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Influence of moderate electric fields on gelation of whey protein isolate

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ABSTRACT

Proteins are one of the food constituents most affected by heating, and some of the changes involve their unfolding, denaturation and gelation. Ohmic heating has often been claimed to improve the quality of foodstuffs due to its uniform heating and (putative) presence of a moderate electric field (MEF). However, this is still subject to discussion, so it is important to determine the effect of ohmic heating and of its MEF upon food constituents. Hence, the aim of this work was to evaluate the effects of MEF on denaturation, aggregation and viscoelastic properties of whey protein isolate (WPI), and compare them with those obtained via conventional heating under identical treatment conditions (up to 30 min at 85 °C). Results have shown that MEF interferes with whey protein unfolding and aggregation pathways at relatively high temperatures. MEF treatments have resulted in WPI solutions possessing more 8 and 10% of native β-Lactoglobulin and α-Lactalbumin, respectively, after 30 s of heating at 85 °C, when compared with a conventional heating method. Protein aggregates from MEF-treated WPI solutions presented a maximum increase in size of 78 nm, whereas conventional heating produced an increase of 86 nm. Unlike in conventional heating, aggregation of whey proteins during MEF was not sufficiently strong to form a true elastic gel network, since decreases in both storage and loss modulus were observed following MEF treatment. Our results suggest that MEF may provide a novel method for production of a whey protein matrix with distinctive gel-forming properties.

1. Introduction

Whey proteins have increasingly been used as nutritional and a functional ingredients in several food formulations, particularly in the form of dry whey, such as whey protein concentrate (WPC) and whey protein isolate (WPI). The functionality of whey ingredients is determined by changes in their physical and chemical properties during manufacture that commonly include such thermal processing as pre-heating, pasteurization or sterilization. β-Lactoglobulin (β-lg) is the most abundant globular protein found in whey, thus overriding functional (e.g. gelation and emulsification) and nutritional properties of whey derivatives (Lefevre, Subirade, & Pezolet, 2005). The effects of heat on denaturation of pure and enriched fractions of β-lg has been extensively reported (Nicorescu et al., 2008). It is known that heating at high temperatures (>60 °C) produces thermal denaturation of globular whey proteins.

The process of denaturation of globular whey proteins is assumed to consist of at least two steps: a partial unfolding of the native protein, and a subsequent aggregation of unfolded molecules (Nielsen, Singh, & Latham, 1996). In particular, unfolding of the native conformation of globular whey proteins at neutral pH leads to exposure of free sulfhydryl groups (SH) and hydrophobic amino acid side-chains, normally occluded within bovine serum albumin (BSA) and β-lg (Kazmierski & Corredig, 2003; Schmitt et al., 2009; Shimada & Cheftel, 1989). With further heat treatment, free SH may rapidly interchange with existing disulfide bonds to generate new inter- and intramolecular disulfide bonds that will engage toward protein aggregation (Fairley, Monahan, German, & Krochta, 1996; Schokker, Singh, Pinder, & Creamer, 2000). The formation of intermolecular disulfide bonds by sulfhydryl–disulfide interchange is considered one of the major mechanisms of protein aggregation, and is mainly governed by formation

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of β-lg oligomers that combine into large aggregates (Havea, Singh, & Creamer, 2002).

However, the mechanism of protein unfolding and aggregation is complex and may be influenced by many factors, such as electrostatic and hydrophobic interactions, hydrogen bonding and disulfide cross-linking (Havea et al., 2002; Kazmierski & Corredig, 2003; Mulvihill, Rector, & Kinsella, 1990). Several studies have shown that the processes of protein unfolding and aggregation occur to different extents depending on the conditions prevailing in the aqueous solution (e.g. pH, ionic strength, ionic calcium, fat content, presence of lactose and protein composition/concentration), and heating treatments (Anema & Li, 2003; Dalgleish & Banks, 1991; Law & Leaver, 2000; Nicorescu et al., 2008; Verheul, Roefs, & de Kruif, 1998).

