Influence of nanoliposomes incorporation on properties of film forming dispersions and films based on corn starch and sodium caseinate.

Alberto Jiménez*, Laura Sánchez-González², Stéphane Desobry², Amparo Chiralt¹, Elmira Arab Tehrany²

¹Instituto de Ingeniería de Alimentos para el Desarrollo. Departamento de Tecnología de Alimentos. Universitat Politècnica de València. Camino de Vera s/n. 46022. Valencia, Spain.
²Laboratoire d’Ingénierie des Biomolécules (LIBio). ENSAIA - Université de Lorraine. 2 avenue de la Forêt de Haye, TSA 40602, 54518 Vandœuvre-lès-Nancy Cedex, France.

(*) Contact information for Corresponding Author

Phone: 34-3877000 ext.83613, Fax: 34-963877369, e-mail: aljimar@upvnet.upv.es
ABSTRACT

The incorporation of potentially antimicrobial volatile compounds (orange essential oil and limonene) into soy and rapeseed nanoliposomes was carried out by encapsulating them through sonication of their aqueous dispersions. Nanoliposomes were added to starch-sodium caseinate (50:50) film forming dispersions, which were dried to obtain films without losses of the volatile compounds. Structural, mechanical and optical properties of the films were analysed, as well as their antimicrobial activity against *Listeria monocytogenes*. The addition of liposomes in the polymeric matrix supposed a decrease of the mechanical resistance and extensibility of the films. The natural colour of lecithin conferred a loss of lightness, a chroma gain and a redder hue to the films, which were also less transparent than the control one, regardless the lecithin and volatile considered. The possible antimicrobial activity of the films containing orange essential oil or limonene was not observed, which could be due to their low antilisterial activity or to the inhibition effect of the encapsulation which difficult their release from the matrix.

Keywords: Starch-sodium caseinate films, nanoliposomes, antimicrobial, encapsulation.
1. INTRODUCTION

Nowadays, it is well known that edible and biodegradable films obtained from biopolymers are able to substitute, at least partially, conventional plastics. The biodegradable plastics, after their useful life, get assimilated by microorganisms and return to the natural ecosystem without causing any pollution or harm to the environment (Maran, Sivakumar, Sridhar & Immanuel, 2013). Polysaccharides and proteins are used in film formulations, since it is possible to obtain transparent, tasteless, odorless and isotropic films by using these polymers (Chick & Ustunol, 1998; Han, 2002; Soliva-Fortuny, Rojas-Graü & Martín-Belloso, 2012). In this sense, one of the most used polysaccharide to obtain films with adequate properties is starch. This biopolymer is a renewable resource, inexpensive (compared with other compounds) and widely available (Lourdin, Della Valle & Colonna, 1995). Starch based films can be formed by using its pure components (amylose and amylopectin; Paes, Yakiments, & Mitchell, 2008), native starch (López & García, 2012), modified starches (López, García & Zaritzky, 2008) and soluble or pregelatinized starch (Pagella, Spigno & De Faveri, 2002). Nevertheless, starch films, as other polysaccharide films, are highly sensitive to moisture action. Furthermore, their mechanical behaviour can vary as a consequence of retrogradation phenomenon throughout time (Fámá, Goyanes & Gerschenson, 2007; Jiménez, Fabra, Talens, & Chiralt, 2012a).

The hydrophilic character of starch films can be modified by different techniques such as surface sterification (Zhou, Ren, Tong, Xie, & Liu, 2009), surface photocrosslinking (Zhou, Zhang, Ma, & Tong, 2008) or by adding hydrophobic compounds to film formulation (Averous, Moro, Dole, & Fringant, 2000; Fang & Fowler, 2003). On the other hand, starch retrogradation has been inhibited by mixing starch with other polymers such as hydroxypropylmethylcellulose (HPMC) or sodium caseinate (Jiménez, Fabra Talens & Chiralt, 2012b,c). Whereas starch-HPMC films showed phase separation in the film, starch-sodium caseinate films were completely homogeneous and showed good functional properties.

Biodegradable films are able to act as carriers of active compounds such as antioxidants or antimicrobials to enlarge the self life of food products where they are applied. Among these compounds, essential oils have a great relevance due to the fact that they can act as antioxidants and antimicrobials at the same time (Ruiz-Navajas, Viuda-Martos, Sendra, Perez-Alvarez, Fernández-López, 2013; Ye, Dai & Hu, 2013). In
general, essential oils are a mix of volatile (85-99 %) and non volatile compounds (1-15 %) (Sánchez-González, Vargas, González-Martínez, Cháfer & Chiralt, 2011a) in which the volatile fraction is composed by terpenes, terpenoids and other aromatic and aliphatic components with low molecular weight (Smith-Palmer, Stewart, Fyfe, 2001; Bakkali, Averbeck, Averbeck & Idaomar, 2008). Previous studies reported antimicrobial activity of films containing different essential oils such as those obtained from bergamot (Sánchez-González, Cháfer, Chiralt & González-Martínez, 2010a; Sánchez-González, Cháfer, Hernández, Chiralt & González-Martínez, 2011b), lemon (Sánchez-González, González-Martínez, Chiralt & Cháfer, 2010b; Iturria, Olabarrieta, Martínez de Marañón, 2012) or sweet and bitter orange (Iturriaga et al., 2012). However, isolate terpenes (limonene, geranyl acetate and alpha-pinene) have been found to promote the growth of *Listeria monocytogenes* in biofilms structures (Sandasi, Leonard & Viljoen, 2008), whereas the antimicrobial activity of essential oils has been attributed to the synergism between different terpenes, which would improve their activity against bacteria (Gallucci, Oliva, Casero, Dambolena, Luna, Zygadlo & Demo, 2009; Piccirillo, Demiray, Silva Ferreira, Pintado & Castro, 2013) and fungi (Edris & Farrag, 2003).

