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Effect of freezing on the viscoelastic behaviour of whey protein concentrate suspensions

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The effect of freezing on viscoelastic behaviour of whey protein concentrate (WPC) suspensions was studied. Suspensions with total protein content of 5% and 9% w/v were prepared from a commercial WPC (unheated suspensions). A group of unheated suspensions was treated at two temperatures (72.5 and 77.5 °C) during selected times to obtain 60% of soluble protein aggregates (heat-treated suspensions). Unheated suspensions and heat-treated suspensions were frozen at −25 °C (frozen unheated and frozen heat-treated suspensions). Frequency sweeps (0.01–10 Hz) were performed in the region of linear viscoelasticity at 10, 20, 30, 40, and 50 °C. Mechanical spectra of all studied suspensions at 20 °C were similar to viscoelastic fluids and complex viscosity increased with the frequency (ω). Elastic (G') and viscous (G'') moduli were modelled using power law equations ($G' = a\omega^x$, $G'' = b\omega^y$), using fitted parameters a, x, b, and y for statistical analysis. Exponent y was the most influenced by freezing, indicating the existence of a higher degree of arrangement in frozen unheated suspensions and a lower degree of arrangement in frozen heat-treated suspensions. Only characteristic relaxation times (inverse of the crossover frequency) of suspensions with 5% w/v of total protein content were significantly influenced by freezing. Time–temperature superposition was satisfactory applied in unheated whey protein concentrate suspensions only in the range of high temperatures (30–50 °C). However, this principle failed over the complete temperature range in most of the frozen suspensions. It is possible that freezing produced an increase in the susceptibility to morphological changes with temperature during the rheological measurements.

1. Introduction

Whey proteins are used as ingredients in food industry and they are worldwide commercialized as isolates (WPI) and concentrates (WPC). Whey protein concentrates contain between 20% and 89% of protein, being WPC with 35% of protein content the most common product (Tunick, 2008). Whey proteins have functional properties like solubility, emulsification, gelation, and foaming capacity (Ennis & Mulvihill, 2000; de Wit, Hontelez-Backx, & Adamse, 1988). In addition, whey proteins have the advantages of high nutritive value and are generally recognized as safe status (Bryant & McClements, 1998).

Sometimes, heat treatments are used to improve whey protein functionality. When whey protein suspensions are heated over the denaturation temperature, the unfolding of the tertiary structure of the protein molecule (especially α-lactalbumin and β-lactoglobulin) is produced. This phenomenon allows both the exhibition of hydrophobic sites and the sulfhydryl/disulfide interchange reactions that induce the formation of aggregates (Anema, 2009). During heat treatment at high protein concentrations (over 10% or 12%), depending on pH, ionic composition, and temperature, a gel may be formed (Walstra, Geurts, Noomen, Jellema, & van Boekel, 1999). Nevertheless, at low protein concentration aggregates can remain in solution.

Heat-treated whey protein suspensions containing soluble protein aggregates can be used as ingredient in food processing due to their specific functional properties. Vardhanabhuti and Foegeding (1999) found that suspensions containing soluble whey proteins aggregates, obtained by heating whey protein isolate suspensions at 80 °C, exhibited high viscosity and had flow behaviours similar to those of hydrocolloids. Also, heat-treated whey protein suspensions can form gels at low temperatures by

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addition of NaCl or $CaCl_2$ (Bryant & McClements, 2000) and by pH lowering (Abd El-Salam & El-Etriby, 1996).

Rheological properties are useful in process engineering calculations, equipment design and optimization. In addition, they can be used for product quality control and sensory assessment of foods (Rao, 1999). Viscoelastic behaviour of whey protein suspensions have been investigated by several research groups (Ikeda & Nishinari, 2001a; Ikeda, Nishinari, & Foegeding, 2001; Lizarraga, De Piante Vicin, Gonzalez, Rubiolo, & Santiago, 2006). Also, the viscoelastic behaviour of heat-treated whey protein concentrate suspensions containing soluble protein aggregates was analysed (Meza, Verdini, & Rubiolo, 2009).

Williams, Landel, and Ferry (1955) developed the time–temperature superposition principle (WLF principle) that equates the effect of time and temperature on rheological properties. This technique, which is sometimes applicable to synthetic polymers and biological materials, has not been widely applied to foods (Steffe, 1996). Isothermal linear viscoelastic data, obtained from frequency sweeps at several different temperatures, are shifted along the frequency axis and overlaid to obtain a single master curve at the reference temperature chosen conveniently (Ferry, 1980). Time–temperature superposition principle was used to investigate the viscoelastic behaviour of bovine serum albumin suspensions (Ikeda & Nishinari, 2000), but it has not been applied in whey protein concentrate suspensions.

