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Development and evaluation of novel flavour microcapsules containing vanilla oil using complex coacervation approach

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ABSTRACT

A novel flavour microcapsule containing vanilla oil (VO) was developed using complex coacervation approach, aimed to control release of VO and enhance its thermostability for spice application in food industry. Viscosity of chitosan (CS) and VO/CS ratio were optimised for fabrication of microcapsules. The flavour microcapsules were evaluated by scanning electron micrograph (SEM), laser confocal microscopy (LSCM), particle size analyser, infrared spectrometer (FT-IR), thermal analysis and controlled-release analysis. The microcapsules were in spherical with good dispersibility when moderate viscosity CS was used. 94.2% of encapsulation efficiency was achieved in VO/CS ratio of 2:1. The FT-IR study proved chemical cross-linking reaction occurred between genipin and chitosan, but a physical interaction between CS and VO. A core–shell structure of microcapsule was confirmed by LSCM, which was beneficial to improve the thermostability of VO in microcapsule. Moreover, VO could be remained about 60% in the microcapsules after release for 30 days, which demonstrated the flavour microcapsules had good potential to serve as a high quality food spice with long residual action and high thermostability.

1. Introduction

Flavours play important roles in consumer satisfaction and influence further consumption of foods (Madene, Jacquot, Scher, & Desobry, 2006). However, the key factors that limit flavour industry development are the long residual action and thermostability of flavour and fragrance (Xiao, Yu, & Yang, 2011). Vanilla is one of the valuable natural flavour plants, it is known as the 'flavour queen', which is widely used in food, cigarettes, high-grade cosmetic and pharmaceutical products, etc. (Brunschwig et al., 2012; Helena et al., 2013). In food industry, vanilla was used as flavour enhancer for wine, tea, chocolate, candy, pastry, dairy products and others foods to make them rich with cream and sweet scent. Green vanilla beans are odourless and flavourless. Characteristic vanilla flavour in beans is only formed during curing process, which results in 2% vanillin and over 170 other compounds with delicate sweet fragrance (Mark, Dignum, & Rob, 2002). There are a lot of methods for vanilla curing, but they include at least four stages, including deactivation of enzymes, fermentation, drying and maturing. The curing process usually last for more than six months, and most of the flavour compositions lose during this process because the volatility and instability of the flavour, which lead to the low yield and high price of vanilla flavour (Mark et al., 2002). Therefore, it is necessary to develop a new vanilla flavour with long residual action and high thermostability for further applications.

Vanilla oil is extracted from the vanilla plant, specifically, vanilla oil comes from the plant's green fruit, it is widely used in perfume, body oil and other holistic health or beauty treatments. However, because the effective component of vanilla oil is volatile heavily and is not stable in the presence of air, light, moisture and high temperature, which make the flavour of vanilla oil not sustainable and limits its application. Therefore, how to effectively reduce the volatility and improve the thermostability of vanilla oil is the critical in solving those challenges and expand its applications.

Recently, microencapsulation technology has been widely applied in pharmaceutical, food, agricultural pesticide, cosmetic, textile, and other related fields. It provides an effective method in controlled release, or protection of active substances (Kashappa & Hyun, 2005; Madene et al., 2006; Brisa, Cinta, Gemma, Ricard, & Tania, 2012). Microcapsules offer food process means with which to protect sensitive food components, ensure against nutritional loss, utilise otherwise sensitive ingredients, controlled-release, mask or preserve flavours, and transform liquids into easily handled solid ingredients (Wen, Chih, & Gao, 2006; Huang, Wei, & Wang, 2010; Jain, Kesharwani, Gupta, & Jain, 2012). Reactive, sen-

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sitive, or volatile additives (vitamins, culturelle, flavours, etc.) can be turned into stable ingredients through microencapsulation. With carefully fine-tuned controlled-release properties, microencapsulation is no longer just an added-value technique, but the source of totally new ingredients with matchless properties (Yang, Song, Li, Fan, & Yang, 2004; Herrero, Del Valle, & Galan, 2006; Polavarapu, Oliver, Ajlouni, & Augustin, 2011). The microencapsulation of vanilla oil brings many advantages: (1) provides the means of converting liquids to solids (2) altering colloidal and surface properties; (3) providing environmental protection and, (4) controlling the flavour release for a long time. Hence, to develop flavour microcapsules by microencapsulation of vanilla oil can confer special attributes to vanilla oil, and enhance its value in certain specialised application.

