Food Hydrocolloids 77 (2018) 152–162

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Nanoencapsulation of D-limonene within nanocarriers produced by pectin-whey protein complexes

Sanaz Ghasemi, Seid Mahdi Jafari*, Elham Assadpour, Morteza Khomeiri

Faculty of Food Science and Technology, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

ARTICLE INFO

Article history:
Received 12 August 2017
Received in revised form
22 September 2017
Accepted 22 September 2017
Available online 26 September 2017

Keywords:
D-limonene
Complex nano carriers
Whey protein
Pectin
Encapsulation

ABSTRACT

D-limonene as a volatile compound is widely used in the food flavorings and it is chemically unstable in the presence of air, light, moisture, and high temperatures. Biopolymer nanocomplex formation between negatively charged polysaccharides and positive proteins is a novel encapsulation technology for protecting of bioactive components. In this study, D-limonene was nanoencapsulated with whey proteins (4, 6 and 8% w/w), and pectin (0.5, 0.75 and 1% w/w) at different pH values (3, 6 and 9). Our results revealed that nanocomplexes prepared with 4% WPC and 1% pectin in pH = 3 had the lowest stability and highest viscosity as well as the highest L* which was selected as the optimum treatment. Atomic force microscopy images showed the morphology of these WPC-pectin nanocomplexes as spherical nanoparticles with an average size of 100 nm. The encapsulation efficiency was determined to approximately 88%. Results of dynamic light scattering for produced nanocomplexes loaded with D-limonene revealed a particle size of about 160 nm and a zeta potential of −0.53 mV. It was found that a ratio of 4 to 1 between WPC and pectin came up with the highest complex formation.

1. Introduction

D-Limonene is one of the important flavors which is used extensively in the food industries. This flavor is extracted from citrus peels. It is volatile in the presence of light, air, moisture and high temperatures and chemically, it is an unstable compound (Madene, Jacquot, Scher, & Desobry, 2006). So nowadays, the researchers are interested to apply some techniques for protection of this volatile compound from decomposition and degradation during processing and shelf life (Jun-xia, Hai-yan, & Jian, 2011). Encapsulation is one of the most important technologies, which is applied in the food industry in order to improve chemical stability of food bioactive ingredients, targeted release of them into the product and preventing adverse interactions with food components.

Different types of biopolymers are capable of binding and encapsulating flavors (both water-soluble to water-insoluble) and creating molecular complexes including polysaccharide-polysaccharide, protein-protein, and protein-polysaccharide (nano) complexes. Polysaccharide-protein nanoparticles are taken more into consideration than pure single biopolymer nanoparticles, due to the synergistic combination between the functional groups of various biopolymers and their higher chemical and colloidal stability (Matalanis, Jones, & McClements, 2011; Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998). So, complexation is a highly desirable and promising nanoencapsulation technique for protection of flavors for engineered delivery to enable subsequent fortification of food products (Assadpour & Jafari, 2017; Assadpour, Jafari, & Maghsoudlou, 2017; Esfanjani, Jafari, Assadpoor, & Mohammadi, 2015). When proteins and polysaccharides are mixed together in a liquid medium, four types of complexes can occur depending on the charge of both biopolymers and effective factors such as pH and ionic strength: Incompatibility and co-solubility that occurs in pH > pI; and soluble and insoluble complexation (coacervation) that occurs in pH < pI (Weinbreck, Nieuwenhuijse, Robijn, & de Kruif, 2004). This complex formation is controlled by environmental conditions and biopolymers features as a function of intrinsic and extrinsic molecular key parameters including molecular weight, concentration, steric conformation, size homogeneity, charge amount and distribution, ion type, ionic strength, pH, temperature, shearing effect, pressure, solvent quality, and various molecular interactions within or between different biopolymers (Goh, Sarkar, & Singh, 2008, pp. 347–376).

The complexation of proteins and polysaccharides such as β-lactoglobulin (β-Lg) and pectin can be applied to encapsulate hydrophilic and hydrophobic nutraceuticals including different vitamins, flavors, essential oils, fatty acids, etc. (Zimet & Livney,

* Corresponding author.
E-mail address: smjafari@gau.ac.ir (S.M. Jafari).

