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Enhancing rosemary oil-in-water microfluidized nanoemulsion properties through formulation optimization by response surface methodology

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ABSTRACT

Nanoemulsions formulated with rosemary essential oil and a mixture of food-grade surfactants were prepared by microfluidization. The influence of HLB values and surfactant/oil ratio (R) on mean droplet sizes and polydispersity have been studied and analysed by response surface methodology. The optimum was found for HLB = 10.5 and R = 1, obtaining the lowest volumetric mean diameter (d_v = 2.88 nm). Taking into account these results, the effect of rosemary oil concentration on the droplet size distributions and physical stability of the nanoemulsions was investigated. All emulsions with dispersed phase concentrations in the 1–10 g/100 g range showed destabilization by Ostwald ripening. The nanoemulsions that presented better physical stability were those formulated with 5 and 7.5 g/100 g of rosemary essential oil. This work demonstrates the capacity of rosemary oil to form stable nanoemulsions with potential applications as delivery systems and food preservatives.

1. Introduction

During the last decade, consumer demand for natural products has increased remarkably. Natural essential oils, which are obtained from plants, are mixtures of different compounds, both volatile and non-volatile. The importance of essential oils for health, beauty and well-being is well-known from ancient times. Rosemary (*Rosmarinus officinalis*) essential oil has been used as a bactericide and as an anti-oxidant (Barbosa-Pereira, Aurrekoetxea, Angulo, Paseiro-Losada, & Cruz, 2014; Bolumar, Andersen, & Orlien, 2011; Takala, Vu, Salmieri, Khan, & Lacroix, 2013). Nevertheless, rosemary essential oil needs some protection in order to improve its bioavailability and uptake since it possesses high volatility and it is vulnerable to some environmental effects. One very effective way to preserve it is to prevent contact between rosemary essential oil and oxygen by forming oil-in-water nanoemulsions (Turasan, Sahin, & Sumnu, 2015).

Nanoemulsions, also referred to in the literature as submicron emulsions, are emulsions with droplet sizes from 2 to 200 nm (Solans, Izquierdo, Nolla, Azemar, & Garcia-Celma, 2005). The occurrence of droplets in the nanoscale confers some important properties, such as optical clarity, high physical stability, and the ability to increase the bioavailability of lipophilic functional components. These features make them very attractive for some commercial applications; mainly food and cosmetic products. The production of nanoemulsions requires at least one, and often both, of the following: (1) a high concentration of surfactant, and (2) a large energy input. Nanoemulsions are usually developed using low-energy or high-energy emulsification methods (Mason, Wilking, Meleson, Chang, & Graves, 2006). High-energy emulsification methods, such as Microfluidizers or high-pressure valve homogenizers, have several advantages including the ability to develop ultrafine nanoemulsions and the control of droplet size distributions. Microfluidizers are effective at producing nanoemulsions since extremely intense disruptive forces can be generated (shear, turbulence, and cavitation) (Jafari, Assadpoor, He, & Bhandari, 2008). Furthermore, this procedure is suitable for a wide range of oils and surfactants and it is easy to scale up to industrial production. Obviously, microfluidization needs a coarse emulsion to pass through the interaction chamber(s), where the coarse emulsion is split into two channels which are made to impinge on one another at high shear. The droplet size distribution obtained from microfluidization is known to be function of the number of cycles and the homogenization pressure (Jo & Kwon, 2014; Salvia-Trujillo, Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2013).

One of the most important challenges that must be overcome during the formulation and development of essential oil-in-water nanoemulsions is their tendency for destabilization by Ostwald ripening. However, nanoemulsions with high physical stability can be obtained by means of an appropriate selection of system components and

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composition. In the food industry, the choice of surfactant is very important due to the fact that it must be able not only to create and stabilize the dispersed phase droplets, but also be biodegradable and non-toxic. In this study, all the surfactants used fulfil these requirements.

Surfactant hydrophilic-lipophilic balance (HLB) and surfactant/oil concentration ratio are important variables that must be taken into account in order to formulate nanoemulsions. The HLB number is a semi-empirical scale for selecting surfactants and the development of emulsions. An optimum value of HLB for a selected oil phase can be obtained using blends of surfactants with a wide range of HLB values. Furthermore, an optimum surfactant/oil concentration ratio not only avoids destabilization effects but also influences the mean droplet sizes and droplet size distributions. As far as cosmetic and food applications are considered, studies concerning the optimization of the nanoemulsion formulation are required (Amselem & Friedman, 1998; El-Aasser & Sudol, 2004; Solans et al., 2005; Solans & Kunieda, 1996; Sonneville-Aubrun, Simonnet, & L'alloret, 2004). A multivariate statistical method, such as a response surface methodology (RSM), is a very powerful tool for the development of new systems (Musa et al., 2013; Luis M; Pérez-Mosqueda, Trujillo-Cayado, Carrillo, Ramírez, & Muñoz, 2015). This can link the processing variables with response variables in order to obtain a mathematical model that fits the behaviour emulsions. In addition, it allows the development of new systems to become more effective.