Due to its volatile nature, essential oils can evaporate from film forming dispersions during drying, thus reducing its effectiveness in dried films. The encapsulation of essential oils could be a solution to maintain their usefulness for a longer time, by a control release of the compounds. The encapsulation of a hydrophobic compound in an aqueous dispersion requires the utilization of amphiphilic substances such as lecithin. Recently Zhang et al. (Zhang, Arab Tehrany, Kahn, Ponçot, Linder & Cleymand, 2012) have obtained very stable lecithin nanoliposomes by means of sonication, in order to incorporate them in chitosan films.

The aim of this work was the development of starch-sodium caseinate films containing nanoliposomes as carriers of antimicrobial compounds (orange essential oil and D-limonene). The influence of the nanoliposomes addition with and without antimicrobials in the properties of film forming dispersions (surface tension and rheological properties) and films (mechanical, optical and antimicrobial properties) was studied.

2. MATERIALS AND METHODS
2.1. Materials

Corn starch was purchased from Roquette (Roquette Laïsa España, Benifaió, Spain) and sodium caseinate (NaCas) was supplied by Sigma (Sigma–Aldrich Chemie, Steinheim, Germany). Glycerol (99.5 % AnalR NORMAPUR), chosen as plasticizer, was provided by WVR International. To form nanoliposomes, rapeseed and soy lecithins were obtained from The Solae Company (Solae Europe, Geneva, Switzerland) and Novastell (Etrepigny, France), respectively. Furthermore, D-Limonene stabilized (purchased from Acros Organics, Geel, Belgium) and orange essential oil (supplied by Laboratoires Mathe, Maxeville, France) were chosen as antimicrobial compounds. BF3 (Boron trifluoride)/methanol (99 %) and chloroform (99.8%), used in gas chromatography, were obtained from Bellfonte-PA (USA) and Prolabo-VWR (Italy) respectively. Hexane (95%) and methanol (99.9%) were obtained from Carlo-Erba (France) meanwhile acetonitrile (99.9%) was obtained from Sigma (Sigma–Aldrich Chemie, Steinheim, Germany). These organic solvents were analytical grade reagents.

2.2. Preparation and characterization of nanoliposomes

Nanoliposomes were obtained by modifying the method of Zhang et al. (2012). 2 g of lecithin were added in 38 g of distilled water and then stirred for 5 h. After this step, the mixture was sonicated at 40 kHz and 40% power for 300 s (1 s on and 1 s off). Sonication step was carried out by using a sonicator (Vibra Cell 75115, Bioblock Scientific, Illkirch, France). In the case of formulations containing antimicrobials (2 g), these compounds were added directly to the lecithin aqueous dispersions previously to sonicate. The amount of antimicrobials (2 g) included in the formulations favoured their proper retention in the nanoliposome core, avoiding their loss by evaporation.

2.2.1. Fatty acids composition

Fatty acid esters (FAMEs) were prepared as described by Ackman (Ackman, 1998). The separation of the FAMEs was carried out on a Shimadzu 2010 gas chromatograph Perichrom (Saulx-lès-Chartreux, France), equipped with a flame-ionization detector. A fused silica capillary column was used (60 m, 0.2 mm i.d. ×0.25 µm film thicknesses, SPTM2380, Supelco, Bellefonte, PA, USA). Injector and detector temperatures were set at 250 °C. A temperature program of column initially set at 120 °C for 3 min, then rising to 180 °C at a rate of 2 °C/min and held at 220 °C for 25 min. Standard mixtures (PUFA1, from marine source, and PUFA2, from vegetable source;
Supelco, Sigma–Aldrich, Bellefonte, PA, USA) were used to identify fatty acids. The results were presented as triplicate analyses.

2.2.2. Lipid classes

The lipid classes of the different fractions were determined by Iatroscan MK-5 TLC-FID (Iatron Laboratories Inc., Tokyo, Japan). Each sample was spotted on ten Chromarod S-III silica coated quartz rods held in a frame. The rods were developed over 20 min in hexane/diethyl ether/formic acid (80:20:0.2, v:v:v), then oven dried for 1 min at 100 °C and finally scanned in the Iatroscan analyzer. The Iatroscan was operated under the following conditions: flow rate of hydrogen, 160 ml/mn; flow rate of air, 2 L/mn. A second migration using a polar eluant of chloroform, methanol, and ammoniac (65:35:5) made it possible to quantify polar lipids. The FID results were expressed as the mean value often separate samples. The following standards were used to identify the sample components:

- Neutral lipids: 1-monostearoyl-rac-glycerol, 1,2-dipalmitoyl-sn-glycerol, tripalmitin, cholesterol.
- Phospholipids: L-a-phosphatidylcholine, 3 sn-phosphatidylethanolamine, L-a-phosphatidyl-L-serine, L-a-phosphatidylinositol, lyso-phosphatidylcholine, sphingomyelin.

All standards were purchased from Sigma (Sigma–Aldrich Chemie, Steinheim, Germany). The recording and integration of the peaks were provided by the ChromStar internal software.

2.2.3. Nanoliposomes size measurement

Size of nanoliposomes was determined by using a Malvern Zetasizer Nano ZS (Malvern Instruments, Worcestershire, U.K.) considering the method of Zhang et al. (2012). Samples were diluted in distilled water (1:100) and measured at 25 °C. At least five replicates were considered for each formulation.

2.2.4. Electrophoretic mobility

Electrophoretic mobility of nanoliposomes was measured in the aqueous dispersion by means of a Malvern Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK) at 25 °C. Dispersions were diluted to a particle concentration of 0.01 % using deionised water.
2.2.3. Surface tension

Surface tension of nanoliposomes aqueous dispersions (and film forming dispersions) was measured by using a Krüss K100 tensiometer (Krüss GmbH; Hamburg, Germany) equipped with a platinum plate. All measures were taken in triplicate at 25 °C.

2.3. Preparation and characterization of film forming dispersions

Seven different film forming dispersions based on corn starch, sodium caseinate and glycerol as plasticizer were prepared. Corn starch was dispersed in cool water to obtain 2 % (w/w) polysaccharide dispersions. These were maintained, under stirring, at 95 °C for 30 min to induce starch gelatinization. Sodium caseinate was dissolved directly in cool distilled water (2 % w/w). Afterwards, both hydrocolloids were mixed to obtain dispersions with a starch:protein ratio of 1:1. This ratio was used on the basis of a previous study carried out by Jiménez et al. (2012c) who observed no starch crystallization in this mixture. After this step, a controlled amount of glycerol was added (hydrocolloid:glycerol ratio was 1:0.25). In the case of dispersions containing nanoliposomes, 10 g of nanoliposome solution were added to 90 g of hydrocolloid dispersions. Then the mixtures were maintained 1 hour under stirring at 300 rpm to disperse nanoliposomes.

