

2.2.3. Cheese coating and sampling

Sealed cheese bars were stored at 4ºC before processing. Immediately after opening the cheese bars, identical cylindrical pieces (diameter: 1.5 cm, height: 2.4 cm) were cut in order to make reproducible experiments.

Cheese pieces were immersed into the corresponding nanoemulsion for 1 min, and allowed to dry at room temperature for 5 min. The addition of $CaCl_2$, which acts as cross-linker was not needed since cheese contains calcium itself. On the other hand, the uncoated pieces were immersed into ultrapure water following the procedure explained above. Lastly, 50 grams of either coated or uncoated cut cheese were packed polypropylene (PP) trays (ATS packaging, Barcelona, Spain) of 170 mm length x 25 mm height x 110 mm width, using a tray sealer (Basic V/G, Ilpra systems, Barcelona, Spain). Afterwards a cover film made of polyamide and polyethylene (Tecnopack, Girona, Spain) was used to heat seal the trays. Lastly, sealed trays were stored at 4ºC during 15 days.

Separate trays were prepared with cheese pieces inoculated with *Staphylococcus aureus* to evaluate the antimicrobial effect of the antimicrobial coating and to assess the changes in quality attributes along 24 days of refrigerated storage. Two trays of each set were prepared, and two replicates for each sealed package were performed.

2.2.4. Water Vapor Resistance (WPR) and weight loss

Water vapor resistance (WVR) of cheese pieces was evaluated gravimetrically at 25 ºC using a modified version of ASTM standard method E96-00 (ASTM, 2000). The method and experimental set up following to determine the water loss of coated cheese pieces was described by García et al. (1998). Cheese cylinders were placed in small test cups (internal diameter of 2.7 cm and depth of 1.3 cm) and weighed in an analytical laboratory scale (AT261 Delta Range, Mettler Toledo, Barcelona, Spain). Initial weights of coated cheese pieces were 8.3 ± 0.12, 8.4 ± 0.23 and 8.5 ± 0.20g for coatings with 1.5% OEO, 2.0% OEO and 2.5% OEO, respectively and 7.8 ± 0.14 g for those uncoated. Cheese cylinders were placed in sealed chambers that were equilibrated at 33% RH with a saturated $MgCl_2 \cdot 6H_2O$ solution (Panreac Quimica SA, Barcelona, Spain) at 25 ºC. Cups weights were recorded at 60 min intervals during 6 h. Weight loss was calculated by difference using equation (2) and plotted versus time. Data were analyzed by lineal regression to obtain the slope (d_s/d_t) of the curve in g/s. Water activity of the coated and uncoated cheese pieces (0.974 ± 0.001 and 0.947 ± 0.001, respectively) was measured twice for each sample with a water activity meter (Acqualab CX-2, Decagon Devices Inc., Pullman, WA).

$$WL = W_i - W_f \qquad \textbf{eq. (2)}$$

where WL is the weight loss of cheese pieces (in mg); W_i and W_f, are initial and final weights of cheese pieces (in mg).

Afterwards, WVR of the coatings was calculated using a modified Fick's first law equation as described in equations (3), (4) and (5) (Ben-Yehoshua et al., 1985; Kaya & Kaya, 2000; Park & Chinnan, 1995; Rojas-Graü et al., 2007):

$$\Delta C = (P_i - P_a)/(R_c \cdot T) \qquad \textbf{eq.(3)}$$

$$A = \pi \cdot r \cdot (2 \cdot h + r) \qquad \textbf{eq.(4)}$$

$$WVR = (A \cdot \Delta C)/(d_s/d_t) \qquad \textbf{eq.(5)}$$

where P_i - P_a is the difference in water vapor pressure (Pa) inside and outside of the cheese piece (P_i = a_w of the cheese x P_0 – that is the vapor pressure of liquid water at 25ºC and P_a = partial water pressure in the environment with 33.3% RH at 25ºC, in Pa x P_0); ΔC is the concentration of gas (g/cm^3) inside and outside the cheese piece at time t; A is the exposed area of cylindrical cheese pieces (5.65 cm^2) taking into account that only one of the bases was in contact with the environment; (d_s/d_t) is the rate of gas exchange (slope) in g/s; Rc is the universal gas constant(3.46 L·mmHg/K g) and T is the temperature in degrees Kelvin.

2.2.5. Antimicrobial efficiency of edible coatings

2.2.5.1. Inoculum preparation

A strain of *Staphylococcus aureus* (CECT 240) (University of Valencia, Spain) was provided by the culture collection of the Department of Food Technology, University of Lleida, Spain. The *Staphylococcus aureus* culture was kept in slant tubes with Tryptone Soy Agar (TSA) (Biokar Diagnostics, France) at 5 ºC. The strain was then inoculated in Tryptone Soy Broth (TSB) (Biokar Diagnostics, France) and incubated at 35 ºC, 200 rpm for 6 h, to obtain cells in stationary growth phase. The inoculum concentration was diluted from 10^8 CFU/mL to 10^6 CFU/mL for cheese inoculation.

2.2.5.2. Antimicrobial activity against inoculated Staphylococcus aureus

Cheese pieces (10 g) were inoculated with 50 µL aliquots of the culture and left dry for 20 min. Cheese pieces were coated with nanoemulsions that contained OEO, packed in heat sealed PP trays and stored at 4ºC for 15 days. In order to corroborate that no one of the other components of edible coating had also antimicrobial activity, nanoemulsions containing corn oil instead of OEO in the same concentrations were prepared and applied onto cheese pieces.

The content of each tray (10 g) was put into a sterile stomacher bag (Strainer bag stomacher® lab system, Seward, UK) with 90 mL of buffer peptone (Biokar Diagnostics, France). Bags were homogenized for 1 min in a Stomacher blender (BagMixer®, Interscience, France). Serial dilutions with saline peptone (Peptic Digest of meat USP, Biokar, Diagnostics, France) were prepared and

spread onto Baird-Parker agar (BP) (Biokar Diagnostics, France). Plates were incubated at 37 ºC for 48 h and colonies were counted. Results were expressed as $\log_{10}$ CFU/g.

