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Influence of pH on the dry heat-induced denaturation/aggregation of whey proteins

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ABSTRACT

The effect of pH on the heat-induced denaturation/aggregation of whey protein isolate (WPI) in the dry state was investigated. WPI powders at different pH values (6.5, 4.5, and 2.5) and controlled water activity (0.23) were dry heated at 100 °C for up to 24 h. Dry heating was accompanied by a loss of soluble proteins (native-like β-lactoglobulin and α-lactalbumin) and the concomitant formation of aggregated structures that increased in size as the pH increased. The loss of soluble proteins was less when the pH of the WPI was 2.5; in this case only soluble aggregates were observed. At higher pH values (4.5 and 6.5), both soluble and insoluble aggregates were formed. The fraction of insoluble aggregates increased with increasing pH. Intermolecular disulphide bonds between aggregated proteins predominated at a lower pH (2.5), while covalent cross-links other than disulphide bonds were also formed at pH 4.5 and 6.5. Hence, pH constitutes an attractive tool for controlling the dry heat-induced denaturation/aggregation of whey proteins and the types of interactions between them. This may be of great importance for whey ingredients having various pH values after processing.

1. Introduction

Although whey proteins are widely used as food ingredients due to the nutritional and textural properties they add to food products, food technologists have developed processes to extend their functionalities. These processes include enzymatic (Kim et al., 2007; Panyam & Kilara, 1996; Rabiey & Britten, 2009), chemical (Kidwai, Ansari, & Salahuddin, 1976; Morgan et al., 1999) and physical modifications (Considine, Patel, Anema, Singh, & Creamer, 2007; Gulzar, Croguennec, Jardin, Piot, & Bouhallab, 2009; Patel, Singh, Havea, Considine, & Creamer, 2005). Amongst these processes, heat treatment in solution under controlled physicochemical conditions was extensively studied (Croguennec, O'Kennedy, & Mehra, 2004; Donato, Schmitt, Bovetto, & Rouvet, 2009; Schmitt, Bovay, Rouvet, Shojaei-Rami, & Kolodziejczyk, 2007) and a correlation between the structural modifications of whey proteins and the quality of the final product was established (Alting, Hamer, De Kruif, Paques, & Visschers, 2003; Alting et al., 2004; Havea, Watkinson, & Kuhn-Sherlock, 2009). In contrast, only a limited number of studies deal with the dry heating of whey proteins (Enomoto et al., 2007, 2009; Ibrahim, Kobayashi, & Kato, 1993; Li, Enomoto, Ohki, Ohtomo, & Aoki, 2005), and it has been shown that the structural modifications in proteins during dry heating cannot be extrapolated from results obtained in solution (Povey et al., 2009).

Dry heating is known to be an efficient tool to modify the functionalities of egg white proteins, such as improvements in gelling, foaming and emulsifying properties (Desfougeres, Lechevalier, Pezennec, Artzner, & Nau, 2008; Kato, Ibrahim, Watanabe, Honma, & Kobayashi, 1989; Matsudomi, Takahashi, & Miyata, 2001; Mine, 1997). It has been shown that only minor modifications in the secondary structure of the protein and a slight increase in the accessibility of thiol groups and hydrophobic patches, resulting in the formation of soluble aggregates linked with intermolecular disulphide bonds and also other covalent bonds, may improve the functional properties of egg white proteins (Kato, Ibrahim, Watanabe, Honma, & Kobayashi, 1990; Matsudomi et al., 2001; Watanabe, Nakamura, Xu, & Shimoyamada, 2000). Dry heating is usually conducted at pH 7–9, which is the natural pH range for egg white proteins (Matsudomi et al., 2001; Mine, 1996, 1997). Some results indicate that dry heating at acidic pH values can also modify protein functionalities (Desfougeres et al., 2008; Li et al., 2005); however, structural modifications in these conditions were inadequately described.

