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Effect of spray drying and freeze drying on the immunomodulatory activity, bitter taste and hygroscopicity of hydrolysate derived from whey protein concentrate

Jing-Jiao Ma [a], Xue-Ying Mao [a,*], Qian Wang [a], Shu Yang [a], Dan Zhang [a], Shang-Wu Chen [a], Ying-Hui Li [b]

[a] College of Food Science and Nutritional Engineering, Key Laboratory of Functional Dairy, Ministry of Education, China Agricultural University, 100083 Beijing, China
[b] China Astronaut Training Center, Beijing, China

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ABSTRACT

The main aim of the present work was to obtain microencapsulated whey protein concentrate hydrolysate (WPCH) in order to reduce its bitter taste and resistance to hygroscopicity without impairing its immunoregulatory activity by spray drying or freeze drying with whey protein concentrate (WPC) and sodium alginate (SA) as carriers. To attenuate its bitter taste, the WPCH were encapsulated with WPC or the mixture of WPC and sodium alginate (WPC/SA). The splenocyte proliferation activity, hygroscopicity, bitter taste and morphology of non-encapsulated WPCH and encapsulated WPCH were evaluated. Results revealed that WPCH could significantly enhance splenocyte proliferation activity compared with WPC itself. Both spray drying and freeze drying with or without carrier material addition did not exert negative effect on the immunomodulatory activity of WPCH. The bitterness, determined by taste dilution analysis method, of both WPC-encapsulated WPCH and WPC/SA-encapsulated WPCH was significantly lower than that of the original non-encapsulated WPCH. Morphological analysis showed that freeze drying process could not encapsule WPCH as spray drying did. All of these indicated that spray drying with WPC or the mixture of WPC and SA (WPC/SA) as carriers was beneficial for reducing the bitter taste and hygroscopicity without impairing the immunoregulatory activity of whey protein hydrolysate.

1. Introduction

Unhealthy lifestyles and stress may have a negative cumulative effect on immune system, which increases the risk for cardiovascular, metabolic and autoimmunity diseases, even cancer. Peptides derived from whey proteins could modulate a variety of immune functions including splenocyte proliferation, cytokine secretion, antibody production and phagocytic activity (Gauthier, Pouliot, & Saint-Sauveur, 2006). Saint-Sauveu, Gauthier, Boutin, and Montoni (2008) reported that peptides derived from whey protein isolate stimulated splenocyte proliferation in the presence of ConA and significantly increased IFN-γ section. Other researches also indicated that hydrolysate of whey protein isolate could enhance splenocyte proliferation activity (Fan, Gong, Qu, Miao, & Liu, 2012; Mercier, Gauthier, & Fliss, 2004). Milk protein derived peptides possessed antihypertensive, antimicrobial and antioxidant activities besides immunomodulatory effect (Hernández-Ledesma, Ramos, & Gómez-Ruiz, 2011; Korhonen & Pihlanto, 2006; Mao, Nan, Li, & Ni, 2005; Wang et al., 2012).

However, bioactive peptides produced during hydrolysis process may possess bitter taste and high hygroscopicity due to the release of hydrophobic and hygroscopic amino acid residues, which frustrates their direct utilization to food processing (Kato, Rhue, & Nishimura; 1989; Yang et al., 2012). Thus, it is imperative to find an appropriate processing technology to reduce the bitterness and hygroscopicity of peptides. Several methods have been tried to reduce the bitterness of protein hydrolysate, such as removal of hydrophobic peptides, the key determinant of bitterness, along with hydrolysis process (Komai, Kawabata, Tojo, Gocho, & Ichishima, 2007) or reducing bitterness by processing technology (Favaro-Trindade, Santana, Monterrey-Quintero, Trindade, & Netto, 2010). To find a practical and widely used processing approach to mask or reduce the unpleasant bitter taste of protein hydrolysate without impairing its biological activities is of great importance.

* Corresponding author. Tel.: +86 1062738684.
E-mail addresses: maoxueying@cau.edu.cn, wumaoxy@163.com (X.-Y. Mao).

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Microencapsulation technology has been widely used for decades to encapsulate food ingredients such as flavors, lycopene and lipids (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). Spray drying is the most extensive microencapsulation technology due to its wide availability of equipments, low processing costs and good stability of final products (Gharsallaoui et al., 2007). It is very important to choose proper carriers for microencapsulation, which affect encapsulation efficiency and microcapsule stability (Goula & Adamopoulos, 2005). Several carriers such as gums, maltodextrin, soy protein isolate, pectin and β-cyclodextrin were successfully applied in microencapsulation process to attenuate the bitterness and hygroscopicity of the products (Fuchs et al., 2006; Lopez-Rubio & Lagaron, 2012; Mendanha et al., 2009; Yang et al., 2012). Alternatively, the high nutritional value and excellent functional properties of food proteins allow them to be good candidates of carrier materials (Landy, Druaux, & Voilley, 1995). Milk proteins and soy proteins are the most commonly used proteins for encapsulation. The bitterness, hygroscopicity and stability of casein hydrolysates that encapsulated by complex coacervation with soybean protein isolate (SPI)/gelation were reduced (Favaro-Trindade et al., 2010). Whey proteins have been successfully used as carriers to encapsulate anhydrous milk fat by spray drying and the encapsulation yield was higher than 90% (Young, Sarda, & Rosenberg, 1993). The mixtures of maltodextrin and WPC were used as carriers in the microencapsulation of flaxseed oil by spray drying, and they performed well in protecting the active substances against oxidation during storage (Carneiro, Tonon, Grosso, & Hubinger, 2013). In addition, freeze drying can ensure the physicochemical and bioactive stability of peptides, but it usually takes an enormous amount of time, labor and expenses (Wang, 2000). Therefore, the effect of spray drying instead of freeze drying on the bioactivity of peptides needs further study.

