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Freeze drying technique for microencapsulation of *Garcinia* fruit extract and its effect on bread quality

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ABSTRACT

Microencapsulation is an enduring technology for protection and controlled release of food ingredients. The *Garcinia cowa* fruit rinds are rich source of (−)-hydroxycitric acid (HCA), which is reported to have various health benefits. But, HCA is hygroscopic in nature and thermally sensitive. Hence, *G. cowa* fruit extract was microencapsulated using three different wall materials such as whey protein isolate (WPI), maltodextrin (MD) and combination of whey protein isolate and maltodextrin (WPI + MD in 1:1 ratio) by freeze drying at 30% concentration. The microencapsulated powders were evaluated for their impact on bread quality and free HCA concentration. The microcapsules exhibited wider particle size range of 15–100 μm and HPLC analysis showed that all the three encapsulates yielded higher free (above 85%) and net (above 90%) HCA recovery. Moreover, bread with WPI encapsulates exhibited higher volume, softer crumb texture, desirable colour and sensory attributes and had higher free HCA concentration. This indicated that WPI has excellent encapsulation efficiency than other two wall materials during bread baking.

1. Introduction

Microencapsulation is a technology for packing solids, liquids or gaseous materials in miniature, sealed capsules to release their contents at controlled rates under specific conditions. It protects the core from adverse environmental conditions, improve shelf life of a product and promote controlled release (Shahidi and Han, 1993). Freeze drying is most suitable technique for dehydration of all heat-sensitive materials and also for microencapsulation (Desai and Park, 2005). It is a multistage operation stabilizing materials through four main operations such as freezing, sublimation, desorption and finally storage (Mascarenhas et al., 1997). The efficiency of protection or controlled release mainly depends on the composition and structure of wall material (Young et al., 1993). Most commonly used wall materials are gum arabic, maltodextrin, emulsifying starches, whey protein, etc. Whey protein possessed unsurpassed nutritional quality and inherent functional properties that meet the demands of encapsulation. Maltodextrin are used as encapsulating material due to their water solubility, low viscosity and low sugar content (Avaltroni et al., 2004). Gum arabic is also an effective encapsulating agent due to its protective colloid functionality. But the cost, limited supply and quality variations of gum arabic have restricted its use for encapsulation and became an another area of research to find alternative biopolymer with same or higher encapsulation efficiency (You-Jin et al., 2003). The blends of biopolymers as wall materials can increase the encapsulation efficiency and shelf life of microcapsules due to interaction of their properties (Perez-Alonso et al., 2009). Hence, the combination of whey protein and maltodextrin were chosen to analyse their encapsulating properties in comparison to them as a single wall material.

During last decades, scientific interest in functional foods has increased due to its protective effects on various degenerative diseases. *Garcinia cowa* is one of the functional foods due to its excellent dietary source of (−)-hydroxycitric acid (HCA) in their fruit rinds (Jena et al., 2002a). Various studies reported that, HCA regulated fatty acid synthesis at a dosage of 1.32 mmoles/kg body weight of rat (Sullivan et al., 1977), lipogenesis at 1500 mg/day (Kovacs and Westerterp-Plantenga, 2006), appetite at 1320 mg/day (Thom, 1996), and weight loss at 1200–2800 mg/day (Ramos et al., 1995; Preuss et al., 2005). It was also attributed to cardioprotection, anti-diabetic effect, correct conditions of lipid abnormalities and enhance endurance in exercise (Jena et al., 2002b). The published literatures had reported several mechanisms of HCA action on weight management such as by inhibition of ATP-citrate lyase, which slows the production of fatty acids, cholesterol, and triglycerides with the net effect of reduced fat production and storage (Clouatre and Rosenbaum, 1994). It also regulates body weight

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through inhibition of malonyl-CoA formation in fatty acid synthesis pathway and regulation of leptin and insulin plasma levels (Hayamizu et al., 2003). But, the native form of HCA (free HCA naturally available in fruit) is thermally sensitive and become lactonized during evaporation and drying (Krishanmurthy et al., 1982), which is biologically less active. Moreover, the hygroscopic nature of HCA makes it unsuitable for its use in tablets, capsules or as powders. Microencapsulation of *Garcinia* fruit extract can convert them into shelf stable particulate powder. Moreover, it prevents the undesirable interactions of bioactive compounds with the carrier food matrix, when incorporated in food stuffs (Champagne and Fustier, 2007). In the previous study, Pillai et al. (2012), investigated the effect of spray drying conditions and wall (whey protein isolate) to core (*Garcinia* fruit extract) ratio on microencapsulation efficiency. Then, microcapsules were incorporated in pasta and found that spray-dried at 90 °C outlet temperature and 1.5:1 wall-to-core ratio exhibited higher antioxidant activity as well as better cooking and sensory characteristics. Incorporation of microencapsulated bioactive compounds into foods is essential to increase their intake and only few studies have performed and analysed their effect on food quality. Moreover, bread is an appropriate foodstuff to incorporate microencapsulates.