2.3.1. Rheological behaviour

The rheological behaviour of the film forming dispersions was analyzed in triplicate at 25 °C by means of a rheometer (Malvern Kinexus, Malvern Instruments, Worcestershire, U.K.) with a coaxial cylinder sensor. Flow curves were obtained after resting the sample in the sensor for 5 min at 25 °C. The shear stress (σ) was measured as a function of shear rate (γ) from 0 to 1000 s⁻¹ and up and down curves were obtained. When samples showed non-Newtonian behaviour, the power law model was applied to determine the consistency index (k) and the flow behaviour index (n).

2.4. Preparation and characterization of films

Films were obtained by casting. Film forming dispersions were gently poured (88.84 g of solids/m²) over PET Petri dishes (85 or 140 mm diameter) resting on a leveled surface. The dispersions were allowed to dry for approximately 48 h at 45 %
RH and 20 ºC. Dry films could be peeled intact from the casting surface. Seven kinds of films were prepared: without nanoliposomes (control), with lecithin nanoliposomes (Rap or Soy), with limonene-lecithin nanoliposomes (Rap-lim or Soy-lim) and with essential oil-lecithin nanoliposomes (Rap-oil or Soy-oil).

2.4.1. Film conditioning

Before tests, all samples were conditioned in a desiccator at 25 ºC and 53 % RH, by using magnesium nitrate-6-hydrate saturated solutions (Sigma–Aldrich Chemie, Steinheim, Germany) for one week, when the analyses were carried out.

2.4.2. Mechanical properties

A Lloyd instruments universal testing machine (AMETEK, LRX, U.K.) was used to determine the tensile strength (TS), elastic modulus (EM), and elongation (E) of the films, according to ASTM standard method D882 (2001). EM, TS, and E were determined from the stress-Hencky strain curves, estimated from force-distance data obtained for the different films (2.5 cm wide and 10 cm long). At least four replicates were obtained for each formulation. Equilibrated film specimens were mounted in the film-extending grips of the testing machine and stretched at a deformation rate of 50 mm/min until breaking. The relative humidity of the environment was held constant at 53 % during the tests, which were performed at 25 ºC.

Measurements of film thickness were carried out by using an electronic digital micrometer (0–25 mm, 1 µm).

2.4.3. FTIR analysis of films

Fourier transform infrared spectroscopy was used to study the presence of interactions between components in conditioned films in total attenuated reflection mode (ATR-FTIR). Measurements were carried out at 25 ºC by using a Tensor 27 mid-FTIR Bruker spectrometer (Bruker, Karlsruhe, Germany) equipped with a Platinum ATR optical cell and an RT-Dla TGS detector (Bruker, Karlsruhe, Germany). The diaphragm during analysis was set at 4 mm whereas the scanning rate was 10 kHz. For the reference (air) and each formulation 154 scans were considered from 4000 to 800 cm⁻¹, with a resolution of 4 cm⁻¹.

After measurements, data were treated by using OPUS software (Bruker, Karlsruhe, Germany). Initial absorbance spectra were smoothed using a nine-points
Savitsky-Golay algorithm as well as elastic baseline correction (200 points) was applied to spectra. These were then centered and normalized using the mentioned software.

2.4.4. Optical Properties

To evaluate the films transparency, the Kubelka-Munk theory was considered for multiple scattering to the reflection spectra (Hutchings, 1999). When the light passes through the film, it is partially absorbed and scattered, which is quantified by the absorption (K) and the scattering (S) coefficients. Internal transmittance (Ti) of the films was quantified using Equation 1. In this equation $R_0$ is the reflectance of the film on an ideal black background. $a$ and $b$ parameters are calculated by Equations 2 and 3 where $R$ is the reflectance of the sample layer backed by a known reflectance ($R_g$). The surface reflectance spectra of the films were determined from 400 to 700 nm with a spectrocolorimeter CM-5 (Konica Minolta Co., Tokyo, Japan) on both a white and a black background. All measurements were performed at least in triplicate for each sample on the free film surface during its drying.

$$ T_i = \sqrt{(a - R_0)^2 - b^2} \quad \text{Equation 1} $$

$$ a = \frac{1}{2}\left(1 + \frac{R_0 - R + R_g}{R_g R}ight) \quad \text{Equation 2} $$

$$ b = (a^2 - 1)^{1/2} \quad \text{Equation 3} $$

Colour coordinates of the films, $L^*$, $C_{ab}^*$ (Equation 4) and $h_{ab}^*$ (Equation 5) from the CIELAB colour space were determined, using D65 illuminant and 10° observer and taking into account $R_\infty$ (Equation 6) which correspond with the reflectance of an infinitely thick layer of the material.

$$ C_{ab}^* = \sqrt{a^{*2} + b^{*2}} \quad \text{Equation 4} $$

$$ h_{ab}^* = \arctg\left(\frac{b^*}{a^*}\right) \quad \text{Equation 5} $$
Finally, to evaluate the colour differences between the different films and control film, Equation 7 was used.

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

**2.4.5. Scanning Electron Microscopy (SEM)**

Microstructural analysis of the films was carried out by SEM using a scanning electron microscope (Hitachi S-4800, Japan). Film samples were maintained in a desiccator with P$_2$O$_5$ for two weeks to ensure that no water was present in the sample. Then, films were frozen in liquid N$_2$ and cryofractured with a pre-chilled razor to observe the cross-section of the samples. Fractured film pieces were then mounted on a SEM tube and observed using an accelerating voltage of 10 kV.

