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

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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.

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 (Bonilla, Atarés, Vargas, & Chiralt, 2012; Adorjan & Buchbauer, 2010; Salvia-Trujillo, Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 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, Soliva-Fortuny, & Martín-Belloso, 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).

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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, Izquierdo, Nolla, Azemar, & García-Celma, 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, Cassani, Martín-Belloso, & Soliva-Fortuny, 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; 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 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-Graü, Avena-Bustillos, et al., 2007; Rojas-Graü, Tapia, Rodríguez, Carmona, & Martin-Belloso, 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).

The aqueous phase was prepared by solving sodium alginate in ultrapure water at 70 °C for 3 h. After reaching room temperature, mandarin fiber was added to alginate solution and mixed using a laboratory high-shear homogenizer (T25 digital Ultra-Turrax, IKA, Staufen, Germany) at 9600 rpm for 3 min. Ultimately, the aqueous phase was filtered in order to remove the fiber in excess. An accurate amount of the lipid phase consisted of the mixture of OEO and Tween 80 at room temperature was added to the aqueous phase, and blended with the high-shear homogenizer at 11,000 rpm for 2 min, leading to coarse emulsions. Lastly, nanoemulsions were formed passing the respective coarse emulsion through a microfluidizer (M110P, Microfluidics, Massachusetts, USA) at 150 MPa for 5 cycles.

2.2.2. Physicochemical characterization of emulsions and nanoemulsions

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

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

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

2.2.2.2. Apparent viscosity and whiteness index. Viscosity measurements (mPa·s) were performed by using a vibro-viscometer (SV-10, A&D Company, Tokyo, Japan) vibrating at 30 Hz, with constant amplitude and working at room 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° 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, Cháfer, Albors, Chiralt, & González-Martínez, 2008):

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

2.2.3. Cheese coating and sampling

Sealed cheese bars were stored at 4 °C 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