LWT - Food Science and Technology 73 (2016) 458–466

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Development of a novel functional drink from all natural ingredients using nanotechnology

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ARTICLE INFO

Article history:
Received 29 January 2016
Received in revised form
7 June 2016
Accepted 22 June 2016
Available online 23 June 2016

Keywords:
Functional drink
Nanotechnology
Sodium caseinate
Pectin
Antioxidant activity
Nano spray drying

ABSTRACT

In this work, nanotechnology was applied to formulate a novel functional drink from all natural food ingredients and a complete formulation of nutrients, including both hydrophilic and hydrophobic vitamins as well as mineral salts, was developed. Peppermint oil dissolved in propylene glycol together with fat-soluble vitamins was first emulsified in aqueous phase with sodium caseinate as natural emulsifier to obtain nanoemulsion. Then sodium caseinate was coated with pectin to form nanocomplex particles, via electro-deposition induced by pH adjustment and heating treatment. The formulations were systematically optimized in terms of various physicochemical properties, including particle size, polydispersity index, and zeta potential. The optimal formulations, which were prepared at pH 4.5, exhibited the particle size around 250 nm with PDI < 0.25, and the zeta potential of −22 mV. The functional drink was able to retain the antioxidant activity of encapsulated nutrients during storage at both refrigeration and room temperature for up to 15 days. The functional drink was also spray dried to obtain powders with excellent redispersibility in water. The present study suggest that this nanotechnology-enabled formulation is able to simultaneously incorporate both hydrophilic and hydrophobic nutrients as well as mineral salts in a single product with great potential of health-promoting benefits.

1. Introduction

Functional foods are defined as the food products that have advantageous physiological effects beyond their basic function to satisfy hunger and provide necessary nutrients for humans. In the last decades, consumer demands in the field of functional foods have significantly increased due to the increasing desire for a healthy life style. The global functional food market is estimated to be at least $33 billion in of 2000 (Hilliam, 2000) and reached nearly $61 billion in 2004 (Benkouider, 2004), and continues to increase in recent years. Functional foods are usually manufactured and processed by either enriching or fortifying with vitamins and/or minerals and other natural antioxidants, such as polyphenols. Examples of functional foods include, but are not limited to, prebiotics, probiotics, functional drinks, functional cereals, bakery products, spreads, etc., among which functional drinks or beverages are the major market share constituting more than 30% of total sales in the US and Europe (Menrad, 2003). However, despite the continuous sale growth in this field, the functional food segment is characterized by a high rate of product failure, due to the limitations in manufacturing technology to develop high-quality functional food products (Siro, Kapolna, Kapolna, & Lugasi, 2008).

As the advancement in the area of food science and technology in recent years, many novel techniques are now becoming available for food industry to overcome many technological obstacles. Nanotechnology and encapsulation are the two emerging technologies that enable food scientists to achieve many innovations in the segment of functional food production. Particularly, encapsulation of lipophilic vitamins into nanoemulsion or polymeric nanoparticles can greatly increase their stability and shelf-life when incorporated into functional food products. For instance, vitamin E nanoparticles with an average size of 100 nm obtained by high pressure homogenization and had a better stability and a 9-fold prolonged shelf-life compared to the commercial vitamin E beverage products (Chen & Wagner, 2004). The small size of the nanoemulsion ensured a weak light scattering of final product and therefore addition of vitamin E nanoemulsion did not visibly alter the appearance of the final beverage product. High transparency is a very favorable and appealing characteristics of nanoemulsion for

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http://dx.doi.org/10.1016/j.lwt.2016.06.050
0023-6438/

applying them as nutrient carriers in beverages industry. A variety of many other nutrients have been studied by encapsulation and nanotechnology that exhibited great potentials in their applications in functional foods (Luo, Teng, & Wang, 2012; Luo et al., 2013; Teng, Luo, & Wang, 2013).

Casein is a milk protein that has numerous applications in the encapsulation of nutrients and drugs, owing to its unique capability to self-assemble into stable micellar structure in aqueous phase (Elzoghby, Abo El-Fotoh, & Elgindy, 2011). Pectin is a naturally-occurring anionic polysaccharide with excellent gelling properties and can stabilize casein micelles in acidified milk to fulfil delivery applications whenever an acidic condition is needed (Laurent & Boulenguer, 2003). Pectin is able to adsorb onto casein micelles at pH 3.5–5.0 and form complex microparticles, driven by electrostatic attractions between the carboxylate groups of pectin and cationic amino acid residues of casein (Rediguieri, de Freitas, Lettinga, & Tuinier, 2007; Tuinier, Rolin, & De Kruif, 2002). It is further demonstrated in a recent study that by modulating acidification rate and controlling casein/pectin concentration, nanoscale casein/pectin complex particles were formed with a diameter of 200–400 nm (Luo, Pan, & Zhong, 2015). With rutin, a natural flavonoid, being studied as a model lipophilic bioactive compound, the casein/pectin nanocomplex showed promising features for oral delivery of nutrients that can be incorporated into an acidic functional beverage or drink.