**2.4.6. Microbiological analysis**

To perform the microbiological analysis, a modification of the method proposed by Kristo, Koutsoumanis & Biliaderis (2008) was considered. *Listeria monocytogenes* (CIP 82110), supplied by the Collection Institut Pasteur (CIP, France), was regenerated (from a culture stored at -80 ºC) by transferring a loopful of bacteria into 10 ml of Tryptone Soy Broth-Yeast Extract, (TSB-YE, Biokar Diagnostics, Beauvais, France) and incubating at 37 ºC overnight. Subsequently, a 10 µl aliquot from the overnight culture was then transferred to 10 ml of TSB-YE and grown at 37 ºC until the end of the exponential phase of growth. This culture, appropriately diluted, was then used for inoculation of the agar plates in order to obtain a target inoculum of approximately $10^3$ CFU/cm$^2$. Tryptone soy agar (TSA, Biokar Diagnostics, Beauvais, France) was used as a model solid food system. Aliquots of TSA (20 g) were poured into Petri dishes. After the culture medium solidified, diluted overnight culture was inoculated on the surface. The different test films of the same diameter as the Petri dishes (containing or not nanoliposomes) were placed on the inoculated surface. Inoculated and uncoated TSA Petri dishes were used as control. Petri dishes were then covered with parafilm to avoid dehydration and stored for 7 days at 10 ºC.
Microbial counts on Palcam agar base (Biokar Diagnostics, Beauvais, France) plates were examined immediately after the inoculation and after 1, 4 and 7 days of storage. To this end, the agar was removed aseptically from Petri dishes and placed in a sterile plastic bag with 100 ml of Tryptone salt broth (Biokar Diagnostics, Beauvais, France). The bag was then homogenized for 150 s in a Stomacher blender 400 (Interscience, Saint-Nom-La-Breteche, France). Serial dilutions were made and poured onto Palcam agar base. The dishes were incubated during 24 h at 37 °C before colonies were counted. All tests were performed in duplicate.

2.5. Statistical Analysis
Statgraphics Plus for Windows 5.1 (Manugistics Corp., Rockville, MD) was used for carrying out statistical analyses of data through analysis of variance (ANOVA). Fisher's least significant difference (LSD) was used at the 95% confidence level.

3. RESULTS AND DISCUSSION

3.1. Characteristics of nanoliposomes
3.1.1. Fatty acids analyses
The main fatty acid composition is shown in Table 1. The percentage of total polyunsaturated fatty acids was the highest in soy lecithin. The high proportions of fatty acids were C18:2 n-6 (52.27 %), found in the polyunsaturated fatty acids class, C18:1 n-9 (21.49 %) in the monounsaturated fatty acids class and C16:0 (17.07 %) in the saturated fatty acids class for soy lecithin. The largest amount of fatty acid was a monounsaturated fatty acid, in regards to rapeseed lecithin, the percentage of C18:3n-3 (6.60 %) was important in the polyunsaturated fatty acids class. The fatty acid most present was C18:1n-9 (56.51 %) found in the monounsaturated fatty acids class.

3.1.2. Lipid classes
The lipid classes of lecithins were separated by thin-layer chromatography (Iatroscan). Moreover, the percentage of triacylglycerols (TAG) contained in lecithins were respectively 37.75±0.1 and 18.15±0.2% for rapeseed and soy lecithins. However, the percentage of polar fraction showed that soy lecithin was richer in polar lipids with 84.76 ±0.6% which was 62.26±0.8% for rapeseed lecithin.
3.1.3. Size of nanoliposomes

The size analysis of nanoliposomes is of interest because of its impact on different properties of the films and its stability and capacity to release the entrapped compounds in the liposome core. Different authors (Pérez-Gago & Krochta, 2001; Bravin, Peressini & Sensidoni, 2004) have related the particle size of lipids in the film forming emulsions with different properties of the films such as mechanical or barrier properties. Low particle size is generally desired since small particles increase the tortuosity of the structure thus improving the barrier capacity and provides a more homogeneous structure. The particle size of nanoparticles (or nanoliposomes) has to be controlled since they can be toxic for humans and for the environment. In this sense, different authors estimated the toxicity of different nanoparticles such as silver nanoparticles (Lankveld, Oomen, Krystek, Neigh, Troost-de Jong, Noorlander, Van Eijkeren, Geertsma, De Jong, 2010), TiO$_2$ nanoparticles (Clément, Hurel, Marmier, 2013) or ZnO nanoparticles (Hsiao & Huang, 2011). Unfortunately, there are no works concerning the toxicity of active compounds loaded-nanoliposomes.

The mean particle diameter of rapeseed and soy nanoliposomes without and with antimicrobial compounds is showed in Table 2. The obtained values are in the same order as those found by Zhang et al. (2012) and differences may be related with the different time of sonication. They obtained stable nanoliposomes by using 180 s of sonication whereas 300 s of sonication were necessary to incorporate completely the antimicrobials into the nanoliposomes in this case.

Size of rapeseed nanoliposomes ranged between 146 and 150 nm without significant differences between them. However, soy nanoliposomes showed different sizes depending on the core compounds. Antimicrobial loaded-nanoliposomes showed lower sizes. The addition of hydrophobic compounds seemed to favour the compactness of soy nanoliposomes by improving the orientation of amphiphilic molecules of soy lecithin through the interactions with the oil compounds. Previous studies (Zhang et al., 2012) showed the formation of vesicles for the major part of lecithin molecules (soy and rapeseed) with some remanent droplets, when applying sonication in water dispersion in similar conditions. So, the formation of vesicles can be expected in this case, although the incorporation of the essential oil or limonene could imply the formation of a different structure due to the change in the balance of the interaction forces in the mixture. Spherical micelles could entrap in their core the incorporated non-polar compounds and a reduction in their size can occur.
3.1.4. Electrophoretic mobility

The electrophoretic mobility values of soy and rapeseed nanoliposomes containing solutions are shown in Table 2. The study of the surface charge of the particles is of interest since it affects the stability of the nanoliposomes, specially in the studied solutions in which the viscosity is too low. The electrophoretic mobility of nanoliposomes containing solutions ranged between -3.21 and -3.36 $\mu$m·cm·V$^{-1}$·s$^{-1}$ for rapeseed nanoliposomes and between -3.89 and 3.99 $\mu$m·cm·V$^{-1}$·s$^{-1}$ for soy nanoliposomes. These values are in agreement with values reported by Zhang et al. (2012). According with obtained results and those found by Arab Tehrany, Kahn, Baravian, Maherani, Belhaj, Wang & Linder (2012), rapeseed and soy lecithins contain different type of phospholipids such as phosphatidylserine, phosphatidic acid, phosphatidylglycerol, phosphatidylinositol, phosphatidylcholine and phosphatidylethanolamine. These components are negatively charged at neutral pH (except phosphatidylcholine which is not charged) thus being responsible of the negative electrophoretic mobility of liposomes (Chansiri, Lyons, Patel & Hem, 1999). The incorporation of the essential oil and limonene slightly increase the particle charge which agrees with the induced changes in the micellar structure.

