Journal of Food Engineering 117 (2013) 545–550

Contents lists available at SciVerse ScienceDirect

Journal of Food Engineering

journal homepage: www.elsevier.com/locate/jfoodeng

Comparative evaluation of the antioxidant efficacy of encapsulated and un-encapsulated eugenol-rich clove extracts in soybean oil: Shelf-life and frying stability of soybean oil

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ARTICLE INFO

Article history:
Available online 29 November 2012

Keywords:
Eugenol-rich clove extract
Encapsulation
Spray dryer
SC-CO_2 extraction
Soybean oil

ABSTRACT

Microencapsulation of eugenol-rich clove extract obtained from clove buds by supercritical carbon dioxide (SC-CO_2) extraction was carried out in maltodextrin and gum arabic matrices using spray dryer. Microencapsulated powder with maximum encapsulation efficiency of 65% was obtained with 1:4.8:2.4 of clove extract: maltodextrin: gum arabic. The morphology of the encapsulated powder was determined from SEM photographs; while its phytochemical properties such as total phenolic content, total eugenol content and antioxidant activity were determined by biochemical assays. Food application in soybean oil was designed using the encapsulated clove powder as a source of natural antioxidant. Comparative evaluation of the antioxidant efficacy of encapsulated clove extract, un-encapsulated clove extract and commercial antioxidant BHT, individually administered in soybean oil, established encapsulated clove extract as a promising natural antioxidant in the same.

1. Introduction

Eugenol ($C_{10}H_{12}O_2$; 4-allyl-2-methoxy phenol) is a phenylpropene, an allyl chain-substituted guaiacol. It is a colorless to light yellowish fluid found in essential oils of spices such as cinnamon (Mallavarapu et al., 1995), bay leaf (Abaul et al., 1995), nutmeg (Mallavarapu and Ramesh, 1998), basil (Leal et al., 2006) and clove (Bhuiyan et al., 2010). Eugenol has considerable therapeutic potency owing to its ability to denature proteins and can react with cell membrane phospholipids changing their permeability (Briozzo, 1989). There are reports on biological activities of eugenol including antifungal (Manohar et al., 2001; Gayoso et al., 2005), antibacterial (Cai and Wu, 1996; Friedman et al., 2002), antioxidant (Ogata et al., 2000) and anti-inflammatory (Daniel et al., 2009).

Among the different sources, the current investigation uses essential oil of clove as the source of eugenol. Cloves are the aromatic dried flower buds of *Eugenia caryophyllata* L. Merr. & Perry (syn.: *Syzygium aromaticum*), the essential oil of which contains ~88.58% eugenol (Chaieb et al., 2007). Conventionally eugenol is extracted from clove buds by steam distillation and solvent extraction which incur problems such as thermal degradation, hydrolysis, water solubilization of constituents and most importantly environmental cum health hazards (Wenqiang et al., 2007) and therefore has limited applicability in food systems. In our previous investigation, we have optimized different procedures such as liquid, subcritical and supercritical carbon dioxide (SC-CO_2) extractions, steam distillation and solvent extraction for isolating eugenol from clove buds. We found that SC-CO_2 clove extracts have the best synergism of therapeutic and phytochemical properties. These were successively administered as natural antioxidants to soybean oil. 30-day shelf life and frying stability study of the soybean oil has demonstrated promising potential in replacing synthetic antioxidant such as butylated hydroxyl toluene (BHT) (Chatterjee et al., 2012).

However, clove oil exhibits sensitivity to light, heat and oxygen and has a short storage life under inappropriate conditions of storage (Shaikh et al., 2006). We have observed that at high temperature (~130 °C) of frying, there was a considerable decrease in antioxidant activity and other phytochemical properties of the clove extracts. Hence, it is assumed that if the clove extract is protected using microencapsulation technology, prior to its application in soybean oil, it would show better stability. Also the clove extracts in its native liquid form have disadvantages in portability and commercial viability as a natural antioxidant. Therefore, microencapsulation of clove extracts would possibly improve these aspects.

Microencapsulation of eugenol has been reported by Shinde and Nagarsenker (2011) using gelatin–sodium alginate complex coacervation system. Wang et al. (2006) used dried brewers' yeast (*Saccharomyces cerevisiae*) cells as wall material for encapsulation

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http://dx.doi.org/10.1016/j.jfoodeng.2012.11.016

of clove oil; while Jing et al. (2010) used β-cyclodextrin for the same. Nonsee et al. (2011) have encapsulated clove oil in edible hydroxylpropyl methylcellulose film and used it as an antimicrobial agent. Ponce et al. (2011) have encapsulated the same in lactose matrix for preservation of lettuce leaves.

The objectives of the current investigation are to encapsulate the eugenol-rich clove extracts obtained by SC-CO_2 extraction in maltodextrin and gum arabic matrices using spray dryer. The encapsulated clove powder was then characterized in terms of encapsulation efficiency and phytochemical properties such as eugenol content, phenolic content and antioxidant activity. Food application was designed using the encapsulated clove powder as a natural antioxidant in soybean oil. Comparative evaluation of the antioxidant efficacy was carried out for soybean oil with encapsulated clove extract, un-encapsulated clove extract and with BHT individually, for a storage period of 30 days with an aim to improve the shelf life and frying stability of the oil.

2. Materials and methods

2.1. Materials

Clove buds (*Syzygium aromaticum* Linn) were purchased from a local market of Jadavpur, Kolkata, India. National Research Centre on Seed Spices, Tabiji Farm, Ajmer, India have authenticated that only this species of clove is cultivated and commercialized in India. Specialty chemicals such as eugenol (99% pure), 1, 1-diphenly-2-picrylhydrazyl (DPPH), maltodextrin, gum arabic, BHT and gallic acid were procured from M/s Sigma, India. Nutrient broth powder, peptone water and nutrient agar powder were procured from M/s Oxoid, UK. All chemicals, solvents and buffers used in the work were of AR grade.