2.2.6. Quality assessment of coated cheese pieces

2.2.6.1. Microbial stability

Psychrophilic bacteria and molds and yeasts growth in cheese pieces was examined along 24 days under refrigeration. Cheese samples of 10 grams randomly chosen in aseptic conditions were put into sterile bags with 90 mL of peptone buffer (Biokar Diagnostics, France). Bags were homogenized for 1 min in a Stomacher blender (BagMixer®, Interscience, France). Serial dilutions with saline peptone (Peptic Digest of meat USP, Biokar, Diagnostics, France) were prepared, and 100 µL were spread onto Plate Count Agar (PCA) (Biokar Diagnostics, France) and Cloranfenicol Glucosa Agar (CGA) (Biokar Diagnostics, France) for psychrophilic bacteria and molds and yeast counts, respectively. PCA plates were incubated at 4 ºC for 15 days, whereas CGA plates were maintained at room temperature during 5 days. Afterwards, colonies were counted and the results were expressed as $\log_{10}$ CFU/g.

2.2.6.2. Headspace gas analysis

The composition of the headspace of each tray was analyzed with a gas chromatograph (Varian 490-CG) equipped with a thermal conductivity detector (Micro-GC CP 2002 gas analyzer, Chromatography Systems, Middelburg, The Netherlands). A 10 mL sample was automatically withdrawn from the tray headspace atmosphere and injected in the gas chromatograph. The oxygen (O_2) content expressed in percentage was analyzed with a 10 m packed column (CP-Molsieve 5Å, Varian, Middelburg, The Netherlands) at 60 ºC and 100 kPa. For quantification of carbon dioxide (CO_2) expressed in percentage, and ethanol (C_2H_5OH) concentration reported in ppm, a column PoraPLOT Q (Varian, Middelburg, The Netherlands) (10 m x 0.32mm, df = 10 mm) held at 70 ºC and 200 kPa was used.

2.2.6.3. Color (WI)

The color of coated and uncoated cheese pieces was measured with a colorimeter (CR-400, Konica Minolta Sensing Inc., Osaka, Japan) set up for illuminant D65 and 10º observer angle and calibrated with a standard white plate. Measurements were taken at room temperature. CIE L^*, a^* and b^* values were determined and the Whiteness Index (WI) was calculated through equation (1).

2.2.6.4. Texture profile analysis (TPA)

TPA of cheese pieces was carried out using a texture analyzer (TA-TX2, Stable Micro Systems, Goldaming, UK) equipped with a 5 kg load cell and the 36R probe, operating with two compression-decompression cycles and 2 $mm \cdot s^{-1}$ of crosshead speed (Diamantino et al., 2014). The hardness, cohesiveness, gumminess, elasticity, adhesiveness and chewiness of the cylindrical cheese pieces were calculated according to Szczesniak (2002). Eight replicates of each tray were performed at each sampling time.

2.2.7. Statistics

All the experiments were assayed in duplicate, and at least three replicate analyses were carried out for each parameter. SigmaPlot 11.0 Systat Software was used to perform the analysis of variance. Tukey test was chosen to determine significant differences among treatments, at a 5% significance level.

Correlation analyses were performed with statistical analysis software (JMP Pro 12, Statistical Discovery™, North Carolina, USA).

3. Results and discussion

3.1. Particle size and ζ-potential of nanoemulsions

Microfluidization process caused a droplet disruption leading to nanoemulsions with smaller droplet sizes than their respective coarse emulsions (particle sizes over 700 nm) (Table 1). In fact, the smallest size (169 ± 23 nm) was obtained in nanoemulsions with 2.0% of OEO, whereas those with

OEO percentages of 1.5% or 2.5% w/w led to particle sizes of 214 ±14 nm or 337 ±79 nm, respectively. The obtained results are in accordance with previous research in which the average droplet diameter of nanoemulsions containing OEO and polysaccharides such as pectin or alginate, were also in the nanorage scale (Guerra-Rosas et al., 2016; Salvia-Trujillo et al., 2014).

Differences in particle size may be related to the observed polydisperse particle distributions in all nanoemulsions (Figure 1). There was a minor peak in the macro range that indicates the presence of particles with greater size, which may cause an increase in the mean particle size values. These minor peaks could be the outcome of an excess or a lack of OEO. When the concentration of OEO is too low, two possible phenomena can occur: the excess of surfactant molecules adsorbed at the interface of droplets may repel biopolymer chains and/or these biopolymer chains may bind free surfactant molecules rather than those that are in the surface of oil droplets (Goddard, 2002; Neumann, Schmitt, & Iamazaki, 2003). On the other hand, an excess of OEO droplets might cause the coalescence phenomenon in which two or more small oil droplets come in contact forming a single bigger droplet (Klang, Matsko, Valenta, & Hofer, 2012).

Regarding the electrical charge of oil droplets, all nanoemulsions showed ζ-potential values lower than -30 mV regardless the concentration of OEO used (-35 ± 5 mV, -47 ± 3 mV and -42 ± 3 mV for 1.5%, 2.0% and 2.5% of OEO, respectively), whereas coarse emulsions had higher ζ-potential (from -14 to -22 mV) (Table 1). It is known that when ζ-potentials are below -30 mV, the electrical charge of droplets is strong enough to assume that repulsive forces between droplets are predominant in the system, keeping them stable (Heurtault, Saulnier, Pech, Proust, & Benoit, 2003). Therefore, nanoemulsions obtained in the current work presented higher stability than coarse emulsions regardless the concentration of OEO.

Despite the fact that a neutral or slightly negative electrical charge was expected at the oil-water interface, according to the non-ionic low-mass nature of Tween 80 (Hsu & Nacu, 2003), the presence of the anionic groups of sodium alginate and mandarin fiber molecules dispersed in the

aqueous phase has a strong influence in the ζ-potential values. When emulsions are exposed to mechanical treatment such as microfluidization, it might cause the opening of the biopolymer chain by mechanical shear, releasing free hydroxyl and carboxyl groups from their molecular structures available to bind with water (Chen et al., 2013). These deprotonated alcohols or carboxylic acids ($R\text{-}O^-$ or $R'CO_2^-$, respectively) contributed to increase the negative charge in the interface of the droplets. Therefore, the higher the biopolymer concentration, the higher was the presence of $R\text{-}O^-$ or $R'CO_2^-$ and the more negative ζ-potential values.

