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Fabrication of whey proteins aggregates by controlled heat treatment and pH: Factors affecting aggregate size

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ABSTRACT

Protein aggregates can be formed by heating globular proteins above the thermal denaturation of the proteins. These aggregates can be used as delivery systems or to modulate the physicochemical and sensory properties of food products. In this study, the effects of heat treatment (15 min at 80 °C), pH adjustment (between 4 and 9), aging at different temperatures (between 10 and 60 °C) and the rate of acidification (pH decreased from 6.25 to 4 at 5 different rates) on whey proteins aggregates were studied. The results indicated that these treatments led to creation of aggregates with different sizes (0.72–5.1 μm). Heat treatment and reducing the pH down to nearly 4 led to an increase in the size of aggregates. At pH < 7, by increasing the aging temperature, the size of aggregates increased (without heat treatment). In the heat treated samples with pH < 9, increasing the aging temperature led to an increase in the size of aggregates. Also larger aggregates were formed by increasing acidification rate. The amount of insoluble whey proteins aggregates increased as a result of applying heat treatment, decreasing pH and increasing aging temperature. The results of this study can be used for fabrication of whey proteins aggregates with specific size and functionality.

1. Introduction

Protein aggregates have useful application in the food, personal care products, and pharmaceutical industries, for example, as encapsulating agents, texture modifiers, or lighting agents. Protein aggregates may be used to encapsulate various chemically labile bioactive agents, such as fish oils or vitamins. These aggregates may also be used to simulate the textural and optical characteristics of lipid droplets, and are therefore useful as fat replacers [1].

Whey is an excellent source of functional proteins that can be used in food industries. Whey consists of water soluble part of milk which is separated during cheese making. Whey proteins are usually composed of globular proteins and β-lactoglobulin, α-lactalbumin and serum albumin are the main proteins in whey. In recent years, whey proteins (WP) have been used in many food formulations. Since functional properties of these proteins (such as solubility, distribution ability, emulsifying, gel formation, digestibility and hydrolysis) are related to their structure. Therefore chemical or physical modification can improve WP functional properties [2–6]. Among the various treatments of WP (such as heat treatment, chemical modification, pH treatment, alternating electric field, supercritical carbon dioxide treatment, high hydrostatic pressure treatment, etc.) heat treatment and changing the pH of WPs solution are methods that could have a broader and easier application on industrial scale.

Heat treatment of WP leads to protein denaturation. Depends on pH, solution composition, ionic strength, etc. several reactions such as polymerization or covalent and non-covalent (electrostatic and hydrophobic) reactions occur when a WP solution is heated [7]. As a result of these interactions, WPs are denatured and then by exposing of thiol groups, reactions between exposed free thiol groups and disulfide bonds lead to aggregation. Aggregation depends on the repulsive and attractive forces between particles, hence the size and the distribution pattern of WP aggregation depend on pH, heating temperature and protein concentration [7,8].

As mentioned above, in addition to heating temperature pH is an important factor which influences whey protein denaturation and aggregation. Close to pI, proteins are in zwitterionic form, which are containing equal amounts of positive and negative charges. In these case, proteins are less likely to be hydrated by aqueous solvent and tends to precipitate or aggregate due to lower electrostatic repulsion at room temperature and at low protein concentrations, the rates of reactions are low and may be negligible at low temperatures, the rate of WP aggregation increases with increasing the temperature which can lead to sedimentation of the proteins at high concentrations [9,10]. Some processes have been already developed for the production of

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microparticulated whey proteins as a fat substitute, i.e. aggregates with diameters between 0.5 and 10 μm can be produced based on heat treatment in the pH-range of 3.5 and 5.0 [11,12]. For example, β-lactoglobulin (β-lg) have an ability to form oligomers, from dimers to octamers, when it is heat treated under controlled pH [13]. However, Aggregation of native β-lg is completely reversed when the pH is increased or decreased away from pI. The rate of aggregation is maximum at pH ≈ 4.6 [9,10].

The structure of aggregates and gels that formed during aging has been investigated in several studies. In these researches it has been found that factors such as pH [7,9], protein concentration [7], time and temperature of heating [14–18] affect shape and size of aggregates. Furthermore, during the initial stages of aggregation process small oligomers, including denatured monomers have been observed between pH 6.0 and 8.7 [15].

It is still not clear how the rate of acidification impacts the aggregation process and aggregate size of WP that is essential for attributing new functionalities of WP. In this work, the effect of temperature, pH, and aging time on the solubility and particle size of WP were investigated. The principal aim of this research was to study the effect of different treatments on the solubility and the particle size of WP. For this purpose the effect of thermal treatment (80 °C for 15 min), a wide range of solutions pH (4–9) and aging conditions of WP solution (temperatures from 10 to 80 °C and times from 0 to 120 min) on the size of aggregates and the amount of insoluble aggregates were investigated. Moreover in this research the effects of different acidification rates (from pH = 6.25 to 4) on the formation and size growth of WP aggregates were studied.

