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Sodium caseinate stabilized clove oil nanoemulsion: Physicochemical properties

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ABSTRACT

In the present study, Clove oil nanoemulsions were prepared using sodium caseinate (NaCas-5%) & pectin (0.1%) as coating material by high speed homogenization. Mean particle size, poly dispersity index (PDI) and zeta potential of most stable nanoemulsion was172.1 ± 4.39 nm, 0.152 ± 0.01 and −37 ± 1.93 mV, respectively with an encapsulation efficiency of 88%. Nanoemulsion was stable at all food processing conditions that generally encountered during processing except pH 3.0–5.0. Droplet diameter was increased with increase in storage time from 0th day (172.1 ± 4.39 nm) to 20th day (415.3 ± 23.38 nm) at 25 °C. Scanning electron micrographs showed spherical nanoparticles with slight adherence whereas transmission electron micrographs confirmed the morphology through discrete spherical droplets with defined boundaries of core and coating material. Our results suggest the possibility of clove oil nanoemulsion as delivery system for antimicrobial bioactive substances in food preservation with a green image.

1. Introduction

The essential oils (EOs) are finding extensive applications in food science for their antimicrobial activity against food pathogens and spoilage organisms (Edris, 2007; Elgayyar et al., 2001; Hammer et al., 1999; Smith-Palmer et al., 1998). However, poor water solubility, high volatility, less stability and loss during food processing poses challenges to incorporate these EOs in food system. While including in the foods, EOs needs to be protected to avoid their interaction with food components, to retain their biological activity and also to minimize their possible adverse effect on sensory properties of food (Burt, 2004; Shah, 2011). Encapsulation, particularly nano scale encapsulation of lipophilic components is considered a best alternative as a delivery system as they can overcome most of the drawbacks associated with lipophilic substances (Alamilla-Beltran et al., 2005; Weiss et al., 2009; Moraru et al., 2009; El-Asbahani et al., 2015; Sari et al., 2015). Further, nanoencapsulation has been reported to augment the antimicrobial activity of EOs by reducing the size of delivery system to subcellular size. The reduced size increases the surface area for contacting bacteria and improves the solubility/dispersability of these antimicrobials in aqueous phases which are most preferential locations for the growth of target microorganisms. (McClements et al., 2007; Weiss et al., 2009).

Oil-in-water nanoemulsions can be used to disperse lipophilic antimicrobial components for their incorporation in food system and are receiving the attention of food industry ((Donsi et al., 2011; Ziani et al., 2011) Nanoemulsions having their size between 50 and 500 nm are new class of emulsions and they exhibit much better stability towards gravity separation and aggregation, if size is less than 100 nm, owing to the smaller droplet size (Quintanilla-Carvajal et al., 2010); McClements, 2010). Apart from the size, the use of ingredients governs the functionality of the nanoemulsions. In general, short to long chain triacylglycerides, essential oils, and flavour oils have been used to produce nanoemulsions for food and beverage applications (Ahmed et al., 2012; McClementsand Rao, 2011). Clove (*Syzygiumaromaticum* L.)and oils extracted from it has been extensively used pharmaceutical and food industries. The major component of clove oil is eugenol (>70%) and the other constituents include beta-caryophyllene, alpha-humelene, and eugenyl acetate (Moon et al., 2011). The clove oil components have analgesic, anti-inflammatory, and antimicrobial properties (Chaieb et al., 2007). Eugenol which is heavier than water (density:1.067 g/ml at 25 °C) (Ntalli et al., 2010) has strong antioxidant (Ogata et al.,

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2000) and antimicrobial properties (Pavithra, 2014). It has been reported that encapsulated or unencapsulated eugenol can effectively scavenge free radicals in soybean oil during storage (Chatterjee and Bhattacharjee, 2013).

Sodium caseinate (NaCas), which is processed product of casein is commonly used as an emulsifier in the food industry. Casein is a heterogeneous protein and is composed of four principal proteins (αs_1, αs_2, β and k caseins) and these proteins have a strong tendency to associate with each other to form casein micelles (~250 kDa) and that are in equilibrium with free casein molecules (~25 kDa) (Dickinson et al., 1998). αs_1-casein and β-casein constitute the major fraction of total protein and are mainly responsible for the emulsifying properties of NaCas. Caseinate stabilized emulsions tend to have better stability during heating; because of its structure which does not affected by the heat-induced conformation changes as that of globular proteins (Hunt and Dalgleish, 1995; Srinivasan et al., 2002). Due to the combination of electrostatic and steric stabilization mechanisms NaCas provide protection to the emulsion droplets at neutral pH (Dickinson et al., 1998).