3.2. Apparent viscosity and whiteness index (WI)

Apparent viscosity values of nanoemulsions regarding coarse emulsions increased after microfluidization (Table 1), probably due to the non-Newtonian behavior of the pair mandarin fiber – alginate (Lundberg et al., 2014). This behavior let biopolymer structures hold additional water after a shear stress, increasing their gel-forming capacity, which consists of the formation of a gelatinous mass through water absorption (Dikeman, Murphy, &Jr, 2006; L. Wang et al., 2015). Regarding the viscosity values of both coarse emulsions and nanoemulsions, significant differences ($P<0.05$) between 1.5% w/w OEO emulsions and those with more concentration of OEO (2.0 and 2.5% w/w) were observed. The lower the percentage of OEO, the higher the viscosity of emulsions probably due to aggregation phenomena between biopolymers and surfactant molecules (Neumann et al., 2003). In this regard, apparent viscosity of emulsions might be strongly influenced by the presence of alginate and mandarin fiber dispersed in the aqueous phase.

The whiteness indexes (WI) of emulsions significantly decreased ($P<0.05$) in all cases after microfluidization process (Table 1). In fact, nanoemulsions have been defined as slightly turbid systems because small droplets scatter light weakly. Therefore, with the increase of droplet size, the light scattering is stronger and the WI of emulsions tends to be higher (Acevedo-Fani, Salvia-Trujillo, Rojas-Graü, & Martín-Belloso, 2015; McClements, 2002).

On the other hand, nanoemulsions with a concentration of OEO over 2% w/w scattered the light significantly ($P<0.05$) more intensely than those with less concentration of OEO, causing an increase in the WI of the former (McClements, 2011; Salvia-Trujillo et al., 2013). This is because the emulsion appearance is highly determined by the physicochemical characteristics of oil droplets such as their size, refractive index or concentration. Specifically, if the concentration of oil droplets rises, L^* value also increased (Table 1) and hence, WI of emulsions become higher (eq.1) (Chantrapornchai, Clydesdale, & McClements, 1999).

3.3. Water Vapor Resistance (WVR) and water loss of coated-cheese pieces

As it is shown in Table 2, the highest weight loss was observed in cheese pieces with coatings containing 1.5% and 2.0% w/w of OEO (205.4 ± 0.1 mg and 216.4 ± 0.2 mg, respectively) without significant differences ($P<0.05$). However, the uncoated cheese presented the lowest weight loss value (156.5 ± 0.1 mg). The fact that the presence of a coating increased the weight loss of cut cheese can be explained by the higher water activity (a_w) of coated cheese pieces, compared with those uncoated(0.974 versus 0.947 , respectively). In the coated product, the concentration of water on the matrix surface is higher, so it is easily captured by saturated $MgCl_2 \cdot 6H_2O$ solution situated in a sealed chamber, causing a greater loss of water from the coating. Regarding the different coatings, those with the highest content of EO resulted more effective as barriers showing a weight loss of 195.3 ± 0.2 mg, which is lower than in the case of using coatings with less concentration of OEO.

Coated cheese pieces exhibited greater values of WVR, compared to those uncoated (Table 2). Moreover, as the concentration of OEO in nanoemulsions increased, the WVR of coatings was higher. Although, polysaccharide-base coatings exhibit limited water vapor barrier ability owing to their hydrophilic and hygroscopic nature (Gennadios, Hanna, & Kurth, 1997), OEO-based coatings, reducing water loss and avoiding cheese dehydration. On the other hand, there was a solid correlation ($r=0.73$) between the water loss of coated product and calculated WVR values of

nanoemulsion-based coatings. It suggested that those edible coatings that presented a higher WVR, experimented a lower water loss.

3.4. Antimicrobial efficiency of edible coatings against inoculated *Staphylococcus aureus*

The effectiveness of the antimicrobial edible coatings in inhibiting *Staphylococcus aureus* growth inoculated on low-fat cut cheese during refrigerated storage is shown in Figure 2. Initial microbial load (10^6 CFU/g) inoculated on the surface of cylindrical cheese pieces decreased 0.9 ± 0.1 log CFU/g on average, just after applying the different coatings or after their submersion in ultrapure water (uncoated cheese pieces).

The antimicrobial effectiveness of coatings with 1.5%, 2.0% or 2.5% w/w of OEO applied on the surface of cylindrical cheese pieces was compared with the formulations loaded with corn oil at the same concentrations confirming the lack of antimicrobial activity of coatings in absence of OEO (Fig.2).

The concentration of OEO used in the formulation of edible coatings had a significant effect on their bactericidal activity against inoculated *Staphylococcus aureus* over time. The microbial population decreased 1.4 and 1.5 log CFU/g in coated cheese pieces containing 2.0 % or 2.5% w/w of OEO, respectively, during 15 days of refrigerated storage. However, coatings with an OEO concentration of 1.5% w/w were not effective in reducing *Staphylococcus aureus* population.

OEO can alter the fatty acid composition of cytoplasmic membranes of pathogenic and spoilage microorganisms (Pasqua, Hoskins, Betts, & Mauriello, 2006). Specifically, carvacrol, the major active compound from OEO, is able to modify the cell membrane of Gram-positive bacterial species such as *Staphylococcus aureus* (La Storia et al., 2011). Nevertheless, the antimicrobial activity of coatings against this pathogen was found to be dependent on the concentration of carvacrol contained in them (Kuorwel et al., 2011). Therefore, neither in coated-cheese pieces with

an OEO concentration of 1.5% w/w nor in uncoated cheese pieces or in those with the coating based on corn oil, was possible to inactivate or inhibit the growth of *Staphylococcus aureus*.

3.5. Microbial growth and quality changes along storage.

3.5.1. Psychrophilic bacteria, molds and yeast

Figure 3 shows the growth of psychrophilic bacteria (A) and molds and yeast (B) in uncoated and coated cheese pieces during refrigerated storage. The results revealed that coatings with a concentration of OEO higher than 2.0% w/w clearly had an antimicrobial effect. However, a concentration of 1.5% w/w of OEO was not enough to inhibit the development of neither psychrophilic bacteria nor molds and yeast in cheese pieces.

