Food Hydrocolloids 51 (2015) 512–518

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Effects of superfine grinding and microparticulation on the surface hydrophobicity of whey protein concentrate and its relation to emulsions stability

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ARTICLE INFO

Article history:
Received 28 January 2015
Received in revised form
18 May 2015
Accepted 22 May 2015
Available online 9 June 2015

Keywords:
Whey protein concentrate
Superfine grinding
Microparticulation
Surface hydrophobicity
Microrheology
Stability

ABSTRACT

In this study, superfine grinding and microparticulation were employed to increase the hydrophobicity of whey protein concentrate (WPC), which was investigated using 8-anilino-1-naphthalene sulfonic acid. WPC-stabilized emulsions containing 40% and 80% (w/w) oil showed smaller average droplet size when superfine grinding-treated WPC (sWPC) and microparticulated protein (MPP) were used than when WPC was used. The micro-rheology of the emulsions was measured upon diffusing wave spectroscopy; results showed that MPP can form stable emulsions. Moreover, the solid liquid balance of the emulsions significantly decreased from 0.87 to 0.38, with changing states from liquid to solid. The Turbiscan Stability Indexes of sWPC and MPP significantly reduced from 15.23 to 13.58 and 1.17, respectively. The operations of superfine grinding and microparticulation rendered WPC into an ingredient with excellent emulsifying properties. Thus, this ingredient can be utilized in both reduced-fat and high-fat food applications.

1. Introduction

Whey proteins (WPs) are widely used in the food industry because of their excellent functional and nutritional properties. WPs stabilize emulsions through their capability to adsorb at the oil–water interface. The final stability of emulsions before consumption is an important consideration for their utilization in the food industry.

The formation and stability of emulsions largely depend on the molecular interactions of the adsorbed protein and how the interactions are influenced by environmental conditions, such as pH, ionic strength, or temperature (Mustapha, Ruttarattanamongkol, & Rizvi, 2012). Protein surface hydrophobicity is another important factor associated with emulsion stability, and researchers have realized its importance while explaining the surface functionality of proteins (Mitidieri & Wagner, 2002).

Microparticulated whey protein (MWP) has been used as a fat replacer in the food industry to improve the sensory properties and nutritional value of dairy products (Lo & Bastian, 1998), such as ice cream (Yilsay, Yilmaz, & Bayizit, 2006), yogurt (Torres, Janhøj, Mikkelsen, & Ipsen, 2011), and cheese (Ismail, Ammar, & El-Metwally, 2011). To date, the production of microparticulated protein (MPP) involves the application of heat and shear to the proteins (Lucca & Tepper, 1994). The combination of acidification and heat treatment in microparticulation improves the foaming and emulsifying properties of proteins because of an increase in surface hydrophobicity induced by deamidation and denaturation (Moro, Gatti, & Delorenzi, 2001).

Superfine grinding has shown a great potential in producing nutraceuticals and functional foods (Chen, Weiss, & Shahidi, 2006). Some studies reported that superfine protein powder possesses high fluidity, solubility, electric conductivity, and water holding capacity; this finding indicates that superfine grinding improves the quality of food products (Wu, Zhang, Wang, Mothibe, & Chen, 2012; Zhao, Yang, Gai, & Yang, 2009). Nevertheless, insufficient information is available about the properties of superfine grinding-treated WPC (sWPC).

Therefore, this study aims to investigate the effect of superfine grinding and microparticulation on surface hydrophobicity of WPC and its relation to the storage stability of sWPC and MWP-stabilized concentrated cold-set emulsion gels.

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http://dx.doi.org/10.1016/j.foodhyd.2015.05.027
0268-005X/

2. Materials and methods

2.1. Materials

Commercial WPC was purchased from Fonterra Commercial Trading (Shanghai) Co., Ltd. The WPC comprised 80.3% protein (wet basis), 3.5% lactose monohydrate, 3.4% starch, 3.8% fat, 5.1% moisture, and 3.9% ash. Rhodamine B and 8-anilino-1-naphthalene sulfonic acid (ANS) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Commercial soybean oil was purchased from a local store in Tianjin. All chemicals were reagent grade.

2.2. Production of sWPC through superfine grinding

sWPC was ground in a multidimensional swing high-energy nanoball mill (CJM-SY-B Qinhuangdao Taiji Ring Nano-Products Co., Ltd., Hebei, China). Milling ball is composed of zirconia, and the ratio of milling ball and material is 6. To prevent heat denaturation, the working temperature was controlled at 35 °C.

In this study, sWPC-4h and sWPC-8h represent WPC treated through superfine grinding for 4 and 8 h, respectively.

2.3. Production of MPP

Approximately 0.12 g/mL protein solutions (pH 4.5) were heated at 85 °C for 35 min and then homogenized in an ULTRA-TURRAX (IKA) T18 high-speed homogenizer for 6 min at 10,000 r/min. The obtained proteins were lyophilized to yield MPP.