In the present work, an experimental strategy has been used for the development and optimization of a formulation process for nanoemulsions containing a bioactive ingredient. The variables selected were the surfactant/oil concentration ratio and the ratio between surfactants (HLB), since a surfactant mixture is used. In addition, the effects of rosemary oil concentration on the visual properties, physical stability and droplet size distribution were analysed for nanoemulsions with optimized values of surfactant/oil ratio and HLB. These rosemary oil-in-water nanoemulsions could be considered as delivery systems for incorporating active ingredients into many foods, and as food preservatives.

2. Materials and methods

2.1. Materials

Rosemary (*R. officinalis* L.) essential oil, Tween 80 (HLB = 15) and Span 80 (HLB = 4.3) were obtained from Sigma-Aldrich. Deionised water obtained from a water purification system was used for the preparation of all samples. All of the chemicals were used as received.

2.2. Preparation of nanoemulsions

Nanomulsions contain rosemary essential oil as dispersed phase, a mixture of surfactants (Tween 80 and Span 80) as emulsifiers and deionised water.

Firstly, an optimal formulation of rosemary oil-in-water nanoemulsions developed with a Microfluidizer M110P was achieved. The influence of two variables using a second-order experimental design and response surface methodology was investigated. The concentration of essential oil in each nanoemulsion was 5 g/100 g. The two independent variables were the hydrophilic lipophilic balance (HLB) value, using different mixtures of Span 80 and Tween 80, and the surfactant/dispersed phase concentration ratio (R). The central part of the process was carried out in triplicate to calculate the repeatability of the method and to check the fitting quality of the mathematical model. Every independent factor was studied at five different levels (−1.414, −1, 0, +1 and + 1.414) selected on the basis of preliminary experimental work (see Table 1). Nanoemulsions with HLB values from 6 to 15 were prepared using different surfactant/oil concentration ratios (0.1–1). The aqueous phases were prepared by dispersing the required amount of surfactants in deionised water. A total mass of 250 g of the samples was prepared in three steps. Firstly, the essential oil was added slowly for 30 s at 4000 rpm to the aqueous phase using a rotor-stator device (Silverson model L5M), and then a secondary homogenization was carried out for 30 s at 6000 rpm. Subsequently, the pre-emulsions were passed through an air-driven microfluidizer (Microfluidizer M110P, interaction chambers F12Y and H30Z, Microfluidics, USA) at 137.9 MPa for 1–10 cycles. The outlet sample tube of the high pressure homogenizer was cooled with water at 5 °C.

Table 1
Experimental design with three central points.

Sample	HLB	R	HLB	R
I	−1	−1	7.32	0.23
II	1	−1	13.68	0.23
III	−1	1	7.32	0.87
IV	1	1	13.68	0.87
V	−1.414	0	6	0.55
VI	1.414	0	15	0.55
VII	0	−1.414	10.5	0.1
VIII	0	1.414	10.5	1
IX	0	0	10.5	0.55
X	0	0	10.5	0.55
XI	0	0	10.5	0.55

Subsequently, nanoemulsions with the optimum HLB value and surfactant/oil concentration ratio were developed using five different essential oil concentrations from 1 to 10 g/100 g.

2.3. Droplet size distributions of nanoemulsions

The droplet size distribution and mean droplet sizes was determined by dynamic light scattering (DLS) measurements using a Zetasizer® ZS. Volumetric mean diameter (d_v) was used to compare the droplet sizes of different nanoemulsions. Moreover, the polydispersity index (PdI) was used to study the distribution width of droplet sizes.

2.4. Analysis of nanoemulsion physical stability

Physical stability of nanoemulsions with different rosemary oil concentrations was studied and quantified by means of multiple light scattering measurements (Turbiscan Lab Expert) at 25 °C. To visualize variations in the physical stability of nanoemulsions, the transmission (T) profiles were plotted in reference mode using delta-transmission (ΔT), which is the difference between the transmission value for a determined aging time and transmission at zero time. The global Turbiscan Stability Index (TSI) parameter was used to compare emulsion stability. The following equation states the value of TSI:

$$TSI = \sum_j |scan_{ref}(h_j) - scan_i(h_j)| \quad (1)$$

where $scan_{ref}$ and $scan_i$ are the initial transmitted radiation/light and the transmitted radiation/light at a specific time, respectively, and h_j is a specific height in the measuring cell.

2.5. Statistical analysis

Experimental data of the preliminary study were fitted to the following quadratic model:

$$Y = \beta_0 + \beta_1 \cdot HLB + \beta_2 \cdot R + \beta_{12} \cdot HLB \cdot R + \beta_{11} \cdot HLB^2 + \beta_{22} \cdot R^2 \quad (2)$$

where Y is the response variable, β_i are the constant parameters of the model and X_1 and X_2 are the coded factors. The actual factors used were the HLB values and the surfactant/dispersed phase concentration ratio (R) whereas the dependent variables considered were the volumetric mean diameter (d_v) for emulsions aged for 24 h and the polydispersity index (PdI). Statistical analysis of the data was performed with Statistic 10.0 software (StatSoft Inc.) to evaluate the analysis of variance