Indeed, the most effective microbial inhibition of psychrophilic bacteria was obtained for cheese pieces coated by the nanoemulsion with the highest percentage of OEO (2.5% w/w). In this case, the growth began after the 6^{th} day of storage and reached the equilibrium with values lower than 6.7 log CFU/g at the 13^{th} day (Fig.3A). Although coatings with 2.0% w/w resulted effective in slowing down the psychrophilic bacteria growth, it was not enough to stop it. The microbial growth in the cheese pieces with a 2.0% w/w of OEO was lower than in those coated containing 1.5% w/w of OEO, reaching the maximum microbial population at the 17^{th} day with a 7.3 log CFU/g. On the other hand, microbial counts of psychrophilic bacteria in uncoated cheese pieces and in those coated with 1.5% OEO increased until 8.3 log CFU/g from the first day to the 17^{th} day of storage before reaching the equilibrium (Fig.3A).

Regarding molds and yeast (Fig. 3B), the growth started after the 13^{th} day in uncoated cheese pieces and in those coated with the lowest percentage of OEO (1.5% w/w). However, the fungi growth for uncoated pieces was greater than for those coated. In the case of coatings prepared with a 2.0% w/w of OEO, the growth began after 17 days of storage. Lastly, cheese pieces coated with 2.5% w/w of OEO did not show molds and yeast growth at least during 24 days of experiment.

Lipids like EOs have many biological functions in microbial cells (La Storia et al., 2011), however, their effectiveness not only depend on the concentration but also on the active compound(Moore-Neibel et al., 2013). According to the results, both psychrophilic bacteria and molds and yeast were especially sensitive to carvacrol (main component of OEO) (Microbiology & Andrews, 2001). In this regard, OEO coatings may require less concentration of essential oil to extend the microbiological shelf life of low-fat cheese pieces (2.0% w/w) than in the case of using other type of EOs (Kavas & Kavas, 2014).

3.5.2. Headspace gas composition

As it can be appreciated in Figure 4A, coatings based on nanoemulsions with OEO caused a decrease of O_2 consumption compared with the uncoated pieces from the 3th day, probably as a result of the control of the microbial growth (Raybaudi-Massilia et al., 2008). On the contrary, the production of CO_2 increased gradually until reaching the equilibrium because of the gas produced by cheese itself (Acerbi, Guillard, Guillaume, Saubanere, & Gontard, 2016). Nonetheless, significant variations ($P<0.05$) were observed during the first 13 days regarding the concentration of OEO (Fig.4B). Although it was supposed that coatings with a higher percentage of EO ought to have a higher resistance to gas diffusion due to their lipophilic nature (Salvia-Trujillo et al., 2015), the presence of some carbohydrates such as alginate and mandarin fiber contributes to increase gas permeability (Rojas-Graü et al., 2007). This is related to the capability of alginate or mandarin fiber chains of holding water in their structures, which together with the fact that EO concentration was low, could cause a decrease in the ability of coatings to act as barriers to the transport of humidity, gases, and aroma compounds (Espitia, Du, Avena-Bustillos, Soares, & McHugh, 2014; Miller & Krochta, 1997).

Regarding ethanol production, there were not significant differences ($P>0.05$) between cut cheese coated by nanoemulsions containing 1.5% OEO and uncoated pieces (Fig.4C). The same occurred

for 2.0% and 2.5% OEO coatings between them probably because both resulted effective in the control of microbial growth. Moreover, despite the fact that cheese produces CO_2 and ethanol during propionic and acid fermentation due to the action of lactic bacteria (Acerbi et al., 2016; Fröhlich-Wyder et al., 2013), it was possible to observe a fast decrease of ethanol concentration during the first three days of storage before reaching the equilibrium, because the alcohol may react with different derivatives of primary or secondary biochemical processes that occur during cheese shelf life(Mei, Guo, Wu, Li, & Yu, 2015). In this regard, lipolysis reactions lead to several free fatty acids whose esters are able to react with ethanol. In addition, the reaction between some acids such as acetic or lactic acid with ethanol molecule produces the corresponding acetates resulting in the chemical equilibrium of equation (6):

$$RCO_2H + CH_3CH_2OH \leftrightarrow RCO_2CH_2CH_3 \qquad \text{eq.(6)}$$

Therefore, ethanol acts as a reaction intermediate, so its production and consumption are in equilibrium and thus, it is likely that an increase of ethanol could not be detected by gas chromatography.

3.5.3. Color (WI)

As has been discussed before, the optical properties of nanoemulsions depended on OEO concentration, thus when they are applied as edible coatings the color of the coated cheese pieces could be altered. Uncoated cheese pieces exhibited the highest WI value (76.32 ± 0.98) and there were no significant differences during the time (Fig.5A). Regarding coated cut cheese, a concentration of over 2.0% w/w OEO did not affect the WI values during storage time . However, when coatings with less OEO concentration (1.5% w/w) were used, the color of the cheese pieces increased progressively until the 13^{th} day, probably because a lack of oil droplets might cause biopolymer or surfactant aggregation leading to more instable coatings (Neumann et al., 2003).

According to experimental data, the WI was influenced by the rise of the *b** coordinate value, which indicates the increase of yellow color (Fig.5B). The higher the *b** value, the more yellow the

cheese pieces and therefore, the lower the WI (eq.1). The increase of the *b** parameter can be explained by the orange color of mandarin fiber that was incorporated to nanoemulsions. Even though coated pieces seemed to be more yellow than uncoated, they were able to maintain the brightness and the external properties of cheese during the time. In fact, the preservation of the outward appearance of coated cheese pieces is very important in terms of being unnoticed for consumers (Stintzing & Carle, 2004).

3.5.4. Cheese Texture Profile Analysis (TPA)

Coated cheese pieces showed similar values of hardness, cohesiveness, gumminess and elasticity regardless the EO percentage incorporated in nanoemulsions, whereas these parameters were higher in uncoated cut cheese (Fig.6). The highest values of adhesiveness were observed in the uncoated product, and it remains constant during the time (Fig.6D). In the case of coated pieces, adhesiveness values gradually decreased during 24 days.

Cheese hardness usually increases over time as a result of water loss and proteolysis (Bourne, 2002). Hence, the product may require a major force in the process of chewing due to a lack of elasticity (Segnini, Dejmek, & Öste, 1999). In the current work, the percentage of high water-content edible coatings allowed maintaining, not only the elasticity of coated cheese pieces but also cheese pieces softness (Fig. 6C, 6A, respectively).