2. Materials and methods

2.1. Materials

Native WP concentrate powder was purchased from Milei (Milei, Germany) and stored at 18 °C. Composition of the powder was reported: 84.23 ± 0.06% total native protein, 2.28 ± 0.02% ash, and 2.46 ± 0.03% fat.

2.2. Preparation of WP soluble polymers

WP concentrate was dispersed in distilled water under gentle stirring (180 rpm, at room temperature), to a native protein concentration of 8% (w/v) and the pH was adjusted on 7.0 using 1 N NaOH. Sodium azide (0.02%) (w/v) was added to prevent microbial growth. After overnight storage at 4 °C, the dispersions were warmed up to room temperature for further treatments.

2.3. Heat treatment, pH adjustment and aging conditions

Some dispersions were prepared in the previous step was heated at 80 °C for 15 min in a water bath. Then the solutions were rapidly cooled down to the aging temperature in an ice bath and diluted to a protein concentration of 2% (w/v). The WP aggregates were produced by pH-cycling [21]. The pH of WP polymer dispersions (with or without heat treatment) were adjusted to 4, 5.5, 6.25, 7, 8 and 9 by addition of 0.1 N HCl and 0.1 N NaOH solutions (initial pH ≈ 6.25) under gentle stirring (180 rpm).

In order to investigate the effect of aging temperature and the time of aging on the WP aggregate size, dispersions were aged at 10–65 °C for 0–120 min in a shaking water bath after pH adjustment.

2.4. Acidification rate

To investigate the effect of acidification rate on WP aggregates sizes, after preparation of WP dispersions, pH was decreased at five rates of 0, 30, 60, 90 and 120 min (from initial pH ≈ 6.25 to final pH of 4).

2.5. Particle size measurement

Particle size distributions in WPs dispersions were measured using static light scattering (Horiba laser light scattering, Horiba, LA-930, Japan). Before analysis, samples (before or after freeze drying) were diluted with distilled water to yield a final WP concentration of 1% w/v [22]. Measurements were performed for each treatment, immediately after pH adjustment and at 30, 60, 90 and 120 min after aging in a shaking water bath at 10, 35 and 60 °C.

2.6. Determination of insoluble aggregates

Each sample (20 ml) were centrifuged at 10,000*g* for 30 min at 20 °C using a refrigerated centrifuge (Sigma 6K15, Germany). After centrifugation, soluble fraction was removed and dry matter content was measured (using an oven at 105 °C for 4 h). Insoluble fraction of each sample was obtained by subtraction of soluble dry matter fraction from total dry mater of the initial sample [22,23].

2.7. Statistical analysis

A D-optimal RSM design was used to investigate the effects of variables (WP dispersion pH, aging temperature and heat treatment) on the aggregate size and the amount of insoluble aggregates. In this design, 25 runs (Table 1) were proposed by Design-Expert (Version 8.07.1, Stat-Ease Inc., Minneapolis, MN, USA). The analysis of variance (ANOVA) was carried out to determine differences between data means at 95% confidence interval.

3. Results and discussion

3.1. Effect of acidification rate on WP aggregate size

Fig. 1 shows the effect of acidification rate on WP aggregate size (rate of pH reduction from 6.25 to 4 during 0 min (2.25 pH/min), 30 min (0.075 pH/min), 60 min (0.038 pH/min), 90 min (0.025 pH/min) and 120 min (0.019 pH/min)). By increasing the acidification rate, the size of formed aggregates (at pH = 4 and the time of 130 min) increased

Table 1
Different test conditions for production of WP aggregates.

Test number	WP dispersion pH		Aging temperature		Heat treatment (80 °C for 15 min)
	Code	Real unit	Code	Real unit (°C)	
1	−0.40	5.5	−1.00	10	+
2	−1.00	4	1.00	65	−
3	−0.40	5.5	0.00	37.5	+
4	0.20	7	1.00	65	+
5	−1.00	4	1.00	65	−
6	−1.00	4	1.00	65	+
7	−1.00	4	0.00	37.5	−
8	−0.40	5.5	−1.00	10	−
9	−1.00	4	1.00	65	+
10	0.20	7	1.00	65	−
11	0.20	7	−1.00	10	−
12	−1.00	4	−1.00	10	+
13	0.20	7	1.00	65	+
14	0.20	7	1.00	65	−
15	−0.10	6.25	0.00	37.5	−
16	−1.00	4	−1.00	10	−
17	0.20	7	−1.00	10	+
18	0.60	8	1.00	65	+
19	0.60	8	1.00	65	−
20	0.60	8	−1.00	10	+
21	0.60	8	−1.00	10	−
22	1.00	9	1.00	65	+
23	1.00	9	1.00	65	−
24	1.00	9	−1.00	10	+
25	1.00	9	−1.00	20	−