In this work, the major goal is to develop a complete formulation for a functional drink, which consists of both lipophilic and hydrophilic vitamins, minerals, and amino acids, as well as antimicrobial agent, using casein/pectin nanocomplex particles as a delivery system. Specifically, peppermint oil was selected as a natural antimicrobial agent due to its edible and GRAS (generally recognized as safe) status and potent antimicrobial activity (Hammer, Carson, & Riley, 1999). Peppermint oil has been previously studied in nanoemulsion systems which are intended for food safety applications (Liang et al., 2012), but rarely incorporated into functional drink formulations. The first objective was to optimize formulations and fabrication conditions, including two concentrations of casein/pectin and three pHs, and compare their capacity to form nanocomplex for encapsulation of incorporated nutrients. The physicochemical properties, including particle size, polydispersity index (PDI), zeta potential and storage stabilities were comprehensively investigated. The second objective was to study the feasibility of two drying techniques, i.e. freeze drying and nano spray drying, to obtain dry powders and their respective redispersibility. It is worth mentioning that nano spray drying is an innovative spray drying technology that utilizes an array of precise, micron-sized mesh that vibrates at 60 kHz to produce millions of tiny droplets per second which are then dried by a laminar airflow system and collected by electrostatic particle collector. This novel technology has been recently validated for production of protein and polysaccharide nanoparticles for pharmaceutical industries (Lee, Heng, Ng, Chan, & Tan, 2011; Li, Anton, Arpagaus, Belleteix, & Vandamme, 2010; Schmid, Arpagaus, & Friess, 2011).

2. Materials and methods

2.1. Materials

Sodium caseinate from bovine milk and pectin from citrus peel (Galacturonic acid ≥ 74.0%) were purchased from Sigma-Aldrich Corp (St. Louis, MO, USA). AAPH [2,2′-azobis-(2-amidinopropane) dihydrochloride] and ABTS [2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt] were also purchased from Sigma-Aldrich. Propylene glycol (PG), citrate acid and sodium hydroxide were purchased from Fisher Scientific (Pittsburgh, PA, USA). All vitamins, minerals, and branched-chain amino acids (BCAA) were purchased from Hard Eight Nutrition LLC (Henderson, NV, USA). Peppermint oil was obtained from Piping Rock Health Products, LLC (Ronkonkoma, NY, USA). Unless noted otherwise, all reagents and solvents were of analytical grade and used without further purification.

2.2. Preparation of nanoemulsion

The preparation procedure was followed by a two-step process, i.e. nanoemulsion formation and heat-induced complexation process (Fig. 1). The nanoemulsion were first prepared by a high-speed homogenization, followed by a pH-reduction and thermal treatment to form complex nanoparticles. The formulation of nanoemulsion is shown in Table 1. Briefly, fat-soluble vitamins (Vitamin D3 and E) were added to the mixture of 1 mL PG (containing 200 μL peppermint oil) and 4 mL purified water to form oil phase (5 mL). The above oil phase was heated to 80 °C for about 5 min in water bath to help vitamins to dissolve in PG and peppermint mixture completely, followed by cooling down to room temperature. All water-soluble vitamins (vitamin B3, B6, B12 and C) and BCAA were completely dissolved in pectin solution. Then, the pH of the above pectin solution was adjusted to 6.8 followed by addition of NaCas solution, and the total volume was made up to 75 mL with purified water to form aqueous phase. The oil phase was then poured into aqueous phase under continuous homogenization (25,000 rpm) for 3 min at room temperature using a high-speed homogenizer (ULTRA-TURRAX T18, Ika Inc., Wilmington, NC, USA). Then, the pH value of the above nanoemulsion was adjusted to either 5, 4.5 or 4, by addition of 1 M citric acid drop by drop, followed by a thermal treatment at 80 °C for 30 min and then cooling to 4 °C immediately in ice bath. This novel technique has been previously reported to prepare NaCas/pectin nanocomplex particles to encapsulate lipophilic bioactive compounds (Luo et al., 2015). The salts were pre-dissolved in 15 mL 0.33 M citric acid solution, and its pH was adjusted to the same pH as above nanocomplex particles (pH 5, 4.5, or 4, using pre-weighed NaOH pellets) and then pure water was added to make up the total volume to 20 mL (final pH was checked and adjusted, if necessary). The above mixture of salts (20 mL) was then added into the nanocomplex particles (80 mL), and thus the final volume of the enhanced water was 100 mL.

2.3. Characterizations

The particle size, PDI, and zeta potential of nanoemulsion were measured by Zetasizer Nano ZS (Malvern Instruments Ltd, Worcestershire, UK) at 22 °C. Particle size was determined by dynamic light scattering (DLS) at a scattering angel of 173 °C. Samples were diluted 10 times with pure water to avoid any effects of multiple scattering. Polydispersity index (PDI) was measured together with particle size and used to evaluate the homogeneity of nanoparticles in the colloidal dispersion. Zeta potential, which is one of the fundamental parameters to affect the stability of colloidal system, was measured and calculated from electrophoretic mobility of the nanoparticles using the same ZetaSizer Nano ZS.

2.4. Drying and redispersion process

The final samples prepared at pH 4.5 (formulation 1 and 2) were either freeze dried or spray dried. Freeze-drying was performed using a Labconco FreeZone 6 Freeze Dry System (Kansas City, MO, USA), under operation condition of −80 °C/0.014 mBar for 24 h. Samples were first frozen at −80 °C in a freezer for at least 12 h before freeze-drying. Spray drying was performed using a Nano Spray Dryer B-90 (Büchi Labortechnik AG, Flawil, Switzerland) with