Szczesniak (2002) pointed out that gumminess and chewiness (defined as the energy required to masticate a solid food) are mutually exclusive (Bourne, 2002) so, as elasticity remained constant over time, the two properties must vary proportionally. Nevertheless, although coatings did not exert any effect on chewiness, which was maintained constant during the time (data not shown), the gumminess of the uncoated cheese pieces started to increase from the 7^{th} day of storage (Fig.6E). Probably because gumminess, understood as the energy required to disintegrate a semisolid food to a state of readiness for swallowing, is dependent on hardness; hence, if the latter increases, the former does(Bourne, 2002).

On the other hand, the cohesiveness of cheese pieces, defined as the limit point to which the material can deform itself before breaking, did not vary over time regardless the concentration of OEO (Fig.6B). Nevertheless, uncoated pieces showed higher cohesiveness values, which decreases during the time; hence, coated cheese may break easily. The cheese becomes a cohesive material along the time because the particles are closer after the product dehydration (Szczesniak, 2002). However, the edible coatings helped to preserve the water into the food matrix maintaining the cohesiveness, whereby the disintegration of the product is less probable.

Lastly, adhesiveness, which is the work necessary to overcome the attractive forces between the surface of the food and the surface of the other materials in contact (Szczesniak, 2002), did not correlate with the other properties studied by TPA (Segnini et al., 1999). In fact, coated cheese pieces experienced a loss of adhesion, whereas this property remained constant over time in the uncoated product (Fig.6D). Therefore, coated cheese pieces might be less sticky during the days than those uncoated.

4. Conclusions

The combination of mandarin fiber with prebiotic properties, and sodium alginate let the formation of stable OEO-loaded nanoemulsions able to act as edible coatings onto cheese pieces. Thus, the incorporation of fiber to the coatings may become in an interesting alternative for increasing the nutritional value of coated cheese pieces. In addition, edible coatings with at least 2.0% w/w of OEO improved the microbial stability of the cheese pieces, resulted effective in the decontamination of external pathogens such as *Staphylococcus aureus* and preserved cheese outward appearance during the time. As a consequence, the incorporation of nanoemulsions-based edible coatings containing OEO onto low-fat cut cheese extended the shelf life of this product. These results evidence the potential advantages of using OEO as natural antimicrobial within edible coatings acting as preservatives and enhancing the safety, quality and nutritional properties of high perishable low-fat cut cheese.

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608

609

Table 1. Physicochemical properties of coarse emulsions (CE) and their respective nanoemulsions (NE) in terms of Z-average (nm), ζ-potential (mV), Viscosity (mPa·s), Whiteness index (WI) and parameter *L**. Data shown are the means ± standard deviation.

	EO concentration (w/w)	Z-average (nm)	ζ-potential (mV)	Viscosity (mPa·s)	WI	*L**
CE	1.5%	1851 ± 592^{Aa}	-17 ± 3^{a}	341 ± 6^{a}	60.42 ± 0.04^{a}	65.6 ± 0.1^{a}
	2.0%	1442 ± 529^{Ca}	-14 ± 3^{a}	250 ± 4^{b}	63.90 ± 0.05^{b}	69.09 ± 0.09^{b}
	2.5%	707 ± 242^{Eb}	-22.51 ± 1.48^{b}	237 ± 3^{b}	71.4 ± 0.3^{c}	75.7 ± 0.3^{c}
NE	1.5%	214 ± 14^{Bc}	-34 ± 5^{c}	366 ± 4^{c}	59.49 ± 0.16^{d}	62.59 ± 0.21^{d}
	2.0%	169 ± 23^{Dd}	-47 ± 3^{d}	270 ± 6^{d}	60.91 ± 0.17^{e}	62.73 ± 0.21^{e}
	2.5%	337 ± 79^{Fe}	-42 ± 4^{d}	265.00 ± 1.41^{d}	67.80 ± 0.10^{f}	71.17 ± 0.19^{e}

[a,b,c,d,e,f] Means in same column with different letters are significantly different at $p < 0.05$ in terms of comparing OEO concentration.

Table 2. Initial and final weights (g) of cheese pieces, weight loss (%) of coated and uncoated cheese pieces and water vapour resistance (WVR) expressed in s/cm of edible coatings based on oregano essential oil (OEO) from nanoemulsions (NE) applied onto cylindrical cut cheese pieces. Data shown are the means ± standard deviation.

	EO concentration (w/w)	Initial weight (g)	Final weight (g)	Weight loss (%)	WVR (s/cm)
NE	1.5%	8.26 ± 0.12^{a}	8.06 ± 0.12^{a}	2.49^{a}	8.40 ± 0.13^{a}
	2.0%	8.44 ± 0.23^{ab}	8.22 ± 0.24^{ab}	2.56^{a}	8.40 ± 0.24^{a}
	2.5%	8.50 ± 0.20^{b}	8.30 ± 0.18^{b}	2.30^{b}	10.08 ± 0.19^{b}
Uncoated		7.80 ± 0.14^{c}	7.65 ± 0.13^{c}	2.00^{c}	12.06 ± 0.14^{c}

[a,b,c] Means in same column with different letters are significantly different at $p < 0.05$.

