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

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ABSTRACT

The incorporation of potentially antimicrobial volatile compounds (orange essential oil and limonene) into soy and rapeseed nanoliposomes was carried out by encapsulating them trough 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 anti-listerial activity or to the inhibition effect of the encapsulation which difficult their release from the matrix.

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, odourless 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 (Famá, 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, 2012c). Whereas starch–HPMC films showed phase separation in the film, starch–sodium caseinate films were completely homogeneous and showed good functional properties.

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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 an 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, 2011) in which the volatile fraction is composed by terpens, terpenoids and other aromatic and aliphatic components with low molecular weight (Bakkali, Averbeck, Averbeck & Idaomar, 2008; Smith-Palmer, Stewart, Fyfe, 2001). Previous studies reported antimicrobial activity of films containing different essential oils such as those obtained from bergamot (Sánchez-González, Cháfer, Chiralt & González-Martínez, 2010; Sánchez-González, Cháfer, Hernández, Chiralt & González-Martínez, 2011), lemon (Iturriaga, Olabarrieta, Martínez de Marañón, 2012; Sánchez-González, González-Martínez, Chiralt & Cháfer, 2010) 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 et al., 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. (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 Laisa España, Benifaió, Spain) and sodium caseinate (NaCas) was supplied by Sigma (Sigma–Aldrich Chemie, Steinheim, Germany). Glycerol (99.5% AnalaR NORMAPUR), choosen 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 (Etrépagny, France), respectively. Furthermore, D-Limonene stabilized (purchased from Acros Organics, Geel, Belgium) and orange essential oil (supplied by Laboratoires Mathe, Maxeville, France) were choosen 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-Erab (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 (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-snglycerol, tripalmitin, cholesterol.
- Phospholipids: L-a-phosphatidylcholine, 3 sn-phosphatidyleth anolamine, 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.