In this study, MWP represents microparticulated WPC, MPP-4h represents microparticulated sWPC-4h, and MPP-8h represents microparticulated sWPC-8h.

2.4. Determination of particle size distributions

The particle size distributions of WPC, sWPC-4h, and sWPC-8h were determined using a BT-2001 Laser Analyzer (Dandong Bettersize Instruments Ltd., China), with air as a dispersion medium. The particle size distributions of MPP dispersions were determined using a BT-9300S Laser Analyzer (Dandong Bettersize Instruments Ltd., China), with water as a dispersion medium. The laser analyzers were all based on dynamic light scattering. The Stokes–Einstein equation was used to calculate the particle size and distribution.

2.5. Protein surface hydrophobicity (PSH) index

The PSH was determined through fluorescence spectroscopy with an ANS probe in accordance with the method described by Alizadeh-Pasdar and Li-Chan (2000) with slight modifications. The relative fluorescence intensity was obtained using an FL-2500 fluorescence spectrophotometer (Hitachi, Science Systems, Ibaraki, Japan). Fluorescence intensity was recorded at excitation and emission wavelengths of 340 and 500 nm, respectively. The excitation and emission slit widths were 2.5 and 2.5 nm, respectively. The index of surface hydrophobicity was determined from the initial slope of the plot of fluorescence intensity versus protein concentration. Within the protein concentration range used in these experiments, linear relationships were obtained ($R^2 = 0.98–0.99$).

2.6. Steady-state fluorescence spectra

Most proteins contain intrinsically fluorescent amino acid residues, such as tryptophan, tyrosine, and phenylalanine. Tryptophan is by far the most useful of these amino acid residues. Royer (2006) reported that specific local information can be attained by selectively exciting the tryptophan residues at 295 nm or above. The protein solutions (0.05%, w/w protein) were prepared in 5 mM phosphate buffer (pH 7.0). The fluorescence emission spectra of tryptophan were obtained using an FL-2500 fluorescence spectrophotometer (Hitachi, Science Systems, Ibaraki, Japan). Fluorescence intensity was recorded at excitation and emission wavelengths of 295 and 300–400 nm, respectively. The excitation and emission slit widths were 5 and 5 nm, respectively, and the voltage was 700 mV.

2.7. Emulsion preparation

Continuous-phase emulsions were prepared from a fixed concentration (12%, w/w) of proteins at pH 4.5 by stirring the appropriate amount of protein in deionized water for 2 h at room temperature (25 °C) and then storing the mixture overnight at 4 °C. Emulsions containing 40% and 80% (w/w) soybean oil were prepared by mixing appropriate amounts of protein solutions and oil in glass containers by using a high-speed dispersing unit (IKA Ultra Turrax, T25 basic, IKA Works, Inc., NC, USA) at 12,000 r/min for 3 min at room temperature (25 °C). Sodium azide (0.02%, w/w) was added to prevent microbial growth.

2.8. Diffusing wave spectroscopy (DWS)

The microrheology of emulsions was measured using Rheolaser Master (Formulaction, l'Union, France) through DWS. When a laser beam illuminates a fluid sample, the photons penetrating into the sample are backscattered by micro-objects, such as particles, droplets, and fibers, which are suspended in the fluid. A video camera was used to record the dynamic interference patterns of the backscattered waves, often known as “the speckle image.”

Standard numerical algorithms are used to deduce the statistical parameters of the sample from dynamic speckle images; such parameters include MSD as a function of time (Chen et al., 2012). The MSD of these tracer particles is a direct and noninvasive probe of medium properties.

Immediately after preparation, the emulsions were placed in flat-bottomed cylindrical glass tubes (140 mm, height; 16 mm, diameter), and the first measure of backscattered light intensity was performed.

2.9. Emulsion stability

Emulsion stability was monitored through visual inspection with the optical scanning instrument Turbiscan ASG (Formulaction, l'Union, France) for kinetic stability studies. This instrument is composed of a detection head that moves up and down along a flat-bottomed cylindrical cell. The detection head facilitates vertical scans of the entire length of the sample. For opaque systems such as emulsions, the apparatus is used to measure the backscattered light intensity of the sample as a function of sample height and time. The prepared emulsions were placed in flat-bottomed cylindrical glass tubes (140 mm, height; 16 mm, diameter), and the first measure of backscattered light intensity was performed. The tubes were stored at 25 °C, and backscattered light was scanned every hour for 24 h.

In this part, the dynamic of stability curve d_i was calculated using Eq. (1) to draw a d_i portrait along the time:

$$\mathrm{d_n} = \frac{\sum_{h=0}^{H} |scan_n(\mathrm{h}) - \mathrm{scan}_{n-1}(\mathrm{h})|}{H} \quad (1)$$

where n is the number of scanning, scan is the light intensity, h is the height per 40 μm, and H is the height of the samples in the cell.

The Turbiscan Stability Index (TSI) is a statistical parameter used to estimate the suspension stability (Wiśniewska, 2010). The TSI was obtained as the sum of all processes occurring in the studied