Whey protein ingredients are usually obtained from whey with different pH values, mainly acidic or neutral, and pH is known to affect both the type and kinetics of chemical reactions taking place during preparation and subsequent processing such as dry heating (Povey et al., 2009). In this work, we studied the dry heating (100 °C for up to 24 h) of whey proteins under controlled water activity (a_w 0.23) at three different pH values (2.5, 4.5, and 6.5) in order to better understand the effect of pH on the denaturation/aggregation mechanism of whey proteins in the dry state.

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The physicochemical parameters of the powders were selected as follows: An a_w of 0.23 corresponds to the a_w of whey protein powders produced on an industrial scale; acidic pH values were chosen to cover the diversity of commercially produced whey (pH 6.5 and 4.5) and extended to pH 2.5 since whey proteins exhibit interesting structural behaviours when heated in solution at this pH (Bolder, Hendrickx, Sagis, & van der Linden, 2006; Oboroceanu, Wang, Brodkorb, Magner, & Auty, 2010). In addition, at selected pH values (6.5, 4.5, and 2.5), the overall charge of the whey proteins is negative, neutral and positive, respectively.

2. Material and methods

2.1. Materials

The spray-dried WPI (Prolacta, Lactalis Ingredient, Bourgbarré, France) contains 90.1 ± 1.0% proteins (w/w, determined by the Kjeldahl method) of which 82% is β-lg and 18% is α-La (determined by reversed-phase chromatography), 6.7 ± 0.2% moisture (determined by air drying), 0.88 ± 0.08% lactose (determined by the Lactose/D-Galactose enzymatic method, Boehringer Mannheim, Darmstadt, Germany), 0.324 ± 0.016% calcium, 0.146 ± 0.012% sodium (determined by atomic absorption spectroscopy), 0.017 ± 0.002% chloride, 0.056 ± 0.005% succinate, 0.020 ± 0.002% sulphate, 0.037 ± 0.002% phosphate, and 0.043 ± 0.006% citrate (determined by ionic chromatography). Approximately 50% of β-Lg and 25% of α-La was lactosylated as assessed by mass spectrometry according to the protocol previously reported (Gulzar et al., 2009). Once reconstituted at 10 g l^{-1}, the WPI solution had a pH of 6.5. The protein solubility at pH 7.0 and pH 4.6 (see Section 2.4.2 for details) was 97 ± 3% and 93 ± 3% respectively. Glycine was from Across Organics (Geel, Belgium); all other chemicals were from Sigma–Aldrich (Saint–Quentin–Fallavier, France).

2.2. Preparation of powders

Spray-dried WPI was dissolved in distilled water at a protein concentration of 15% and the solution was adjusted to three different pH values (2.5, 4.5, and 6.5) by using HCl. The solutions were then lyophilised. The samples containing 10 g of powder were stored for two weeks in a desiccator containing a saturated salt (CH_3CO_2K) solution in order to maintain a water activity of 0.23. The water activity of the powder was checked using an a_w meter (Novasina RTD 200/0 and RTD 33, Pfäffikon, Switzerland).

2.3. Preparation of samples

Powders with three different pH values and an a_w of 0.23 were heated at 100 °C for 0, 8, 16 or 24 h in hermetically-sealed bottles. Subsequently, all the powders were reconstituted at 10 g l^{-1} in distilled water containing an adequate concentration of NaCl (0.11, 0.115, and 0.12 M for dry heated powders at pH 2.5, 4.5, and 6.5, respectively in order to compensate for salts [chloride and sodium ions] introduced during powder preparation and reconstitution at pH 7). All samples reached the same final ionic strength of 0.12 M after reconstitution and pH adjustment at 7 by adding 1 N NaOH (Solutions 1). Solutions 1 were centrifuged at 10,000*g* for 15 min using an Eppendorf 5415C Micro Centrifuge (Scientific Support, Hayward, California) in order to remove insoluble aggregates at pH 7. The supernatants (Solutions 2) contained soluble aggregates, residual native and "native-like" proteins. The pH of Solutions 1 were lowered to 4.6 using 1 N HCl. Acidified samples were centrifuged at 10,000*g* for 15 min using an Eppendorf 5415C Micro Centrifuge (Scientific Support, Hayward, California). Centrifugation resulted in the removal of both soluble and insoluble aggregates at pH 7; subsequently, supernatants at pH 4.6 (Solutions 3) with only residual native and "native-like" proteins (soluble proteins at pH 4.6) were recovered. All samples were prepared in duplicate.