The aim of the present study was to evaluate the *in vitro* splenocyte proliferation activity of whey protein concentrate hydrolysate (WPCH), and the potential of whey protein concentrate (WPC) or whey protein concentrate/sodium alginate (WPC/SA) mixture as carriers to attenuate or mask the bitter taste and reduce the hygroscopicity of WPCH without obvious negative effect on its immunoregulatory activity. The effect of spray drying and freeze drying process on microencapsule formation and *in vitro* splenocyte proliferation activity of the resultant WPCH were also investigated.

2. Material and methods

2.1. Materials

Commercial whey protein concentrate (WPC 80) was obtained from Hilmar Ingredients (Hilmar, CA, USA). Commercial alcalase (E.C.3.4.21.62, endoproteinase from *Bacillus licheniformis*) with a declared activity of 2.4 Ansonunits (AU) per gram was provided by Novo Nordisk Biochem Inc. (Franklinton, NC, USA). Sodium alginate was obtained from Xilong Chemical Co., Ltd. (Guangdong, China). Female BALB/c mice, 6–8 weeks old, were obtained from HFK Bioscience Co., Ltd. (Beijing, China). RPMI-1640 medium, fetal bovine serum (FBS) and penicillin/streptomycin were obtained from Gibco Invitrogen Co. (Auckland, NZ, USA). Concanavalin A (ConA) and 5-Methylphenazinium methyl sulfate (PMS) were obtained from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium), inner salt (MTS) was obtained from Promega Co. (Madison, WI, USA). Trypan blue was obtained from Cellgro-Mediatech Inc. (Washington, DC, USA). All other reagents used in this study were analytical grade chemicals.

2.2. Preparation of whey protein concentrate hydrolysate

Whey protein concentrate was solubilized in demineralized water (50 g/mL). The pH of the protein solution was adjusted to 8.0 and then mixed with alcalase. Hydrolysis conditions were: pH 8.0, temperature 50 °C, enzyme to substrate ratio of 1:20 (w/w). During the hydrolysis process, the mixtures were maintained at pH 8.0 by the addition of 1 mol/L NaOH. At different hydrolysis periods (0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 h), the hydrolysate solutions were collected and heated in an 85 °C water bath for 20 min to inactivate the protease. After cooling to room temperature, the hydrolysates were centrifuged at 4000 g for 15 min. Then the supernatants were collected, lyophilized and regarded as whey protein concentrate hydrolysate (WPCH).

2.3. Preparation of encapsulated and non-encapsulated whey protein hydrolysates

Whey protein hydrolysate (WPC) and the mixture of WPC and sodium alginate (WPC/SA) (34:1, w/w) were used as carriers, respectively. The ratio between carrier and WPCH was 70:30 (w/w). Initially, WPC and sodium alginate were separately solubilized in distilled water. Then WPCH solutions were added and mixed under mechanical agitation with a motor stirrer (JJ-1, Ronghua, Jiangsu, China) at room temperature for at least 2 h to ensure complete dissolution.

The obtained solutions were treated in the following three ways. i) Spray-dried: Before spray drying process, the solutions were concentrated to solids content of 20% by a rotary evaporator. A spray dryer (YC-015, Yacheng, Shanghai, China) was applied for spray drying process. The concentrated solutions were fed into the dryer through a peristaltic pump at a flow rate of 1000 mL/h. The flow rate of compressed air was 0.7 m^3/h. The inlet air temperature and outlet air temperature were maintained at 200 °C and 90 ± 5 °C, respectively. The obtained products through spray drying were defined as non-encapsulated WPCH (S-1), WPC-encapsulated WPCH (S-2) and WPC/SA-encapsulated WPCH (S-3). ii) Freeze-dried: the solutions were dried directly with a freeze dryer (LGJ-12, Songyuanhuaxing Technology Co. Ltd., Beijing, China). The obtained products through freeze drying were defined as non-encapsulated WPCH (L-1), WPC-encapsulated WPCH (L-2) and WPC/SA-encapsulated WPCH (L-3). All the freeze-dried and spray-dried powders were collected and packaged in sealed bags until analysis. iii) Mechanical blended: the carriers and WPCH were mixed directly. The obtained solutions were stored at 4 °C for further analysis.

2.4. Evaluation of splenocyte proliferation

Immunomodulatory activity of WPC hydrolysates was evaluated by measuring their *in vitro* splenocyte proliferation activity according to Mao et al. (2005). Briefly, balb/c mice housed in an SPF facility were killed by cervical dislocation after being anesthetized. The spleens were removed using a sterile technique and placed in sterile plates containing RMPI-1640 without fetal bovine serum (FBS). The lymphocyte suspensions were then adjusted to 2×10^6 cells/mL in complete RPMI-1640 medium supplementing with 10% (v/v) FBS and 100 U/mL penicillin/streptomycin.

Spleen cell suspension (100 μL) were added to 96-well tissue culture plate followed by the addition of ConA (10 μL) at the final concentration of 4 μg/mL which had been determined in the preliminary assays and different samples (10 μL). The samples were prepared in RPMI-1640 medium (without FBS). The control wells were added the same volume RPMI-1640 medium instead of the tested samples. Negative control culture wells received RPMI-1640