The purpose of this study was to investigate the emulsifying ability of food grade emulsifier (sodium caseinate) in combination with polysaccharide (pectin) to encapsulate the clove oil in the form of nanoemulsions for possible application in food manufacturing. The nanoemulsion was further studied for their physico-chemical and morphological properties and evaluated its stability during different food processing conditions.

2. Materials and methods

2.1. Materials

Clove oil was procured from Plant Lipids Pvt. Ltd. Kolenchery, (Kerala, India). Sodium Caseinate was prepared from buffalo milk according to the procedure given by Fox (1970). Pectin was obtained from Sisco Research Laboratories Pvt. Ltd., (Mumbai, India). All other solvents and reagents used were of analytical grade.

2.2. Methods

2.2.1. Clove oil nanoemulsion preparation

Clove oil nanoemulsions were prepared by mixing ingredients like clove oil, NaCas, and pectin by using high speed magnetic stirrer at ambient temperature for 20 min and homogenized by using an Ultra TurraxT25 at 15,000 to 24,000 rpm/20 minas shown in Fig. 1. Clove oil was taken as an inner oil phase (O) (1–15% v/v) and the outer aqueous phase was prepared by mixing NaCas (1–15% w/v) and pectin (0.1–1% w/v) with millipore water at pH 7.0.

2.2.2. Stability criteria for the selection of stable nanoemulsion

Stability (sedimentation or phase separation) of freshly prepared nanoemulsions were measured by centrifugation at 3500 rpm (Kubota, Tokyo, Japan) for 30 min at 5°Cand heating at 80°Cfor 30 min followed by centrifugation for 30 min at 5 °C.The most stable nanoemulsion (5% clove oil, 5% sodium caseinate and 0.1% pectin) was chosen for further studies.

2.2.3. Physico-chemical characterization

The mean particle size (Z-average), poly dispersity index (PDI), and zeta-potential of the clove oil nanoemulsion was measured using dynamic light scattering method (Malvern Instruments Ltd., U.K). Zeta potential, the electrical charge on the emulsion droplets was determined under holder temperature of 25 °C and electric voltage 3.9 V by the same instrument. Diluted samples (1:50) were used for the experiments to avoid multiple scatterings.

2.2.4. Encapsulation efficiency

The encapsulation efficiency of nanoemulsion was analyzed by combining two methods with slight modifications:solvent extraction followed by the estimation total phenolic content by using Folin-Ciocalteu's reagent (Zheng and Wang, 2001). Hexane and nanoemulsion (1:1 v/v) were mixed for 3 to 5 min to facilitate the solubilization of loosely bounded or free clove oil in hexane and then kept for 10 min for layer separation. Two layers were separated as upper layer with free clove oil in hexane and lower layer with encapsulated clove oil. The upper hexane layer with free clove oil was collected to calculate the encapsulation efficiency by measuring the total phenolic content present in the sample after removal of lower layer. Total phenolic content of freshly prepared nanoemulsion and upper layer of hexane extracted with solvent extraction was analyzed by Folin-Ciocalteu's method given by Zheng and Wang (2001) and encapsulation efficiency was calculated as follows:

$$\textit{Encapsulation efficiency}\ (\%) = \frac{\text{Actual polyphenol content in nanoemulsion (g)}}{\text{Total polyphenol content in fresh nanoemulsion (g)}} \times 100 \quad (1)$$

$$\begin{aligned}\text{Actual polyphenol content in nanoemulsion} &= \text{Total polyphenol content in fresh emulsion} \\ &- \text{Polyphenol content in the upper hexane layer}\end{aligned} \quad (2)$$

2.2.5. Effect of processing conditions

The stability of clove oil nanoemulsion under processing conditions were evaluated against different pH ranges (3.0–7.0); heat treatments like pasteurization (63°C/30 min), forewarming (80°C/30 min), boiling (100°C/10 min) & sterilization (121°C/15 min); and salt concentrations (0.1 − 1 M). After different treatments, the samples were analyzed for particle size and zeta potential.

2.2.6. Effect of storage time

Freshly prepared nanoemulsion was stored at 25°C for one month. The effect of storage time on the stability of nanoemulsion was determined at five days interval by physical observation, and particle size & zeta potential analysis.

2.2.7. Scanning electron microscopy

The surface morphology of nanoparticles of liquid and freeze dried emulsion was confirmed by scanning electron microscopy (SEM) operating at 15 kV.

For fresh nanoemulsion, one drop of 50 time's diluted (in 50 mM, 7.2 pH phosphate buffer) sample was taken on a cover slip