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Resveratrol entrapped niosomes as yoghurt additive

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ABSTRACT

Nanodesign of niosomes containing resveratrol (RSV) was carried out using food-grade surfactants with dodecanol to stabilise the membrane. Niosomes were prepared using a modified thin film hydration method.

A factorial design analysis was carried out to reduce the number of experiments. The response factors were: mean size, polydispersity index (PDI) and entrapment efficiency (EE). Agitation speed and surfactant to dodecanol weight ratio were selected as key parameters for niosomes preparation. Parameter contribution was determined using a statistical analysis of variance (ANOVA).

Niosomes formulated with Span 60 or Maisine 35-1 as surfactants, and dodecanol as stabiliser, were able to incorporate RSV. These niosomes exhibited a small mean size, narrow size distribution, high RSV entrapment efficiency and good stability. RSV addition did not involve changes in the textural properties of regular yoghurt demonstrating that RSV entrapped niosomes are suitable additives in these dairy products.

1. Introduction

Natural products have been widely used to prevent or mitigate various diseases and lately there has been a growing interest in research, development and commercialisation of functional foods, nutraceuticals and dietary supplements (Dewapriya & Kim, 2014; Ortuño et al., 2010; Shahidi, 2009). The term "*nutraceutical*" is defined as any food-based substance that provides health benefits, including prevention and treatment of diseases (Defelice, 1995). This term is commonly used in marketing although there is no regulatory definition, and in certain countries the terms functional food and nutraceutical are used interchangeably. In all cases, the main focus is to improve health and reduce the risk of diseases mainly through prevention (Shahidi, 2009). Moreover, pharmaceutical and biotechnology companies have made significant investments in the discovery and production of nutraceuticals/functional foods (Kalra, 2003; Nelson, 1999).

Resveratrol (RSV) is a natural polyphenol which can be considered as a *nutraceutical* because of benefits such as anticancer activity (Jang & Surh, 2003; Surh et al., 1999), lifespan extension (Howitz et al., 2003), cardioprotection (Hung, Chen, Huang, Lee, & Su, 2000), antioxidant activity (Frankel, Waterhouse, & Kinsella, 1993; Fremont, Belguendouz, & Delpal, 1999), inhibition of platelet aggregation (Bertelli et al., 1995; Chung, Teng, Cheng, Ko, & Lin, 1992) and antiinflammatory activity (Pace-Asciak, Hahn, Diamandis, Soleas, & Goldberg, 1995). Consequently, RSV may prove to be a useful ingredient for functional foods.

RSV is a photosensitive molecule that exists in *cis* and *trans* structural *isomers*, but only *trans*-RSV demonstrates health benefits. The change from the active *trans* isomer to the inactive *cis* isomer is mainly caused by exposure to light. RSV therefore needs to be encapsulated. Encapsulation facilitates control of the rate of RSV release and protects the molecule during digestion, from degradation under pancreatic conditions (Tagliazucchi, Verzelloni, Bertolini, & Conte, 2010). It can also help to mask undesired flavours.

RSV encapsulation studies have been done in pharmaceutical and cosmetic industries with the aims of preventing degradation, increasing its solubility in water, and targeting specific locations in the body by using multiparticulate forms and colloidal carriers (Amri, Chaumeil, Sfar, & Charrueau, 2012; Caddeo, Teskač, Sinico, & Kristl, 2008; Donsi, Sessa, Mediouni, Mgaidi, & Ferrari, 2011; Kristl, Teskač, Caddeo, Abramovic, & Sentjurc, 2009; Lucas-Abellán, Fortea, López-Nicolás, & Núñez-Delicado, 2007; Matos, Gutiérrez, Coca, & Pazos, 2014; Pando, Caddeo, Manconi, Fadda, & Pazos, 2013; Pando, Gutiérrez, Coca, & Pazos, 2013; Peng et al., 2010; Sessa et al., 2014; Teskač & Kristl, 2010; Wang et al., 2011).

Niosomes are vesicles formed by the self-assembly of non-ionic surfactants in aqueous media resulting in closed bilayer structures

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(Uchegbu & Vyas, 1998). These vesicles are commonly used to encapsulate both hydrophilic and lipophilic compounds, for either food, pharmaceutical or cosmetic applications. Hydrophilic compounds are entrapped in the aqueous compartments between the bilayers while the lipophilic components are preferentially located within the surfactant bilayer (Devaraj et al., 2002).

Surfactants are versatile products of the chemical industry, and the large number of available non-ionic surfactants enables the design of niosomes for specific applications (Manosroi et al., 2003). The main advantage of niosomes, with respect to other encapsulation technologies, such as liposomes (Fang & Bhandari, 2010; Gibis, Zeeb, & Weiss, 2014), is their low cost, high stability, and biocompatibility (Kopermsub, Mayen, & Warin, 2011).

Size distribution, stability and entrapment efficiency are the key parameters to obtain optimal niosomal systems. Two interacting variables involved in niosomes preparation (*i.e.* agitation speed and surfactant to stabiliser weight ratio) have to be tested to properly analyse the behaviour of the system and to optimise system parameters. Changing one factor at a time is not an efficient and economic strategy because it does not provide information regarding the optimum location and it does not take into account interactions of parameters. Factorial design and analysis of variance (ANOVA) methodology are appropriate and efficient statistical tools, which make it possible to study the effects of several factors that influence responses by varying them simultaneously within a limited number of experiments (Kincl, Turk, & Vrečer, 2005; Martínez-Sancho, Herrero-Vanrell, & Negro, 2004).