The methods that for fabrication of microcapsules have been widely reported, including the spray drying method, phase separation, interfacial polymerisation and extrusion method, etc. (Ma et al., 2009; Adem et al., 2012). Complex coacervation is a phase separation process based on the simultaneous desolvation of oppositely charged polyelectrolytes induced by media modifications. Complex coacervation is a unique and promising method for microencapsulation because of the very high payloads achievable and the controlled release possibilities based on mechanical stress, temperature or sustained release (Reineccius, 1989). Microcapsules produced by complex coacervation method possess excellent controlled release characteristics, heat resistant properties, and high encapsulation efficiency. However, a challenge issues with complex coacervation is the burst release effect, aggregation and conglutination problems, which is not desirable in applications of most microcapsule. In addition, a toxic aldehyde curing agent is used in most of microcapsule preparation process, such as formaldehyde and glutaraldehyde, which cause the toxic material residue problem in the microcapsule products and also restrict its use in a wide-range application (Yeo, Bellas, Firestone, Langer, & Kohane, 2005; Dong, Ma, Hayat, Liu, & Zhang, 2011). The wall materials for fabrication of microcapsules could be chosen from a wide range. Gelatin and arabic gum are the common used wall materials for complex coacervation approach, but gelatin is quite viscous even in low concentrations, which lead to the aggregation and conglutination problem in microcapsule preparation process (Chan, Lim, & Heng, 2000; Nickerson, Patel, Heyd, Rousseau, & Paulson, 2006). Among different wall materials to prepare controlled release microcapsules, the use of natural polymer chitosan has gained much attention. Chitosan is a biocompatible, biodegradable, cheap and non-toxic polymer; which makes it attractive for applications in medicine and food industry.

The present study focuses on the formulation and evaluation of novel flavour microcapsules containing vanilla oil using complex coacervation approach, with chitosan and arabic gum as the wall material, genipin as a nontoxic curing agent, which aims to controlled release of vanilla oil and to achieve a flavour microcapsule with long residual action, high thermostability and non-toxic characteristics, so as to expand the applications of vanilla oil in food industry.

2. Materials and methods

2.1. Materials

Chitosan (deacetylation degree $\geqslant$90%) were purchased from AK Biotech Ltd. (Jinan, China). Arabic gum was obtained from Guangzhou Southern-Based Companies (Guangzhou, China). Vanilla oil was provided by Natural Medicinal Oil Refinery Company (Nanchang, China). Vanillin, span-83, sodium hydroxide and acetic acid were purchased from Zhanjiang Xinmao Chemical-Glass Com. Ltd (Zhanjiang, China). All other chemicals and reagents were of analytical grade.

2.2. Preparation of flavour microcapsules

1.0% (w/v) of chitosan solution was prepared by dissolving 1.0 g of chitosan in 100 mL of 1% (v/v) acetic acid, and heated in water bath at 50 °C. A proper amount of vanilla oil was added into the chitosan solution. Then, 100 mL arabic gum solution (2.0%, w/v), which was prepared in distilled water, and 0.6 g span-83 were added to the mixture. The system was kept stirring at 5000 rpm for 5 min to form O/W emulsion. To start complex coacervation, the stirring speed was set to 400 rpm, and the pH value of the mixture was adjusted from 6 to 3 with 10 wt% sodium hydroxide solution. After stirring for 30 min, the mixture solution was cooled to 0–5 °C by ice bath, and the pH value was adjusted to 7 with sodium hydroxide solution. After that, 5 mL genipin ethanol solution (25 wt%) was added into the mixture. The microcapsules were collected by centrifugation with $1 \times 10^4 g$ of relative centrifugal force (RCF) for 5 min. Finally, they were freeze–dried at −50 °C for 24 h after being purified with distilled water for two times.

2.3. Characterisation of flavour microcapsules

The surface morphology of microcapsules was examined by scanning electron microscopy (SEM). Dry microcapsules were fixed on metal stubs with double sided tape, and coated with gold by a gold sputter coater in a high-vacuum evaporator. Then Samples were examined on a scanning electron microscope (S-4800, Hitachi, Japan) at an acceleration voltage of 20 kV. FT-IR spectrum of samples were examined on a FT-IR instrument (GX-1, PerkinElmer, USA) from 4000 to 400 cm^{-1}. The particles size and size distribution of flavour microcapsules were determined using a laser particle size analyser (Mastersizer 2000, Malvern, UK). The confocal laser scanning microscope (CLSM) was used to visualise the vanilla oil distribution within the microcapsules and study the internal structure of the flavour microcapsules. Samples were observed with a Leica TCS-SP2 confocal laser scanning microscope at excitation wavelength of 485 nm. The thermal properties of flavour microcapsules were test by differential thermal-thermogravimetric analyser (DTA-TG 209, NETZCH, Germany) at range from 20 to 600 °C at a heating rate of 10 °C/min.

2.4. Ultraviolet standard calibration curve of vanilla oil

Vanilla oil-hexane solutions with different concentration were prepared to measure the absorbance of each solution. The absorbance of vanilla oil was determined at $\lambda = 278$ nm which was the maximum absorption wavelength of vanilla oil. The standard calibration curve was drawn along with x-axis as the concentrations of vanilla oil, y-axis as the amount of absorbance. Equation of the standard curve was $Y = 0.21569X + 0.00512$, correlation coefficient was R = 0.99812, and excellent linearity relationship was obtained.

2.5. Encapsulation efficiency and loading capacity of flavour microcapsules

The encapsulation efficiency and loading capacity of microcapsules were determined by an ultraviolet standard calibration curve method. Firstly, the microcapsules were washed with ethanol for two times to remove the surface oil before extraction. Then, 0.1 g dried microcapsules were added to 100 mL n-hexane and placed in a water bath at 37 °C. The mixture solution was treated with ultrasonic for 1 h and set in a 37 °C shaking table with stirring of 150 rpm to extract the vanilla oil completely from the microcapsules. Then the insoluble substance in the mixture solution was