2.4. Physical analysis

2.4.1. Turbidity measurement

Protein samples were diluted 10 times in 0.12 M NaCl solution. Optical density (OD) at 500 nm was determined before and after centrifugation at pH 7 in spectroscopic plastic cuvettes (1 cm path length) using a Visible Spectrophotometer S1205 (Unico, France). Turbidity was determined by following the equation: $\tau = (2.303 \times OD)/l$, where OD is the optical density of samples at 500 nm and l is the path length of light in the cuvette.

2.4.2. Protein solubility

The protein concentration in solutions 1, 2, and 3 was determined by the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951). Protein solubility at pH 4.6 (Solutions 3) or pH 7 (Solutions 2) was expressed as a percentage of the protein recovered in the supernatants after centrifugation.

2.4.3. Determination of aggregate size

The size of the aggregates was determined by dynamic light scattering using a Zetasizer NanoZS apparatus (Malvern Instrument, Worcestershire, UK), which was equipped with a He/Ne laser working at 633 nm and an attenuator that automatically adjusts the laser intensity to the specific range for scattered light detection. Protein samples were diluted in a phosphate buffer (0.05 M, pH 7, 0.1 M NaCl) and placed in a 10 × 10 mm disposable polystyrene cell (Sarstedt, Germany) equilibrated at 20 °C for measurements. Heated samples were diluted 10 times, while non-heated samples were diluted three times to have sufficient signals for measurement. The intensity of scattering is detected at 173° (backscatter detection) to reduce multiple scattering. The hydrodynamic diameter of the aggregates was calculated using the Stockes–Einstein equation, taking the calculated diffusion coefficient from the fit of the correlation curve. All the samples were measured in triplicate.

2.5. Chemical analysis

2.5.1. Gel permeation chromatography

Soluble proteins at pH 7 were analysed by High Pressure – Gel Permeation Chromatography (HP-GPC) using a TSK G3000 SWXL (300 × 7.8 mm i.d.) column (Phenomenex, Le Pecq, France) connected to a Waters chromatography system (Milford, USA), consisting of a Waters e2695 Separation Module, and a Waters 2489 Dual λ Absorbance Detector and Empower chromatography application software to acquire, process and report chromatographic information. A 0.05 M phosphate buffer at pH 7 containing 0.1 M NaCl was used to equilibrate the column and to elute the proteins at a flow rate of 0.8 mL min^{-1}. Proteins were detected at 214 nm.

2.5.2. SDS–PAGE analysis

SDS–PAGE was performed under reducing (with DTT) and non-reducing conditions (without DTT) using a Mini Protean II system (Bio-Rad Laboratories, A Technologies, Dublin, Ireland) as described by Laemmli (1970) using a 12.5% acrylamide separating gel and 4% concentration gel. Soluble proteins at pH 7 (Solutions 2) were diluted 10-fold with the denaturing buffer (77.975% 0.08 M Tris–HCl, pH 6.8; 20% glycerol; 2% SDS; 0.025% bromophenol blue). The proteins (10 μg) were loaded into the sample slots and separated at 75 V for 30 min and at 150 V for 60 min. Gels were stained with Coomassie Brilliant Blue G250. A low molecular weight marker kit (14.4–94 kg mol^{-1}, Amersham Biosciences,