2.2. Methods

2.2.1. SC-CO_2 extraction of eugenol from clove buds

The studies on optimization of SC-CO_2 extraction of eugenol-rich fractions from clove buds have been previously carried out in our laboratory. For SC-CO_2 extraction, a SPE-ED SFE 2 model of M/s Applied Separations, Allentown, USA, was used. 20 g of ground clove powder (d_p = 0.5 mm) was charged into a 50 ml extraction vessel (SS 316). The flow rate of CO_2 (food grade) was maintained constant at 2 l min^{-1}. The optimized conditions for SC-CO_2 extraction were 60 °C, 250 bar with static and dynamic time of 60 min and 30 min respectively (Chatterjee and Bhattacharjee, 2012). Eugenol-rich clove extract was weighed and stored in an inert atmosphere of nitrogen in screw-capped amber colored glass vials at 4 °C in the dark, until further analyses.

2.2.2. Evaluation of phytochemical properties of the clove extracts

Total phenolic compounds in the clove extract were determined using Folin–Ciocalteu reagent and expressed as mg gallic acid/g of dry clove buds (Spanos and Wrolstad, 1990). The total eugenol content of the clove extract and in the encapsulated powder was carried out by densitometric method (considering eugenol as the reference standard) in accordance to the method described by Bhattacharjee et al. (2012), with little modification. The clove extract was diluted and spotted on aluminum plates coated with silica gel 60 (F_{254}) of dimension 100 × 100 mm by use of Camag Linomat V (M/s Camag, Basel, Switzerland). 20 µl extract was applied to the plates in the form of bands, each 8 mm wide, spacing between consecutive bands being 11.6 mm. Nitrogen gas was used at a low flow rate at 4 bar during spotting. The plates were developed at (23 ± 2) °C in a glass chamber containing toluene:ethyl acetate (=3:1) in which eugenol showed R_f value of 0.6. Spectrum scanning of the bands was carried out in Camag HPTLC unit (TLC scanner III) in the wavelength range of 200–350 nm in which eugenol recorded absorbance maximum at 281 nm. Densitometric studies were thus performed at 281 nm and the areas under the curves of different bands were recorded and the amount of eugenol present in the extracts was determined from the standard curve prepared. The antioxidant activity was determined by measuring the radical scavenging activity of DPPH (Aiyegoro and Okoh, 2010) and expressed as IC_{50} values.

2.2.3. Microencapsulation of clove extract

In the current investigation, the microencapsulation of eugenol-rich clove extract was carried out using Mini Spray Dryer B-290 (M/s Buchi, Switzerland) model in a matrix comprising of maltodextrin and gum arabic. Optimization of batch size and process parameters were set through several experimental trials. The proportion of clove extracts:maltodextrin:gum arabic was varied during preliminary trials and it was found that the ratio of 1:4.8:2.4 of the same gave optimum yield of powder with desirable attributes. 2.5 g clove extracts were added to 100 ml solution containing 12 g maltodextrin and 6 g gum arabic (1:4.8:2.4). The emulsion was created by mixing the solution in an Ultra-Turax homogenizer (M/s Ika, Germany) for 30 s. The inlet temperature was kept at 150 °C and outlet temperature at 86 °C. The air flow rate, sample feed rate and atomization pressure were kept constant at 40 mm, 6.67 ml/min and −45 mbar respectively. The powder obtained was packed in aluminum foils and placed in Ziploc pouches (M/s Johnson, India), flushed with nitrogen and stored at 4 °C until analyzed.

2.2.4. Characterization of encapsulated clove powder

2.2.4.1. Determination of microencapsulation efficiency of the clove powder. For quantifying the total bioactive compounds (TB), the material of the coating structure of the microcapsule was completely destroyed in accordance to the procedure reported by Robert et al. (2010), with little modifications. 500 mg of the encapsulated powder was accurately weighed and added to 50 ml methanol. The dispersion was agitated using a vortex (1 min) and then in an Ultrasonicator (M/s Ika, Germany) for 30 min at 1000 rpm, then centrifuged at 112,000 g for 5 min and finally filtered. The supernatant was analyzed for bioactive compounds such as total eugenol content, total phenolic content and antioxidant activity.

The microencapsulation efficiency (ME) was calculated according to Eq. (1) using the results from total bioactive compounds and theoretical bioactive compounds (Ahmed et al., 2010).

$$\mathrm{ME}\ (\%) = (\text{Total bioactive compounds}/\text{Theoretical bioactive compounds}) \times 100 \quad (1)$$

2.2.4.2. Scanning electron microscopy. The outer structure of the encapsulated powder obtained under optimal conditions was studied by scanning electron microscopy (SEM). The sample was coated with gold using a Vacuum Evaporator (M/s Emitech, Netherlands) and analyzed using ESEM Quantum Mark II (M/s FEI, Netherlands) at 20 kV with a working distance of 9.5 mm. The scanned images were collected digitally using XT Microscope software.

2.2.5. Food application studies using clove extracts

In the current investigation, the food application study entailed shelf life and frying stability studies of soybean oil (most widely consumed edible oil with appreciable ω-3 fatty acid) (Thoenes, 2004) using potato wedges as the model fried food. Since it was opined that the addition of antioxidant could enhance the antioxidative potential of soybean oil (Ghosh et al., 2012); different