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Application of a digital image procedure to evaluate microstructure of caseinate and soy protein acid gels

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ABSTRACT

Acid gelation of proteins is commonly used in the food industry and it can be induced by the addition of glucono-δ-lactone (GDL). The aim of this work was to use textural analysis of images in order to assess possible changes in the microstructure of bovine sodium caseinate (NaCAS) and soy protein isolate (SPI) acid gels. The gelation rate of NaCAS related to the amount of GDL was evaluated. Also, the effect of the presence of NaCAS hydrolysates obtained at different hydrolysis times by the enzyme of *Bacillus* sp. P7 was studied. Finally, SPI acid gels were evaluated in the presence of whey soy protein isolate (WSP) in different ratios. The gel images were obtained by conventional optical microscopy and texture parameters were obtained by using specific programs which were developed in Python language. Shannon entropy, smoothness, mean normalized grey-level variance and uniformity were analyzed as estimators of the texture of the images obtained. Results obtained in the evaluated systems showed that these parameters were able to represent the structural changes in the gel network, as changes in size of pores or in degree of compactness. Also, these results were contrasted with rheological properties of the systems evaluated.

1. Introduction

Preference of customers for healthier industrial products having the same texture and flavour as the traditional ones, has grown over recent years. This fact accounts for the many manufacturers' growing interest in intensifying and diversifying their production lines. However, to develop these food products with the desired texture, another food constituent stabilization is needed, where proteins play a key role due to their functional and interaction properties (Foegeding, Çakır, & Koç, 2010). The use of a simplified model system provides a scientific framework, allowing prediction of the behaviour of a more complex system, and facilitating the development and formulation of new products with the desired characteristics.

Bovine milk proteins are extensively used in the food industry because of their physicochemical, nutritional and functional properties. Bovine caseins can be precipitated at pH 4.6 and may be resolubilized by increasing the pH. If the pH increase is carried out by addition of NaOH it is possible to obtain sodium caseinate (NaCAS), which is widely used in the food industry (Ennis & Mulvihill, 2000; Mulvihill & Fox, 1989). NaCAS are stable against heat treatment which makes them an excellent nutrient (Manski, van Riemsdijk, van der Goot, & Boom, 2007). NaCAS particles are found in aqueous solutions as individual protein molecules, oligomers (NaCAS nanoparticles) or sub micelles of caseins (Farrell et al., 1996). NaCAS assists in the texturing of different foods, for example, it is used in the industry of meat products, sausages and luncheon, due to its heat resistance, adhesiveness and ability to confer juiciness to the product.

In the food industry, proteases have been extensively used in cheese-making, bakery products, preparation of soy hydrolyzes and meat tenderized (Rao, Tanksale, Ghatge, & Deshpande, 1998; Sumantha, Larroche, & Pandey, 2006). Also, commercial proteases have recently been used in the production of protein hydrolysates with promising bioactive properties (Rival, Boeriu, & Wichers,

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2001; Zhu, Zhou, & Qian, 2006). Particularly, enzymatic hydrolysis under mild conditions pH (6–8) and temperature (40–60 °C) allows the obtention of bioactive nutritional components and improved functional properties, such as gelation, emulsification and foaming (Hartmann & Meisel, 2007; Silva & Malcata, 2005).

Many microorganisms produce proteases that are particularly interesting because they have well-established methods of cultivation and provide the industry with a wide variety of proteases suitable for different purposes (Gupta, Beg, & Lorenz, 2002). It has been reported that proteolytic enzymes of *Bacillus* sp. P7, isolated from the intestinal tract of a species of the Amazon basin, *Piaractus mesopotamicus*, produce high levels of extracellular proteases with biotechnological potential which can be grown on a relatively inexpensive media (Daroit, Corrêa, & Brandelli, 2009, 2011). They generate less bitterness in food protein hydrolysates than acidic endopeptidases. Also, their low thermo tolerance is advantageous for controlling their reactivity during the production of hydrolysates (Rao et al., 1998).

NACAS bovine protein hydrolysates, obtained by controlled proteolysis using an extracellular enzyme produced by *Bacillus* sp. P7 showed various biological activities: antioxidant, antibacterial, reducing power and chelating capacity (Corrêa, Hidalgo, Mancilla Canales, Risso, & Brandelli, 2011). Therefore, it is interesting to evaluate the incorporation of such hydrolysates to a protein network which is the basis for the development of a dairy product.

Consumption of soy-based products has grown due to its beneficial effects on health and nutrition (Friedman & Brandon, 2001). The native soy protein isolate (SPI), which has a high nutritional value, presents several functional properties. Approximately 90% of soy proteins are globulins and those that precipitate at pH 4.5 are traditionally called reserve or storage proteins. There are two main fractions of high molecular weights referred to as globulin 7S (β-conglycinin) and globulin 11S (glycinin). They both consist of various subunits that easily associate and dissociate under different conditions of pH, ionic strength and heat treatment (Kinsella, 2001; Pearson, 1983). The protein fraction of whey soy protein isolates (WSP), isoelectric supernatant formed during SPI preparation, consists mainly of low molecular weight components (hemagglutinin, Kunitz and Bowman–Birk antitryptic factors and enzymes such as β-amylase, lipoxygenase and urease) (Sorgentini & Wagner, 1999). Lately, there has been further research about functional properties of soy components that are discarded or generated in the development of soymilk, tofu, isolates and concentrates. If WSP is inactivated, it has a biological value comparable to that of the storage proteins (Kishi & Inoue, 1987).