https://doi.org/10.1016/j.foodhyd.2017.09.030
0268-005X/

2009). So in the present study, whey protein and pectin were applied to nanoencapsulate D-limonene flavor by complexation method. Whey protein concentrate (WPC) is almost an ivory mixture of different globular proteins including β-Lg being the most principal followed by α-lactalbumin. WPC includes both hydrophobic and hydrophilic chains and both charged and uncharged amino acids (Assadpour, Maghsoudlou, Jafari, Ghorbani, & Aalami, 2016a). Pectin is one of the most popular polysaccharides, which is formed from galactronic acid units with alpha-D 1 to 4 connectors and often is esterified with methanol (Wagoner & Foegeding, 2017). There are many investigations about production and application of nano and micro complexes of different proteins like caseinate, β-Lg, zein, gelatin, etc. with anionic polysaccharides such as pectin, Arabic gum, alginate, and carrageenan (Harnsilawat, Pongsawatmanit, & McClements, 2006; Peinado, Lesmes, Andrés, & McClements, 2010; Ye, Flanagan, & Singh, 2006; Zimet & Livney, 2009). In a study by Ilyasoglu and El (2014) revealed that stable protein–polysaccharide complexes can be applied for nanoencapsulation of hydrophobic compounds such eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) to enrich non- or low-fat beverages. They utilized sodium caseinate and gum Arabic for encapsulation of fish oil, as a source of EPA and DHA with multilayered interfacial membranes. Firstly, the optimum conditions for the formation of stable complexes were determined at pH = 4 and at a concentration of 0.1% w/v sodium caseinate and 0.2% w/v gum Arabic x for complex nanoencapsulation of fish oil. Particle size and encapsulation efficiency were obtained almost 232.3 nm and 78.88 ± 2.89%, respectively. Then, the fish oil loaded-nanocomplexes containing 40–50–60 mg EPA + DHA were applied in the fortification of 100 ml fruit juice. After in vitro digestion, the bioaccessibility of EPA, DHA and EPA + DHA were found as 56.16 ± 6.39, 36.25 ± 5.38 and 47.37 ± 10.65 percent, respectively ($p < 0.05$). In another study, Ron, Zimet, Bargarum, and Livney (2010) investigated the encapsulation of vitamin D2 by β-Lg alone or by a complex between β-Lg and pectin. It was found that the nanocomplexes of β-Lg-pectin provided better and more significant protection to the vitamin against degradation. The optimal samples obtained were transparent, and may be suitable for enrichment of non-fat clear acid beverages. Jun-xia et al. (2011) in their study encapsulated sweet orange oil by formation of a complex between soybean protein isolate (SPI) and gum Arabic (GA) as a function of ionic strength, pH, SPI/GA ratio, and core material load. The optimum pH for the formation of SPI-GA complex coacervation was obtained at pH = 4.0. Their results showed that higher ionic strengths decreased the formation of coacervation between two applied biopolymers. The ratio of 1:1 (SPI: GA) had the highest coacervate yield with a core material load of 10%. The results of SEM and GC-MS analysis revealed the spherical particles without any holes on the surface and well retaining the flavour component within microcapsules, respectively which resulted in good production for core material.

In the present work, production of D-limonene-loaded nanocomplexes was studied by low methoxyl pectin-WPC as a function of pH (3, 6 and 9), pectin content (0.5, 0.75 and 1%) and WPC content (4, 6 and 8%). Our main goal was to produce an optimized nanocomplex based on the viscosity, color, and stability. Then atomic force microscopy, encapsulation efficiency, size and zeta potential of particles were analyzed to confirm the successfulness of nanocomplex encapsulation of D-limonene.

2. Materials and methods

Orange peel oil was purchased from Ramsar Citrus Concentrate Co. (Iran) which had approximately 96% D-limonene after analyzing by GC method. Whey Protein Concentrate (WPC; 80 w/w % protein, 3.5% ash, 6% moisture and 0.45 g/cm3 bulk density), citrus low methoxyl pectin (LMP) and maltodextrin (MD; DE = 16–20) as applied biopolymers were obtained from Arla (Denmark), Sigma-Aldrich Co. (USA), and Qinhuangdao (China) respectively. Tween 80 (Sigma), a non-ionic surfactant, was applied as the emulsifying agent. For preparation of all the solutions, deionized water was used.

2.1. Preparation of biopolymer solutions

LMP powder (0.5, 0.75, 1 g) was dissolved in hot deionized water (70 °C) to prepare 100 ml solutions. At the same time, different aqueous solutions of WPC were prepared by dispersing 4, 6 and 8 g of WPC powder into deionized water to obtain 100 ml solutions. To increase the total soluble solids of the samples for obtaining higher powders, 50 g maltodextrin (at constant ratio) was dissolved in deionized water to prepare 100 ml solutions. These solutions were slightly stirred for at least 30 min on a magnetic stirrer (IKA, Germany), and stored overnight at 4 °C to complete hydration of biopolymers (Ghasemi, Jafari, Assadpour, & Khomeiri, 2017).

2.2. Preparation WPC - pectin nanocomplexes loaded with D-limonene

The prepared WPC, LMP, and maltodextrin solutions were mixed together with the same volume and stirred for at least 30 min on a magnetic stirrer. Then, Tween 80 at a ratio of 10% of the total solids was added into the solution and mixed to solve completely. Finally, during homogenization of the prepared aqueous solution by an ultrasonic homogenizer (Iranian Ultrasonic Technology Company, 400 W, 20 kHZ, 12 mm probe diameter) at 25 °C (temperature was controlled by a container of ice and water) and the power of 350 W for 10 min, D-limonene was added into this solution gradually. pH of the solutions was adjusted to predetermined pH values (3, 6, 9) using HCL and NaOH (0.1 and 1 N) (Assadpour, Maghsoudlou, Jafari, Ghorbani, & Aalami, 2016b; Jafari, He, & Bhandari, 2007).

2.3. The stability measurement of nanocomplex solutions

For measuring the stability of nanocomplex solutions, the accelerated method was applied by centrifugation (3k30, Sigma, USA) at the temperature of 25 °C and 20,000 g for 30 min 50 ml of nanocomplex solution (primary nanocomplex volume) was centrifuged and then, the volume of remained stable nanocomplex was determined by a graduated cylinder. Then the percentage of stability was calculated with Eq. (1). (Ghasemi et al., 2017; Zimet & Livney, 2009; Zimet, Rosenberg, & Livney, 2011).

$$\text{Stability}\ (\%) = \frac{\text{The volume of nanocomplex remained stable}}{\text{The volume of primary nanocomplex}} \times 100 \quad (1)$$

2.4. Viscosity measurement of D-limonene-loaded nanocomplex solutions

In order to measure the viscosity of the samples, 16 ml of each sample transferred into the cylinder then the viscosity was determined using a Brookfield viscometer (LVDV Pro II, Brook-field Engineering Laboratories, spindle S00, USA) at the temperature of 25 °C and shear rate of 18.3 1/s.