Ohmic heating (OH) has been receiving increased attention due to its uniform transmission of thermal energy and extremely rapid heating rates that enable high temperatures to be applied without inducing coagulation or excessive denaturation of the constituent proteins (Parrot, 1992). In this sense, this technology provides processed products of higher quality (i.e. where such thermal labile compounds as proteins and vitamins can be preserved) than those obtained by conventional heating technologies (Castro, Teixeira, Salengke, Sastry, & Vicente, 2003; Machado, Pereira, Martins, Teixeira, & Vicente, 2010; Parrot, 1992). During OH treatment, electric current passes through food that behaves as a resistor in an electrical circuit, and heat is internally generated according to Joule's law (De Alwis & Fryer, 1990). OH technology can be distinguished from other electrical heating methods: by a) presence of electrodes contacting the foods; b) frequency range applied (from 50 to 25,000 Hz); and c) unrestricted, and typically sinusoidal waveform. OH is also known by the name of moderate electric fields (MEF) due to the application of an electric field of relatively low intensity (arbitrarily defined between 1 and 1000 V cm^{-1}) aiming at controlling permeabilization of membranes and other non-thermal effects (Machado et al., 2010). However, only a few scientific and technical works have focused on the effects of MEF upon enriched fractions of β-lg (which is the whey protein most susceptible to heat treatments). Recently, it was shown that MEF may lead to protein conformational disturbances during heat-induced denaturation of WPI (Pereira, Souza, Cerqueira, Teixeira, & Vicente, 2010; Pereira, Teixeira, & Vicente, 2011). Hence, is expected that MEF will influence protein aggregation kinetics and gelation, and accordingly affect WPI functional properties. Knowledge of WPI aggregation and gelation behavior upon MEF treatment would be of great significance to the food industry once this whey product is more often used than pure fractions of proteins essentially due to low cost and high availability. Despite WPC being considered the most widespread ingredient, the study of heat-induced aggregation of WPC is usually hindered by the presence of high quantities of fat, lactose and other impurities, which can modify the aggregation behavior and the role of the primary functional proteins (Kazmierski & Corredig, 2003). The objective of this work was therefore to evaluate the formation of soluble whey protein aggregates from WPI, under the presence and absence of MEF at almost neutral pH conditions. Fine-stranded WPI gels were also produced under acidic conditions, and characterized through small amplitude oscillatory dynamic measurements – in an attempt to provide new insights about MEF effects upon non-covalent protein interactions.

2. Material and methods

2.1. Whey protein isolate

WPI powder (Lacprodan DI-9212) used was kindly supplied by Arla Foods Ingredients (Viby, Denmark), and was essentially free of lactose (max 0.5%) and fat (max 0.2%), with a protein content of 91% (of dry weight). Protein composition of WPI powder was determined by reverse-phase high-performance liquid chromatography (RP-HPLC): α-lac 22.8%, BSA 1.7%, β-lgA 44%, β-lgB 30.7% and immunoglobulins (IG) 1.1% on a protein basis. The amount of proteins considered to be in their "native" state (i.e. soluble at pH 4.6) was 85% of the total protein content (Pereira et al., 2011).

2.2. Whey protein solutions

Protein stock solutions of 3 and 10% (w/w) were prepared by dissolving the WPI powder in 50 and 20 mM sodium phosphate buffer (pH = 6), respectively. Both WPI solutions were then stirred continuously overnight at refrigeration temperature (5 °C), to ensure full rehydration. For studies of protein unfolding, denaturation and production of soluble protein aggregates, WPI solution at 3% (w/w) was prepared with final pH adjusted to 6.8 with 1 M of NaOH (Merck, Germany). It has been shown that, when WPI solutions of low protein concentration (1–3%) heated under neutral pH, small amounts of added salts, lead to the formation of soluble protein aggregates via both disulfide and hydrophobic interactions (Purwanti et al., 2011; Ryan et al., 2012; Schmitt, Bovay, Rouvet, Shojaei-Rami, & Kolodziejczyk, 2007). For the development of protein gel, was used the WPI solution at 10% (w/w) and final pH was adjusted to 3.0 with 1 M of HCl (Merck, Germany). Heating WPI with high protein concentration (8–11%) and under acidic conditions can yield a gel stabilized by non-covalent bonding (Aymard, Nicolai, Durand, & Clark, 1999; Otte, Zakora, & Qvist, 2000). Electrical conductivity of the prepared WPI solutions ranged approximately from ca. 1000 to 1500 μS cm^{-1}, which allowed OH effect to be observed.

2.3. Heating units

2.3.1. Conventional heating (COV)

Experiments were performed in a double-walled water-jacketed reactor vessel (3 mm of internal diameter and 100 mm height), as reported previously (Pereira et al., 2010). Treatment temperature was controlled by circulating thermo-stabilized water from a bath (set at the same temperature as that selected for the treatment) in order to better control temperature. A magnetic stirrer was introduced inside the reactor vessel to homogenize the solution and improve heat transfer during the heating cycle. Temperature evolution was measured with a type-K thermocouple (1 °C, Omega, 709, U.S.A.), placed at the geometric center of the sample volume and connected to a data logger (National Instruments, USB-9161, U.S.A.) – working with Lab View 7 Express software (National Instruments, NI Data logger).

2.3.2. Moderate electric fields (MEF)

MEF treatments were performed in a cylindrical glass reactor (30 cm total length and an inner diameter of 2.3 cm), with two stainless steel electrodes placed at each edge isolated by polytetrafluoroethylene (PTFE) caps, as described elsewhere (Pereira et al., 2010, 2011). A gap of 10 cm between the electrodes (the treatment chamber) was used for the experiments. The supplied voltage, and consequently temperature were controlled through the use of a function generator (Agilent 33,220 A, Bayan Lepas, Malaysia; 1 Hz–25 MHz and 1–10 V) connected to an amplifier system (Peavey CS3000, Meridian, MS, USA; 0.3 V–170 V). During heating phase and holding treatment, the MEF applied varied from 15 to 22 V cm^{-1} and 4 to 8 V cm^{-1}, respectively, whereas electrical frequency was of 25 kHz. At this high frequency, (25 kHz), electrochemical reactions at the electrode interface are reduced, thus minimizing potential corrosion and leakage of metals to the