NaCAS and SPI may form gels near their isoelectric point by the addition of GDL (Braga, Menossi, & Cunha, 2006; Campbell, Gu, Dewar, & Euston, 2009). Protein–protein interactions increase when pH decreases due to a decrease in their net charge. As a consequence, if the protein concentration is high enough, the protein aggregation occurs and the gel is formed. Protein gels are responsible for rheological/textural properties of foods, such as elasticity, resistant and hardness (Foegeding, 2007).

The deep understanding of the complex relationship between the different components in food will allow the control and/or monitoring of the micro/nanostructure. Consequently, the texture manipulation of processed foods and the formulation of new products with differential characteristics will be possible. However, the models to predict complex systems need to be continually modified to relate them more closely with the texture of food (Foegeding, Brown, Drake, & Daubert, 2003).

Changes in the gelation process rate may affect the physical properties of the resulting gel, such as texture and water holding capacity. Moreover, the addition of cosolutes modifies the conformation and the intermolecular association of biopolymers. The addition of a component with less efficiency to be linked to hydrogen promotes polymer–polymer association, reducing the polymer–solvent interactions (Ribeiro, Rodrigues, Sabadini, & Cunha, 2004).

In order to interpret the interaction between food composition, texture, aromatization and sensory characteristics it is necessary to take into account the distribution of a phase in a multiphase system (Aguilera, 2006; Renard, van de Velde, & Visschers, 2006). The actual distribution of this phase and the gel microstructure can be investigated by means of microscopic techniques (Donato, Kolodziejcyk, & Rouvet, 2011). Among the available microscopic technique, conventional optical microscopy (COM) has the advantage of requiring easier sample preparation, lower cost of equipment maintenance and a less specialized operator compared to other advanced microscopic techniques like Confocal Scanning Laser Microscopy (CSLM) or Scanning Electron Microscopy (SEM) (Guyomarc'h, Jemin, Le Tilly, Madec, & Famelart, 2009; Mellema, Walstra, van Opheusden, & van Vliet, 2002). In addition, the presence of fluorescent markers in CSLM or the sample preparation in SEM could induce alterations in the microstructure of gels. Therefore the use of a non-invasive technique as COM permits to avoid this drawback.

Image analysis provides a tool for the characterization of protein gels and this study allows us to understand how the gel network is formed and how it is affected by the processing conditions (Langton & Hermansson, 1996). Moreover, Rodriguez-Hernández, Durand, Garnier, Tecante, and Doublier (2003) showed that an increase in the elasticity of gellan systems is related to compactness and interconnectivity of the network by digital image analysis (Rodriguez-Hernández et al., 2003). Also, Pugnaloni, Matia-Merino, and Dickinson (2005) showed that the decrease in pore size implies an enhanced interconnectivity of the network, which increases the gel rigidity in NaCAS gels containing sucrose (Pugnaloni et al., 2005).

To the best of our knowledge, no research has been undertaken on the use of COM to evaluate gel microstructure. Therefore, the aim of this work was to investigate changes in the microstructure of acid gels of NaCAS and SPI due to changes in gelling rate or cosolute addition by the analysis of digital images obtained by COM. Also, the relationship between the microstructure of gels and their rheological properties was analyzed.

2. Materials and methods

2.1. Materials

Bovine sodium caseinate powder, GDL and tris(hydroxymethyl) aminomethane (Tris) were purchased from Sigma–Aldrich Co. (Steinheim, Germany). HCl and NaOH were provided by Cicarelli SRL (San Lorenzo, Argentina).

NaCAS aqueous suspensions were prepared from dissolution of commercial drug in distilled water (isoionic pH) at room temperature. After concentration measurements, 0.15 g/L sodium azide was added as a bacteriostatic agent, and the solutions were stored at 4 °C. Protein concentration was determined by the Kuaye's method (Kuaye, 1994).

The SPI was prepared following the procedures outlined by Sorgentini and Wagner (1999), from defatted soy flour (Solae Latin America, Brazil), which was not heat-treated and was desolventized under mild conditions (90.7 ± 0.2 g/100 g, N × 6.25) (Sorgentini & Wagner, 1999). The WSP was prepared from the supernatant of the isoelectric precipitation (pH 4.5) of SPI proteins. It was adjusted to pH 8 and centrifuged (12,400 × g, 15 min, and 20 °C) to obtain a clarified supernatant. Later, 60 g of ammonium sulphate was added for each 100 mL of supernatant to achieve 90% saturation (Scopes, 1994; Sobral & Wagner, 2009). The resulting precipitate was removed by centrifugation (12,400 × g, 15 min,