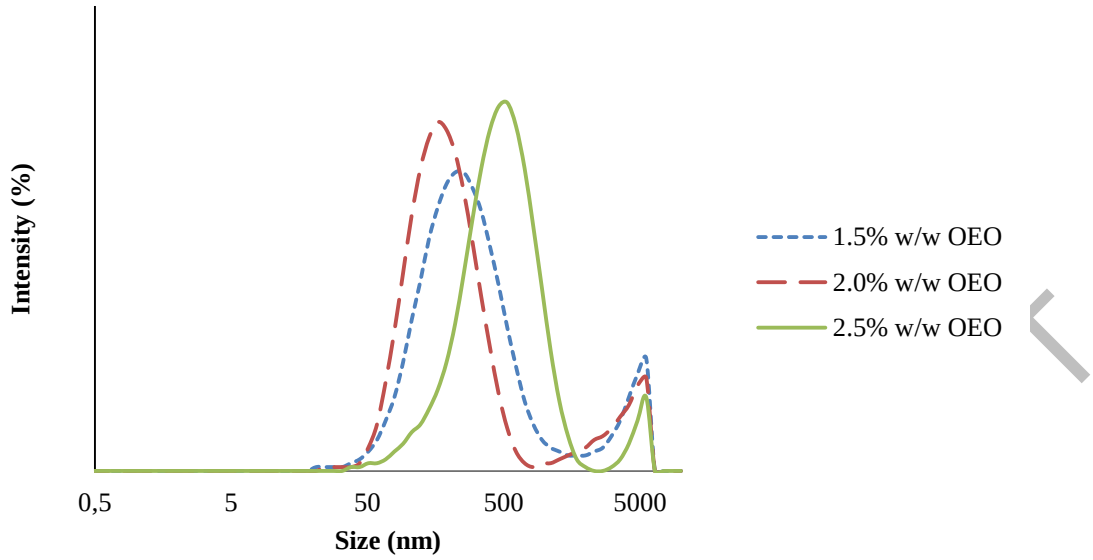


Figure 1. Particle size distribution (nm) of the nanoemulsions with different concentrations of oregano essential oil (OEO).

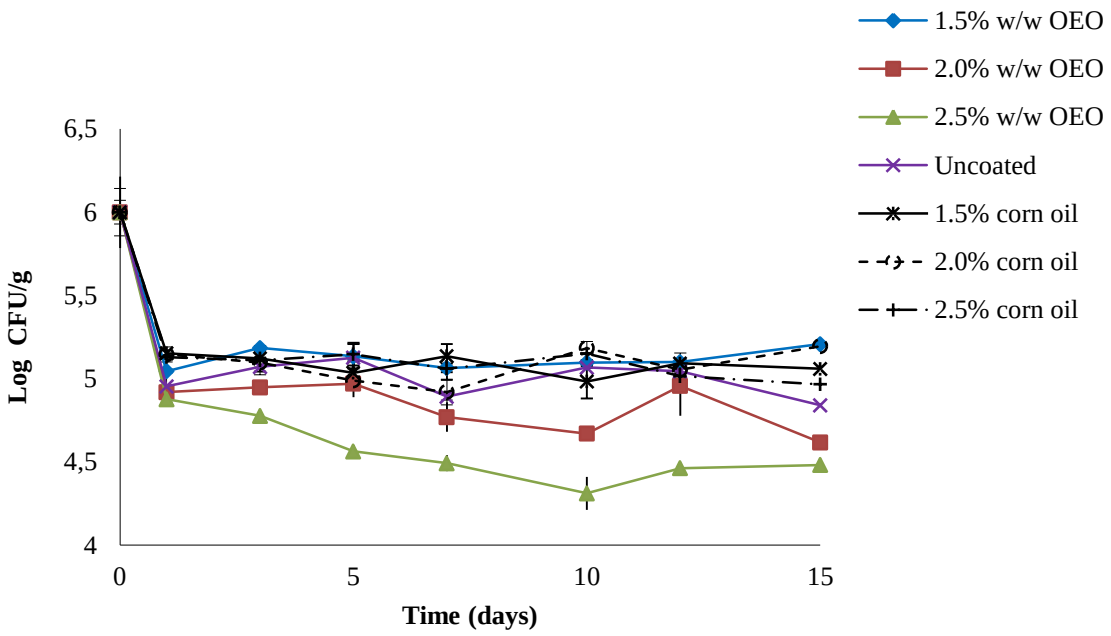


Figure 2. Effect of the edible coatings from nanoemulsions containing different concentrations of oregano essential oil (OEO) against *Staphylococcus aureus* (log CFU/g) inoculated onto cheese pieces. Data shown are the means ± standard deviation.