3.1.5. Surface tension of nanoliposomes dispersions

Lecithins were choosen since its amphiphilic nature allows to incorporate the hidrophobic antimicrobials into the hydrophilic starch-sodium caseinate dispersions. Due to its low molecular weight, these surfactants migrate rapidly to the small terpen droplets that are formed during sonication thus preventing coalescence and flocculation (McSweeney, Healy & Mulvihill, 2008). Surface tension values of rapeseed and soy nanoliposomes containing solutions are showed in Table 2. As expected, the surface tension of nanoliposomes solutions was lower in comparison with pure water whose surface tension is 72 mN·m$^{-1}$ (Walstra, 2003). The obtained values demonstrate the ability of lecithins to form stable nanoliposomes with and without antimicrobials in the aqueous media. Differences in the surface tension values of the dispersions of rapeseed and soy nanoliposomes were found. The surface tension of rapeseed nanoliposomes was lower than for soy nanoliposomes containing solution. This fact can be associated to the total content of polyunsaturated fatty acids (PUFA) with different surface activity. In this sense, as showed in Table 1, the content of PUFA was higher in soy lecithin than in
rapeseed lecithin. Leshem, Landau & Deutsch (1988) related the presence of unsaturations with an important effect on the surface tension. They explained that for a fixed surfactant monolayer area in a completely expanded state, an increase in the number of cis-double bonds cause an increase in the surface tension, in agreement with results found in this work.

The inclusion of orange essential oil and D-limonene did not produce any difference in surface tension for rapeseed nanoliposomes. However, the addition of these compounds significantly reduced the surface tension of soy nanoliposomes containing solutions. This indicates that the addition of these compounds affects the critical micellar concentration and the corresponding minimal surface tension of the soy lecithin. The lower value of the surface tension of rapeseed lecithin with respect to the soy lecithin could be explained, as commented on above, by its lower concentration of PUFA.

3.2. Characterization of the film forming dispersions

3.2.1. Surface tension

Table 3 shows the values of the surface tension of all film forming dispersions under study. The presence of the protein in combination with starch remarkably reduced the surface tension of water (72 mN·m\(^{-1}\) at 25 °C; Walstra, 2003) to 51.1 mN·m\(^{-1}\) as it can be observed for control formulation. This effect is due to the amphiphilic nature of caseinate and is in agreement with the results found by Fabra, Jiménez, Atarés, Talens & Chiralt (2009). The analysis of the surface tension of film forming dispersions are of interest, specially in the food industry, since low values of surface tension would favour the coating of products (Fernández, Díaz de Apodaca, Cebrían, Villarán & Maté, 2006). In the case of film forming dispersions containing nanoliposomes, the surface tension was always lower as compared with the control sample as it was expected by the action of surfactants. Nevertheless, the values did not reach those obtained in the aqueous nanoliposome dispersions, which indicates that protein is present to a great extent in the water-air interface and no total substitution of this occurred when nanoliposomes were added. No notable differences were found between the different formulations with and without antimicrobials, except for the film forming dispersion with soy-orange oil nanoliposomes where the lowest surface tension was obtained. The greater migration of surfactant to the sample surface seems to occur, thus decreasing the surface tension to a greater extent. This fact could be related with a lower stability of liposomes in this case.
3.2.2. Rheological behaviour

The study of the rheological behaviour of film forming dispersions is of interest to have information about the fluid structure and interactions between particles during flow. The analyses of the rheological behaviour of the film forming dispersions were carried out at 25 ºC with a shear rate between 0 and 1000 s\(^{-1}\). All formulations showed newtonian behaviour at low shear rates and a shear thickening or dilatant character from a determined shear rate (see Figure 1). Furthermore, all samples showed non-time dependent behaviour since up and down curves coincided. The change in the rheological behaviour as the shear rate increases can be related with particles (starch and sodium caseinate chains and vesicles) aggregation due to orthokinetic flocculation (Peker & Helvaci, 2007). The aggregates would present sufficient cohesive forces to withstand the shear stress, thus producing shear thickening behaviour (Christianson & Bagley, 1983). The water content entrapped in these aggregates would increase, leading to a greater flow resistance.

The viscosity of studied film forming dispersions in the newtonian domain are showed in Table 3. The obtained viscosities are low, in agreement with the polymer concentrations used, thus indicating that no gels were formed during the film forming dispersions preparation. Although starch-sodium caseinate interactions can take place in determined conditions (Jiménez et al., 2012c), in this case these not lead to a gel formation. No significant differences were found among the Newtonian viscosity values of the different formulations, despite the different total solid contents. In this sense, it is remarkable that the composition of continuous phase is the same in all cases and the volume concentration of the nanoliposomes is relatively low to affect notably the sample viscosity.

The change from newtonian to shear thickening behaviour took place at a shear rate ranging between 238-291 s\(^{-1}\), regardless the type of sample. From these shear rate values, the experimental data were fitted to the Ostwal-de-Waele model (power law). The flow behaviour index (\(n\)) and the consistency index (\(k\)) of film forming dispersions are shown in Table 3. No significant differences in \(n\) values, were found for the different samples; these values being higher than 1, as corresponds to dilatant fluids and the consistency index was also similar for all formulations.
In conclusion, small differences were found between the different film forming dispersions concerning their rheological behaviour and only a decrease of their surface tension was observed for those containing lipids due to the surfactant action of lecithin.