Freezing is widely used for food preservation. However, the formation and growth of ice crystals can produce modifications in physicochemical properties of foods like pH, ionic strength, and solute concentration. Freeze-concentration in the unfrozen fraction of the aqueous phase produces changes in the product, including textural modifications, protein denaturation, and cell membrane destruction (Walstra, 2003). The possibility of using freezing to preserve concentrated whey protein suspensions and the effect of freezing on some of their functional properties was investigated by Bhargava and Jelen (1995). However, very little attention has been paid into the use of freezing as a method to preserve heat-treated whey protein suspensions and only few studies about freeze-texturisation of heat-treated whey protein suspensions were found in literature (Lawrence, Consolacion, & Jelen, 1986). Whey protein suspensions can be used as ingredients in foods that can be frozen, like desserts and cheeses. For this reason, it is interesting to study the effects of freezing on the rheological properties of whey protein suspensions.

In this study, the effect of freezing on the viscoelastic behaviour of unheated and heat-treated whey protein suspensions prepared from a commercial WPC was analysed performing frequency sweeps in the region of linear viscoelasticity.

2. Materials and methods

2.1. Whey protein concentrate

Commercial whey protein concentrate (WPC) obtained from sweet cheese whey provided by a local industry was used (Milkaut S.A., Frank, Santa Fe, Argentina).

2.2. Physicochemical analysis of the WPC

The initial composition of the WPC was determined in laboratory with standardized techniques. The total protein content was considered from total nitrogen content determined by the Kjeldahl method, using a Büchi 430 automatic digestor (Büchi, Flawil, Switzerland), a Büchi 322 distillation unit, and a Mettler DL40RC automatic titrator (Mettler Instrumente AG, Greifensee, Switzerland). Ash content was determined after an overnight incineration in a muffle furnace at 540 °C. Moisture content was measured with a CEM AVC 80 microwave (CEM, Mattheus, NC). Fat content was determined using the Standard International Dairy Federation method (IDF, 1969). Lactose content was defined as the difference between the mass of the sample and the amount of protein, ash, moisture and fat. All compositional analyses were determined in triplicate. The initial composition of the WPC was: lactose 48.8%, protein 38.3%, ash 7.5%, moisture 3.2%, and fat 2.2%.

2.3. Whey protein concentrate suspensions

2.3.1. Unheated suspensions

Whey protein concentrate suspensions at natural pH were prepared to reach 5% and 9% w/v of total protein content. An appropriate amount of WPC was weighed and dissolved in distilled water with vigorous agitation. All suspensions were prepared in triplicate and stored at 5 °C overnight (unheated suspensions). The denatured protein (DP) content of unheated suspensions was $52.8 \pm 1.5\%$ and $47.0 \pm 1.1\%$ for suspensions with 5% and 9% w/v of total protein content, respectively. The codes of samples are shown in Table 1.

2.3.2. Heat-treated suspensions

Glass tubes (160 by 16 mm) containing 10 mL of unheated suspensions were heat-treated at 72.5 and 77.5 °C for different times to produce 60% of soluble protein aggregates expressed as percentage of DP content, using the previously published procedure (Meza et al., 2009). According to this technique, the protein that remains soluble at pH 4.6 (approximately 40%) is the protein that underwent neither denaturation nor aggregation (Li-Chan, 1983; Verheul, Roefs, & de Kruif, 1998; de Wit, 1990).

All suspensions were prepared in triplicate and stored at 5 °C overnight (heat-treated suspensions). Codes of samples are shown in Table 1.

2.4. Freezing

A group of both unheated and heat-treated suspensions was frozen in the freezer (−25 °C) for 24 h and subsequently thawed at 20 °C. These samples were called frozen unheated suspensions and frozen heat-treated suspensions, respectively. The DP content of frozen unheated suspensions was $52.0 \pm 0.6\%$ and $46.4 \pm 1.1\%$ for suspensions with 5% and 9% w/v of total protein content, respectively. All suspensions were prepared and stored at each study condition in triplicate. Codes of samples are shown in Table 1.

Table 1
Codes of samples of whey protein concentrate suspensions.

	Unfrozen samples	Frozen samples	Total protein content (% w/v)	Heat-treatment conditions (time and temperature)
Unheated suspensions	5-unheated	5-unheated-F	5	–
	9-unheated	9-unheated-F	9	–
Heat-treated suspensions	5-72.5	5-72.5-F	5	72.5 °C – 15 min
	9-72.5	9-72.5-F	9	72.5 °C – 40 min
	5-77.5	5-77.5-F	5	77.5 °C – 6 min
	9-77.5	9-77.5-F	9	77.5 °C – 12 min