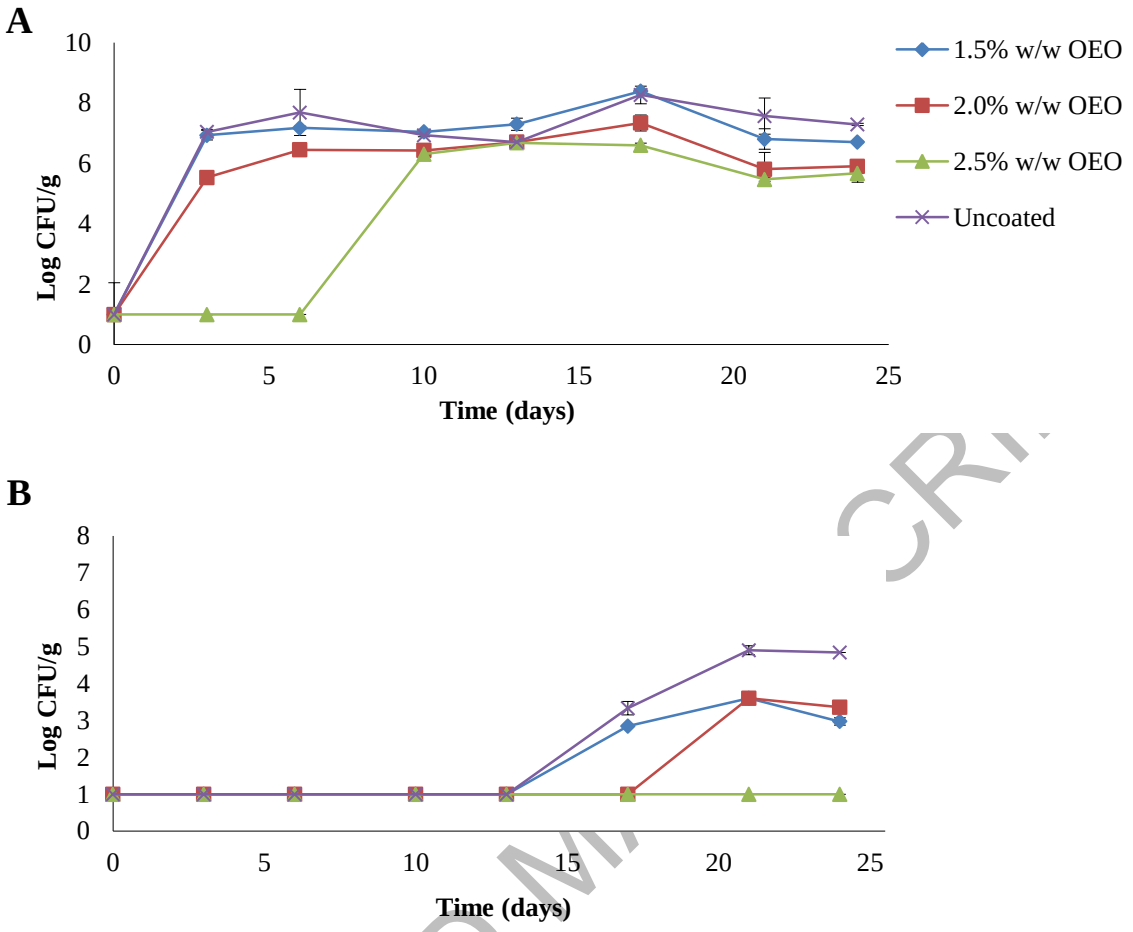


Figure 3. Effect of the nanoemulsion-based edible coatings containing oregano essential oil (OEO) on the microbial growth (log CFU/g) of psychrophilic bacteria (A) and molds and yeasts (B) in cheese pieces. Data shown are the means ± standard deviation.

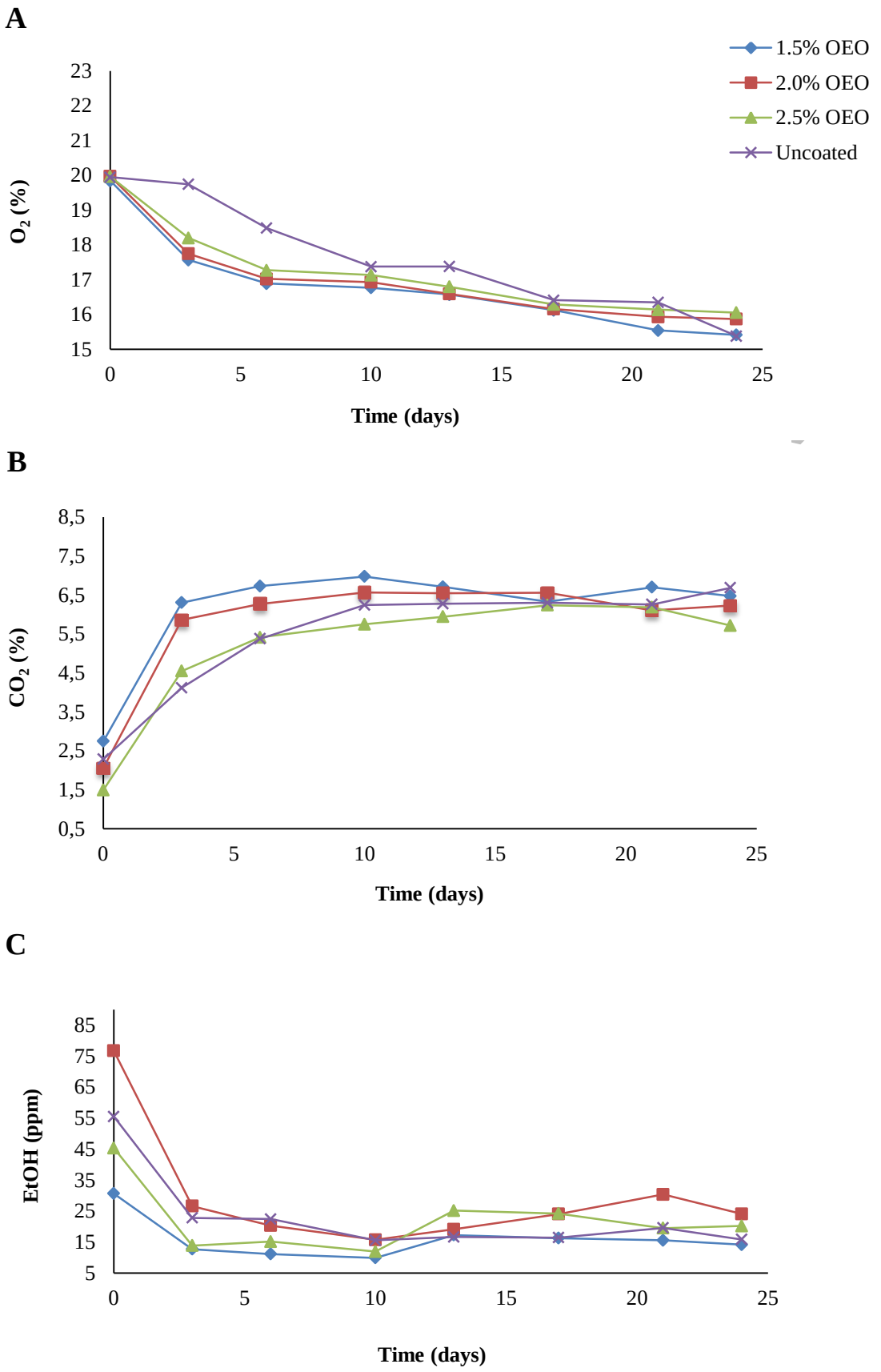
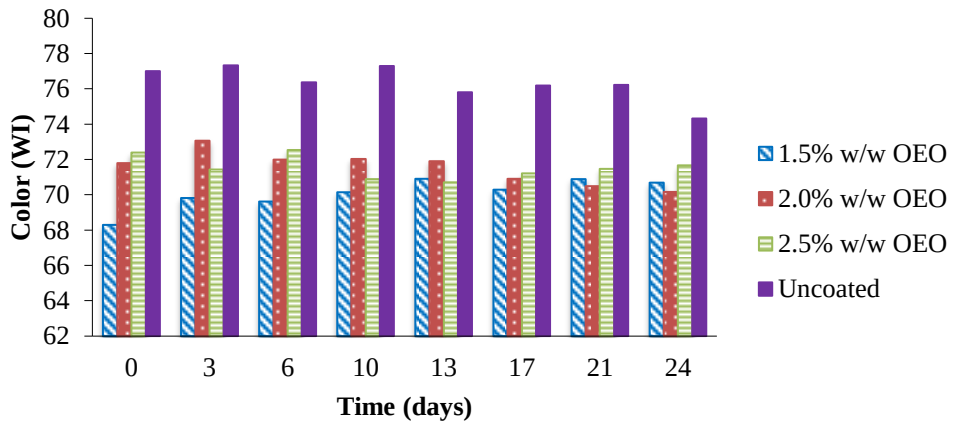


Figure 4. (A) Oxygen (O_2), (B) Carbon dioxide (CO_2) and (C) Ethanol (EtOH) headspace gas concentration of sealed trays containing uncoated or coated cheese samples during storage at 4ºC. Data shown are the means ± standard deviation.