3.3. Characterization of the films

3.3.1. Structural and mechanical properties

Despite the small size of liposomes obtained in aqueous dispersion, when they are incorporated into the film forming dispersions, the changes in the aqueous environment and the establishment of interactions, mainly between surfactants and proteins (Erickson, 1990), can promote significant changes in the structure of lipid particles. In fact the mean size of particles increased when liposomes were incorporated in the film forming dispersions and it could not be measured with the available equipment because they were out the measure range.

A positive aspect of the essential oil incorporation as nanoliposomes was the inhibition of the oil evaporation during the film drying step, which supposes a decrease of the film thickness by the loss of solids, when a constant of solids per surface area was poured in the plate to obtain de film. This has been previously observed in previous works (Sánchez-González et al. 2010ab) and supposes the loss of potentially active compounds of the film. Table 4 shows the values of the film’s thickness, where the increase of this parameter when nanoliposomes were incorporated can be observed, on the contrary that occurs when free essential oil was incorporated in the film. This increase confirms that, not only essential oil was not evaporated but also that the arrangement of the polymer chains with lipids is more open probably due to a different coupling of the components on the basis of the developed interactions.

Figure 2 shows the SEM micrographs of the cross section of control film and those containing liposomes. Control film showed a quite homogeneous structure, but coarser than that obtained by Jiménez et al. (2012c) for films with the same composition. In this work, the authors prepared the film forming dispersions by applying a homogenization step, using a rotor-stator equipment, at 95°C. The high temperature and the shear stress promoted denaturation of the protein and the interaction between polymers, which favours the formation of a more homogenous blend.

When control samples are compared with those containing lipids, a much coarser structure is observed for the latter, which agrees with the increase of size of nanoliposomes when incorporated to the film forming dispersions (data not showed).
and the possibly progress of this increase during the film drying step. During this step, as the water of the system is being removed phase transitions occurs in the lipid association structures which may promote the break of liposomes and the re-restructuration of the molecule association, even giving rise to inverted structures (Krog, 1990; Larsson, K., & Dejmek, 1990). In fact voids of different sizes can be observed in the matrix, which can be associated to the presence of the lipid droplets interrupting the matrix continuity in a size higher than nano-scale.

From the analysis of the stress-Hencky strain curves, elasticity modulus (EM), tensile strength (TS) and elongation at break (E) were determined for each film sample. According to McHugh & Krochta (1994), these parameters are very useful for describing the mechanical properties of a film, and are closely related with its internal structure. Table 4 shows the obtained values for each sample. Film without liposomes presented the highest EM value, in comparison with nanoliposome containing films. The addition of nanoliposomes introduces discontinuities in the matrix, as commented on above, which affects significantly the mechanical resistance of films. The same behaviour is observed for TS values. Considering the EM and TS values of control film it is remarkable that there is a great difference between these values and those obtained obtained by Jiménez et al. (2012c) using the same formulation starch-sodium caseinate, but by applying a heat-homogenization step before the film casting. This could provoke a reduction the mechanical resistance and extensibility of the obtained structure.

Among films containing nanoliposomes, for rapeseed liposomes, elastic modulus significantly decreased when essential oil or limonene are present in the film which could be due to a different release of this compounds in the matrix from liposomes.

As concerns extensibility of the films (E), these can be considered few extensible, in comparison with other films in which starch were blended with other polymers (Phan The, Debeaufort, Voilley & Luu, 2009; Jiménez et al., 2012bc). This low extensibility can be related with the kind of structure generated where the slippage of the chains during the film stretching is more difficult. When heat-homogenization is was applied to starch-sodium caseinate films with the same composition, extensibility is almost 2.5 times higher probably due to the heat induced unfolding of proteins and the more linear entanglement of the chains in the matrix. In this work the heat-homogenization step was not applied to avoid the rupture of nanoliposomes, thus losing the active compounds.

3.3.2. Optical properties
Spectral distribution curves of Ti parameters are plotted in Figure 3. In general, high values of Ti are associated with greater film homogeneity, which gives rise to more transparent films. On the contrary, lower values of Ti are related with a higher opacity of the films. As observed in Figure 3, control film was the most transparent with high values of Ti, in agreement with that reported in previous works for starch and sodium caseinate (Fabra et al., 2009; Jiménez et al., 2012a). The addition of nanoliposomes decreased the transparency of films regardless the type of lecithin and the antimicrobial mainly at low wavelength. This fact is due, in part, to the natural brown colour of lecithins. This produces the absorption of the blue and green light (low wavelength) thus giving rise to a yellow-brown colour in the films. The presence of a dispersed phase in the matrix also contributes to the decrease in the Ti values.

The colour of films as a consequence of nanoliposomes addition, is shown in Table 4, in terms of lightness (L*), chroma (C*ab) and hue (h*ab) parameters, for each formulation. L* and C*ab values for control film varied significantly by the incorporation of nanoliposomes. Incorporation of nanoliposomes provoked a decrease the lightness and the hue and an increase of chroma, due to the colour of lecithins. The film colour become more vivid a redder by the action of lecithin liposomes. To estimate colour differences, ∆E were calculated between control film and the films containing nanoliposomes. These values ranged between 25 and 29, thus indicating that there is a relevant difference of colour between films. Nevertheless no notable differences were found among in films containing nanoliposomes since there are no significant differences in their colour parameters.

3.3.3. Fourier transform infrared spectroscopy

Figure 4A shows the FTIR spectra of the films without and with nanoliposomes and Figure 4B the corresponding spectra of each one of film components, in order to compare the main characteristics peaks of the different components and films. The broad band located at 3300 cm⁻¹ corresponds with vibration modes of OH-groups from the absorbed water (García, Fámá, Dufresne, Aranguren & Goyanes, 2009) and from the polymers themselves (Bourtoom & Chinnan, 2008; Pereda, Amica, Rácz & Marcovich, 2011). The peaks located at 2854 and 2923 cm⁻¹ are related with vibration of –CH₂ and –CH₃ groups (axial carbon-hydrogen bond) (Zhang et al., 2012). The band at 1690-1590 cm⁻¹ corresponds to the amide I vibrations, which is common to proteins (Pereda, Aranguren & Marcovich, 2008; Pereda et al., 2011), as can be seen in Figure 4B for
sodium caseinate. Other main peak observed in Figure 4A, which has been associated with C-O stretching vibrations (Zhang et al., 2012), appeared at 1022 cm\(^{-1}\).