The aim of this work is to formulate RSV entrapped niosomes for oral administration, using a modified thin film hydration method. Dodecanol was selected as a membrane stabiliser, because it has previously been reported that stable niosomes can be prepared with fatty alcohols (Devaraj et al., 2002), instead of cholesterol, which avoids the gel–liquid phase transition of niosomes. By contrast cholesterol may not be suitable for use in functional foods because of potential adverse health effects.

The key parameters involved in niosome preparation (*i.e.* surfactant to dodecanol weight ratio and agitation speed) were optimised by a factorial design of experiments and statistical analysis of variance (ANOVA) to assess their contributions to mean size, polydispersity index (PDI), and entrapment efficiency (EE). Finally, the best formulation for each surfactant was selected for subsequent preparation of yoghurts enriched with RSV. The textural properties of these yoghurts were also analysed.

2. Materials and methods

2.1. Materials

Trans-resveratrol (RSV), with a purity >99%, sorbitan monostearate (Span 60, S60), lauryl alcohol (dodecanol, Dod) and absolute ethanol were supplied by Sigma–Aldrich (USA). Labrasol (Lab) and Maisine 35-1 (Mai) were a gift from Gattefossé (France). Methanol, acetonitrile, 2-propanol and acetic acid of HPLC-grade were obtained from Sigma–Aldrich (USA). Deionised water was used in all experiments.

2.2. Preparation of niosomes

Niosomes were prepared by the thin film hydration method (Baillie, Florence, Hume, Muirhead, & Rogerson, 1985; Bangham, Standish, & Watkins, 1965) with minor modifications, followed by agitation–sonication (Pando, Gutiérrez, et al., 2013). Accurately weighed amounts of surfactant (S60, Lab or Mai) and dodecanol in different weight ratios, from 1:0.5 to 1:1.5, were dissolved in 20 ml of a solution of ethanol containing a known concentration of RSV and placed into a 100 ml round bottom flask. Ethanol was then removed at 40 °C under reduced pressure in a rotary evaporator (Buchi, Switzerland). The dried film was hydrated with 40 ml of deionised water at 60 °C to achieve a RSV concentration of 150 mg/L. The resulting sample was subsequently homogenised (SilentCrusher M, rotor model 22G, Heidolph, Germany) at speeds ranging from 5000 to 15,000 rpm depending on the experiment, and further sonicated for 30 min (CY-500 sonicator, Optic Ivymen System, Spain), using 45% amplitude, 500 W power and 20 kHz frequency.

2.3. Characterisation of RSV entrapped niosomes

2.3.1. Vesicle size and zeta potential measurements

Mean (Z-Average) sizes and PDI of the niosomes were determined via Dynamic Light Scattering (DSL) using a Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK). Three independent samples were taken from each formulation, and each was measured three times at room temperature without dilution.

For determination of zeta potentials (ζ-potential), three independent samples were also taken from each formulation and measure three times at room temperature. Each of them were diluted (1:10 v/v) before measuring vesicle electrophoretic mobility using M3-PALS (Phase Analysis Light Scattering) technique. High absolute values of ζ-potential indicate electrostatic repulsion between vesicles. Such conditions are linked to high stability.

2.3.2. Stability measurements

Stability of the niosomes was determined by measuring backscattering (BS) profiles in a Turbiscan Lab® Expert apparatus (Formulaction, France) provided with an Ageing Station (Formulaction, France). Undiluted niosome samples were placed in cylindrical glass test cells and the backscattered light was monitored as a function of time and cell height for 15 days, every 3 h, at 30 °C. The optical reading head scans the sample in the cell, providing BS data every 40 μm in % relative to standards (suspension of monodisperse spheres and silicone oil) as a function of the sample height (in mm). These profiles provide a macroscopic fingerprint of the niosomes at a given time, providing useful information about changes in vesicle size distribution and/or appearance of a creaming layer or a clarification front with time (Pando, Caddeo, et al., 2013; Pando, Gutiérrez, et al., 2013).

2.4. RSV entrapment efficiency (EE)

Entrapped RSV was separated from free RSV by dialysis. Samples (2 ml) were placed into a dialysis bag, which was immersed in 1000 ml of deionised water at room temperature and stirred at 500 rpm for 2 h. Dialysed and non-dialysed samples were diluted 1:10 (v/v) with methanol to rupture the vesicle membranes enabling extraction of RSV. Later, RSV concentration was determined by chromatography (HP series 1100 chromatograph, Hewlett Packard, USA). The system was equipped with a UV/VIS absorbance detector HP G1315A and a fluorescence detector 1260 Infinity A (Agilent Technologies, USA). A wavelength of 305 nm was used for the UV/VIS detector while fluorescence detector used 310/410 nm of $\lambda_{excitation}/\lambda_{emission}$. The analytical column was a Zorbax Eclipse Plus C_{18} of 5 μm particle size, 4.6 mm × 150 mm (Agilent Technologies, USA).

The mobile phase consisted of a mixture of (A) 100% milliQ-water and (B) 100% methanol with gradient elution at a flow rate of 0.8 ml/min. The step gradient started with a mobile phase of 80% (A) running 100% mobile phase (B) after 5 min for 10 min. The mobile phase (B) was fed for 2 min after each injection to prepare the column for the next sample. The separation was carried out at 30 °C.