A



B

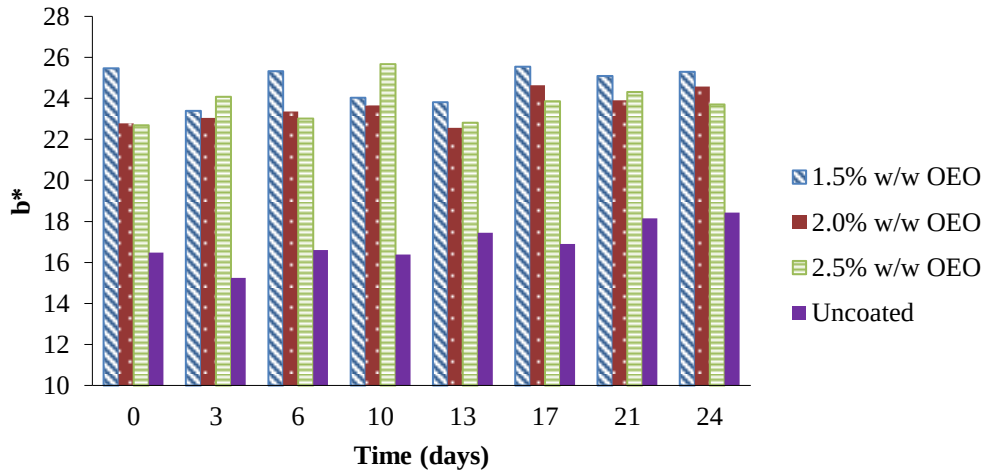


Figure 5. (A) Color changes in terms of whiteness index (WI) values of coated and uncoated cheese pieces during storage. (B)Changes in b* parameter values of coated and uncoated cheese pieces during storage. Data shown are the means ± standard deviation.

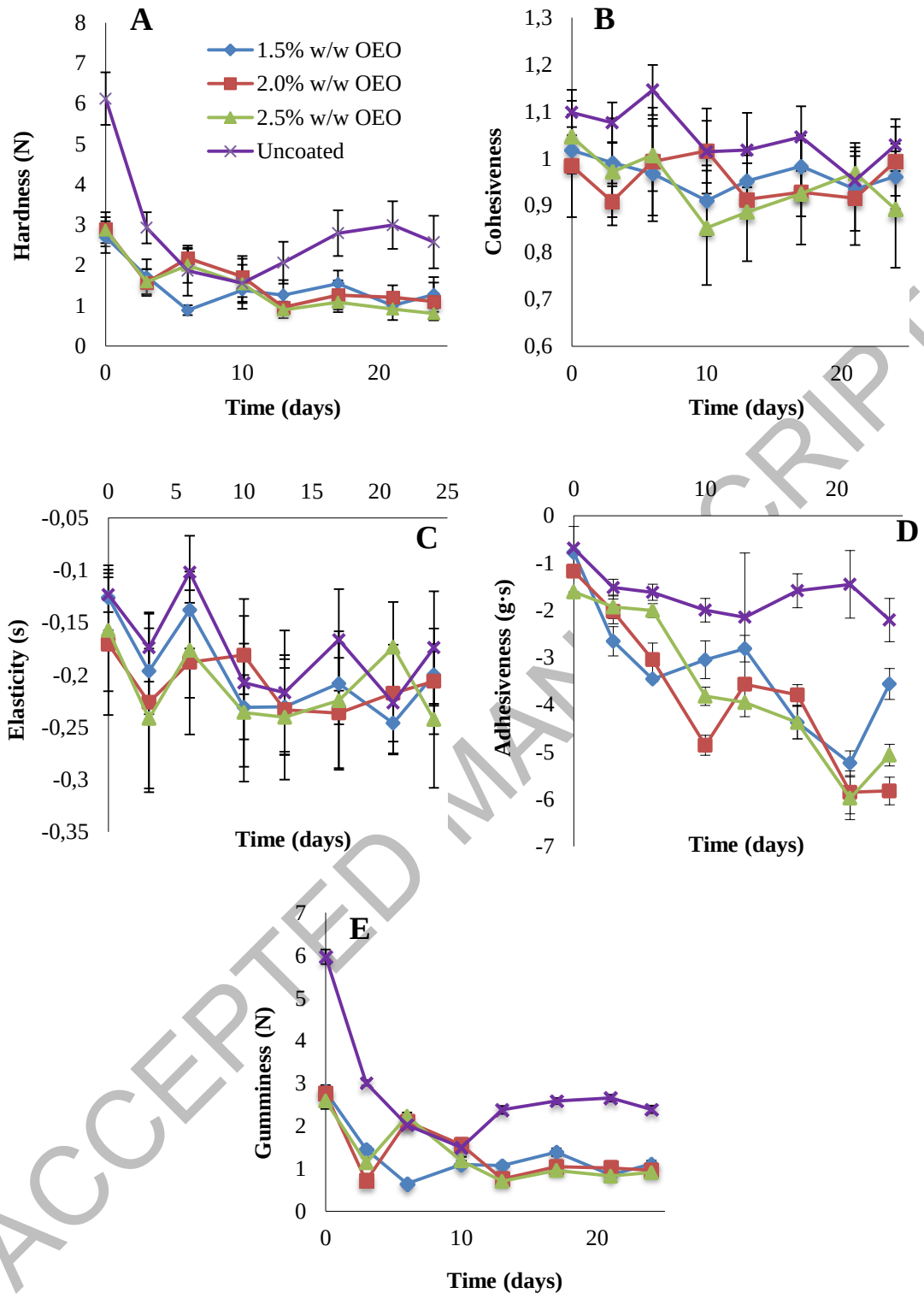


Figure 6.Texture Profile Analysis of coated and uncoated cheese pieces.(A) Hardness (N); (B) Cohesiveness (N·s/N·s); (C) Elasticity (s); (D)Adhesiveness (g·s)and (E) Gumminess (N). Data shown are the means ± standard deviation.