FTIR spectra of the films were very similar, as correspond to their similar composition. Nevertheless, some differences can be drawn. The main difference is the higher intensity of the peak at 1022 cm\(^{-1}\) in films containing nanoliposomes in comparison with the control. As can be observed, in Figure 4B, lecithins presented a broad band around this wavenumber, thus explaining the greater intensity observed in Figure 4A for films containing nanoliposomes. The intensity of peaks at 2854 and 2923 cm\(^{-1}\) also increased with nanoliposomes addition, in agreement with spectra observed in Figure 4B for lecithins and antimicrobial compounds. For films with nanoliposomes, Figure 4A also shows a little peak at 1746 cm\(^{-1}\) which correspond to the C=O stretching (Tantipolphan, Rades, McQuillan & Medlicott, 2007). This group is located between hydrophobic tails and hydrophilic head group of the lecithin molecule. Nevertheless, no displacement of peaks in the film spectra with respect to the characteristic wavelength found for each isolated compound was observed, which indicates that no specific interactions among components can be detected from FTIR spectra.

3.3.4. Antimicrobial activity against *Listeria monocytogenes*

Figures 5A and 5B show the growth curves of *Listeria monocytogenes* on TSA medium without film and on those coated with the different films. Bacteria population increases from 3 to 8 logs CFU/cm\(^2\) at the end of the storage period. The slightly greater microbial growth in plates coated with the different films than in uncoated one can be observed in Figures 5A and B. This indicates that they did not have antimicrobial activity, as expected for starch-NaCas film (control), while contribute to the bacteria nutrients as a consequence of its composition (protein and starch). The incorporation of nanoliposomes did not improve the antimicrobial capacity of films, regardless the type of lecithin and the potentially antimicrobial compound. Only orange oil-soy lecithin nanoliposomes containing film seemed to present a little activity at the end of the storage (day 7). In this case, some more days of analysis would be necessary to evaluate if there is a significant antimicrobial activity. These results could be attributed to the encapsulation of the active compounds in liposomes, which inhibit their release to the plate surface and to a low antimicrobial activity of limonene and orange essential oil. Similar results were reported by Imran (Imran, 2011) since he found that the release of bioactive compounds entrapped onto nanoliposomes is a relatively long process.
Moreover this study revealed that nanoliposome composition is an important factor to take into account to control the release of active compounds. In addition, Imran, Revol-Junelles, René, Jamshidian, Akhtar, Arab-Tehrany, Jacquout & Desobry (2012) observed that the highest antilisterial activity corresponded with films containing both free and encapsulated antimicrobial compound. This result demonstrates that at initial time it is necessary a little amount of free bioactive compound to avoid microbial growth until it was released from the nanoliposomes. Concerning differences observed between antilisterial activity of films with limonene and orange oil nanoliposomes, previous studies reported also a greater effectiveness of the essential oils in terms of antimicrobial activity than the mix of the major components or pure terpens (Gill et al. 2002; Mourey & Canillac 2002). Minor components therefore play an important role, and synergism phenomena occur.

4. CONCLUSIONS

The incorporation of potentially antimicrobial volatile compounds (orange essential oil and limonene) to starch-sodium caseinate blend films was carried out in a effective way to avoid the losses of volatile compounds during the film drying step. Nanoliposomes of soy and rapeseed lecithins were obtained by sonication of their water dispersions. Incorporation of the essential oil and limonene to the liposomes was also effective by using the same method. The addition of lipids in the polymeric matrix supposed a decrease of the mechanical resistance and extensibility of the films. The natural colour of lecithin conferred a loss of lightness, a chroma gain and a redder hue to the films, which were also less transparent than the control one, regardless the lecithin and volatile lipid considered. The possible antimicrobial activity of the films containing orange essential oil or limonene was not observed, which could be due to their low antilisterial activity or to the inhibition effect of the encapsulation which difficult their release from the matrix.

REFERENCES


Table 1. Main fatty acids composition of rapeseed and soy lecithins.

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Rapeseed lecithin</th>
<th>Soy lecithin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>SD</td>
</tr>
<tr>
<td>C14</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C16</td>
<td>7.41</td>
<td>0.01</td>
</tr>
<tr>
<td>C17</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C18</td>
<td>1.31</td>
<td>0.00</td>
</tr>
<tr>
<td>C20</td>
<td>0.36</td>
<td>0.01</td>
</tr>
<tr>
<td>C21</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C22</td>
<td>0.21</td>
<td>0.02</td>
</tr>
<tr>
<td>C23</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SFA</td>
<td>9.29</td>
<td>20.82</td>
</tr>
<tr>
<td>C15:1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C16:1</td>
<td>0.33</td>
<td>0.01</td>
</tr>
<tr>
<td>C17:1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C18:1n9</td>
<td>56.51</td>
<td>0.04</td>
</tr>
<tr>
<td>C20:1n11</td>
<td>0.72</td>
<td>0.04</td>
</tr>
<tr>
<td>C22:1n9</td>
<td>0.25</td>
<td>0.03</td>
</tr>
<tr>
<td>MUFA</td>
<td>57.81</td>
<td>21.49</td>
</tr>
<tr>
<td>C18:2n6</td>
<td>26.32</td>
<td>0.04</td>
</tr>
<tr>
<td>C18:2n3</td>
<td>6.60</td>
<td>0.01</td>
</tr>
<tr>
<td>C20:2n6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C20:3n6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C20:3n3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C20:4n6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C20:5n3 (EPA)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C22:4n6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C22:5n3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C22:6n3 (DHA)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PUFA</td>
<td>32.92</td>
<td>57.68</td>
</tr>
</tbody>
</table>
Table 2. Particle size, electrophoretic mobility and surface tension of aqueous nanoliposomes solutions.