The main objective of this study was to investigate the effect of wall materials types (whey protein isolate, maltodextrin and the combination of whey protein isolate and maltodextrin) on microencapsulation of *G. cowa* fruit extract using freeze drying. Further, these encapsulated powders were incorporated into bread to investigate their impact on bread quality and HCA concentration after bread baking.

2. Materials and methods

2.1. Materials

Dried *G. cowa* fruit rinds were obtained from Assam, India and whey protein isolate (WPI) from British Nutrition (Bangalore, India). Maltodextrin (Dextrose Equivalent value as 20) was acquired from Loba Chemie (Mumbai, India). Wheat flour, sugar, fat, salt and yeast, were procured from local market in Mysore, India. All other chemicals and reagents were of analytical grade.

2.2. Microencapsulation of Garcinia extract

One kg dried fruit rinds of *G. cowa* was soaked overnight in water, autoclaved for 30 min, filtered and the extract was concentrated to 30% (w/w-dry weight solid) using a flash evaporator (Buchi, Switzerland) at 60 °C (less HCA degradation due to short time of evaporation under vacuum). Further, concentrated extract was encapsulated using three different wall materials such as whey protein isolate (WPI), maltodextrin (MD) and combination of whey protein isolate and maltodextrin (WPI + MD). 20 g of each wall material was mixed with the 66.6 g of concentrated fruit extract (containing 20 g of solid) and 46.7 g of water to achieve the 30% total concentration and 1:1 wall to core ratio. All the three samples were stirred gently with magnetic stirrer for 30 min to dissolve solid particles prior to freeze drying. Then the samples were freeze dried using pilot scale freeze dryer at −40 to 30 °C. The entire freeze drying process was carried out in 20 h. The microencapsulated powders were collected, packed in polythene bags and stored in a dessicator.

2.3. Moisture content analysis

The average moisture content of encapsulated powders was measured gravimetrically by oven drying method using hot air oven (Industrial and laboratory tools corporation, Chennai, India). A known mass of the sample (0.5 g) was dried in oven for a period of 12 h at 110 ± 2 °C to a constant mass. From the initial and final weights, moisture content of the samples was calculated on wet basis. The test was performed in triplicate and the average values were used to calculate the final moisture.

2.4. Morphology studies

The morphology of encapsulated freeze dried powders was examined using Scanning Electron Microscope (Leo 435 VP, Leo Electronic Systems, Cambridge, UK). The powders were mounted on the specimen holder and sputter-coated with gold (2 min, 2 mbar). Then transferred to the microscope where its images were observed at 15 kV and a vacuum of 9.75×10^{-5} torr.

2.5. Particle size analysis

Particle diameter of encapsulated powders was measured using a laser light diffraction instrument, Malvern Mastersizer (Malvern Instruments, Malvern, UK). Small amount of sample was suspended in iso-butanol (99.9%) using magnetic agitation, and the particle size distribution was monitored during each measurement until successive readings became constant.

2.6. RP-HPLC analysis of HCA

The HCA content (free and lactone HCA) in the feed liquid (*Garcinia* water extract alone) and the encapsulated powders were analysed using reversed phase HPLC (RP-HPLC) according to the method described by Jayaprakasha and Sakariah (2000). An analytical HPLC (1100 series, Agilent technologies, North Point, Hong kong, manual injector and quaternary pump) equipped with C18 column (4.6 × 250 mm) with 5 μm of pore size was used. 20 μl of each sample was manually injected into the column with 8 mM sulphuric acid as mobile phase. Elution of samples and mobile phase were carried out at a flow rate of 0.7 ml/min at 65 bar pressure and 25 °C column temperature under isocratic conditions. HCA detection was performed by a UV–visible spectrophotometer SPD-6AV at a wavelength of 210 nm. On the injection of each sample, free HCA and lactone HCA were identified by means of their specific retention time and peaks were quantified by comparing peak areas with HCA standard peaks [pure HCA was prepared from the calcium salt of HCA (sigma Chemicals, USA) by using ion exchange method to separate the free HCA]. Then analysis of each sample was carried out in triplicates and the average peak area was used to calculate the HCA content in the feed and microencapsulated powder. Further, standard HCA was heated at 90 °C for 60 min and analysed by RP-HPLC to verify the heat induced lactone HCA formation.

2.7. Microencapsulation efficiency

The free HCA recovery (free HCA alone) and net HCA recovery (free HCA and lactone HCA) during freeze drying were obtained from the HCA content of the feed liquid and encapsulated powders. Hence, the microencapsulation efficiency (net HCA recovery) was estimated using the following formula:

$$\text{Encapsulation efficiency } (\%) = (\text{HCA content in powder}/\text{HCA content in feed liquid}) \times 100 \quad (1)$$

2.8. Bread making

Microencapsulated powders were incorporated into wheat flour along with other ingredients for bread preparation. Wheat flour and *Garcinia* powder (with and without encapsulation) were taken in the ratio of 100: 2 for bread making. Based on the screening study