<table>
<thead>
<tr>
<th></th>
<th>Particle size (nm)</th>
<th>μE (µm·cm/V·s)</th>
<th>ST (mN·m⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rap</td>
<td>146 (1)ᵃ</td>
<td>-3.21 (0.01)ᵃ</td>
<td>26.8 (0.5)ᵃ</td>
</tr>
<tr>
<td>Rap-lim</td>
<td>150 (3)ᵃ</td>
<td>-3.36 (0.03)ᵇ</td>
<td>27.7 (0.2)ᵇ</td>
</tr>
<tr>
<td>Rap-oil</td>
<td>148 (2)ᵃ</td>
<td>-3.31 (0.02)ᵇ</td>
<td>27.9 (0.4)ᵇ</td>
</tr>
<tr>
<td>Soy</td>
<td>188 (5)¹</td>
<td>-3.89 (0.07)¹</td>
<td>31.6 (0.6)¹</td>
</tr>
<tr>
<td>Soy-lim</td>
<td>175 (5)²</td>
<td>-3.98 (0.03)¹²</td>
<td>28.6 (0.7)²</td>
</tr>
<tr>
<td>Soy-oil</td>
<td>159 (5)³</td>
<td>-3.99 (0.02)²</td>
<td>28.0 (1.0)²</td>
</tr>
</tbody>
</table>

ᵃ-c: Different superscripts within the same column indicate significant differences among formulations containing rapeseed nanoliposomes (p < 0.05).

¹-³: Different superscripts within the same column indicate significant differences among formulations containing soy nanoliposomes (p < 0.05).
Table 3. Surface tension and rheological properties of film forming dispersions.

<table>
<thead>
<tr>
<th></th>
<th>Newtonian viscosity ($\cdot 10^3$, Pa·s)</th>
<th>n</th>
<th>K ($\cdot 10^3$, Pa·s$^n$)</th>
<th>ST (mN·m$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.7 (0.1)$^{ab1}$</td>
<td>1.27 (0.01)$^{a1}$</td>
<td>1.47 (0.07)$^{a1}$</td>
<td>51.1 (0.1)$^{a1}$</td>
</tr>
<tr>
<td>Rap</td>
<td>5.8 (0.2)$^a$</td>
<td>1.24 (0.01)$^b$</td>
<td>1.75 (0.14)$^b$</td>
<td>44.9 (0.7)$^b$</td>
</tr>
<tr>
<td>Rap-lim</td>
<td>5.4 (0.2)$^b$</td>
<td>1.25 (0.01)$^{ab}$</td>
<td>1.5 (0.1)$^a$</td>
<td>43.8 (1.7)$^b$</td>
</tr>
<tr>
<td>Rap-oil</td>
<td>5.7 (0.1)$^{ab}$</td>
<td>1.24 (0.01)$^b$</td>
<td>1.75 (0.06)$^b$</td>
<td>43.6 (1.4)$^b$</td>
</tr>
<tr>
<td>Soy</td>
<td>5.62 (0.04)$^{12}$</td>
<td>1.26 (0.01)$^l$</td>
<td>1.52 (0.07)$^l$</td>
<td>44.6 (1.2)$^2$</td>
</tr>
<tr>
<td>Soy-lim</td>
<td>5.5 (0.1)$^{12}$</td>
<td>1.25 (0.01)$^l$</td>
<td>1.5 (0.1)$^l$</td>
<td>46 (1)$^2$</td>
</tr>
<tr>
<td>Soy-oil</td>
<td>5.4 (0.2)$^2$</td>
<td>1.25 (0.01)$^l$</td>
<td>1.6 (0.2)$^l$</td>
<td>42.0 (0.3)$^3$</td>
</tr>
</tbody>
</table>

a-b: Different superscripts within the same column indicate significant differences among formulations containing rapeseed nanoliposomes ($p < 0.05$).

1-3: Different superscripts within the same column indicate significant differences among formulations containing soy nanoliposomes ($p < 0.05$).
Table 4. Mechanical properties and colour of the obtained films.

<table>
<thead>
<tr>
<th></th>
<th>EM (MPa)</th>
<th>TS (MPa)</th>
<th>E (%)</th>
<th>Thickness (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1900 (200)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.6 (1.5)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.9 (0.5)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55 (8)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rap</td>
<td>1300 (200)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.0 (0.7)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.8 (0.2)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>63 (8)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rap-lim</td>
<td>700 (100)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.8 (0.4)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.0 (0.2)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>76 (11)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rap-oil</td>
<td>900 (200)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.1 (1.8)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.0 (0.3)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>89 (12)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soy</td>
<td>900 (100)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>9.6 (1.9)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.7 (0.6)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>66 (7)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soy-lim</td>
<td>1000 (100)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>11 (2)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.8 (0.6)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>71 (11)&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soy-oil</td>
<td>860 (70)&lt;sup&gt;g&lt;/sup&gt;</td>
<td>9.6 (0.3)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.7 (0.1)&lt;sup&gt;g&lt;/sup&gt;</td>
<td>81 (9)&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

L*: Lightness, Cab*: Chroma; hab*: Hue; ∆E: Colour difference in comparison with control film.

EM: Elastic modulus; TS: Tensile strength; E: Elongation at break.

a-c: Different superscripts within the same column indicate significant differences among formulations containing rapeseed nanoliposomes (p < 0.05).

1-3: Different superscripts within the same column indicate significant differences among formulations containing soy nanoliposomes (p < 0.05).
Figure 1. Flow curve of control formulation (without nanoliposomes) showing the change between newtonian and shear-thickening behaviour.
Figure 2. SEM micrographs of the cross-sections of the films. (A-B: Control, C: Rap, D: Rap-lim, E: Rap-oil, F: Soy, G: Soy-lim, H: Soy-oil).
Figure 3. Spectra of internal transmittance (Ti) of obtained films.
Figure 4A. FTIR spectra of the studied films.
Figure 4B. FTIR spectra of isolated components of the films.
Figure 5. Microbial counts as a function of time for samples without films and coated with the different films containing rapeseed liposomes (A) and soy liposomes (B). Mean values and LSD intervals.