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The effect of protein concentration and heat treatment temperature on micellar casein–soy protein mixtures

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ABSTRACT

The objective of this study was to investigate the effect of concentration and temperature on the rheological properties of soy proteins (SP) and micellar casein (MCN) systems. Individual and mixed (1:1) protein systems of 2–15% concentration were prepared and heat treated for 5 min at 40–90 °C. After cooling to 20 °C, their rheological properties were determined using steady-shear rheology. Zeta potential and particle size measurements were also conducted. Both proteins were negatively charged under all experimental conditions, but the absolute values of zeta potential and thus the stability of the protein solutions decreased with temperature and concentration. For SP solutions, viscosity and apparent yield stress increased with concentration. Shear thinning behavior was prevalent, becoming more pronounced with increasing concentration. Heat treatments at $T \geq 80$ °C induced glycinin denaturation, followed by aggregation and network formation when $C \geq 7.5\%$. Heat treatment did not significantly affect viscosity of MCN systems, while increasing concentration resulted in a significant increase in apparent viscosity and apparent yield stress. Most MCN systems exhibited Newtonian flow behavior, with the exception of systems with $C \geq 12.5\%$ treated at $T \geq 80$ °C, which became slightly shear thickening. Mixed SP–MCN systems mimicked the behavior of SP, with most values of rheological parameters intermediate between SP and MCN-only systems. Mixtures of 7.5–12.5% concentration treated at 90 °C displayed local phase separation, low viscosity and apparent yield stress, while 15% mixtures treated at 90 °C showed protein aggregation and incipient network formation. The data generated in this study can be used to develop a range of protein based products with unique flow characteristics and storage stability.

1. Introduction

Despite scientifically proven health benefits of soy, many Western consumers are reticent to adopt soy products in their diet, due to undesirable sensory properties. One way to enhance the acceptability of soy proteins could be to incorporate them in dairy products, which have both highly acceptable sensory properties and health benefits. The consumer studies conducted by Drake and Gerard (2003) indicated consumer interest and significant market potential for soy-fortified dairy foods. In recent years there have been several reports about the fortification of dairy products with soy proteins (Abdullah et al., 2003; Biswas, Chakraborty, & Choudhuri, 2002; Gokce & Gursoy, 2003; Mandal, Bandyopadhyay, & Ghatak, 1996). In most of these cases however, the physical and sensory properties of the final products were negatively affected by the addition of soy. For instance, Drake, Chen, Tamarapu, and Leenano (2000) reported that the addition of soy protein to dairy yogurts resulted in an increase in sensory chalkiness even at 1% addition.

A critical element in designing milk protein–soy protein blends with palatable textures is establishing the range of compositional and processing conditions that promote miscibility and homogeneity. While the interactions between soy proteins and milk serum proteins have been previously studied (Roesch & Corredig, 2005), the interactions between soy proteins and casein, the major milk protein, have not yet been investigated.

Soy proteins are represented by two classes of globular proteins: globulins (90%), which can be extracted using dilute salt solutions, and albumins (10%), which can be extracted by water (Fukushima, 1991). According to their sedimentation rates in a 0.5 M ionic strength buffer at pH 7.6, soybean globulins can be classified in four fractions: 2s (15%), 7s (34%), 11s (41.9%) and 15s (9.1%) (Koshiyama, 1969). The 11s and 15s fractions consist of glycinin and polymers of glycinin (Wolf, 1970), while the 7s fraction contains mostly

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β-conglycinin but also γ-conglycinin, lipoxygenases, α-amylases and hemagglutinins (Nielsen, 1985). The 2s fraction consists of Bowman-Birk and Kunitz trypsin inhibitors, cytochrome C, and α-conglycinin (Wolf, 1970). β-Conglycinin and glycinin represent approximately 80% of the total proteins in soy, and thus have a significant influence on the functional properties of soy protein systems. Glycinin exists as a hexamer with a molecular mass of 360 kDa (Renkema, Knabben, & van Vliet, 2001), while β-conglycinin is a trimeric glycoprotein with a molecular mass of 150–200 kDa (Utsumi, Matsumura, & Mori, 1997). Soy proteins denature upon heating, and in the denatured state they can form gels. In the case of glycinin, gel formation takes place via disulfide bonds and noncovalent bonds such as hydrophobic interactions, ionic and hydrogen bonds (Mori, Nakamura, & Utsumi, 1986). Gelation of β-conglycinin is caused by hydrophobic interactions and hydrogen bonds, with no contribution from disulfide exchange reactions (Nakamura, Utsumi, & Mori, 1986).

Caseins are thermally stable phosphoproteins that precipitate at an isoelectric pH of 4.6. They make up 82% of the true protein in milk and are composed of four individual casein proteins, α_{s1}-casein, α_{s2}-casein, β-casein and κ-casein, in a weight ratio of 3:0.8:3:1 (Schmidt, 1982). Individual casein molecules are disordered and very flexible, mostly due to their high proline content, whose cyclic structure prevents the formation of a highly organized secondary structure (Holt, 1992). In milk, casein is present as micelles, which are spherical and polydisperse particles with a weighted average diameter of about 200 nm (Beliciu & Moraru, 2009; de Kruif, 1998). Approximately two thirds of the micellar volume in solution is caused by hydration of the porous structure, which explains the generally high viscosity of casein suspensions (Dalgleish, 1997). Several casein micelle models have been developed over the last 50 years, but there still is no consensus as to the validity of any singular model (Horne, 2006). Despite some fundamental differences that exist among these models, particularly related to the existence of casein submicelles, there is general agreement related to certain aspects of the casein micelle structure, including the location of κ-casein at the surface of casein micelles and their role in stabilizing the casein micelles (Fox, 2003; Walstra, 1990). This is relevant to the current work, since κ-casein molecules can provide interaction sites between the casein micelles and other molecules. For instance, in heat treated milk the denatured serum proteins bind to κ-casein via disulfide bonds (Beaulieu, Pouliot, & Pouliot, 1999; Dalgleish, 1990; Dannenberg & Kessler, 1988; Singh & Fox, 1985; Singh & Latham, 1993). Under certain conditions, similar interactions could potentially occur between casein and soy proteins.

An important factor that can facilitate or impede the interactions between casein micelles and soy proteins is their miscibility. Miscibility of biopolymers in general and of proteins in particular is thermodynamic in nature and depends on many factors, such as concentration, temperature, and molecular weight (Moraru, Lee, Karwe, & Kokini, 2002; Tolstoguzov, 1991; Zimeri & Kokini, 2003a, 2003b, 2003c). Incompatibility is typical for proteins belonging to different classes within the Osborne classification, such as casein and soy globulins. Even proteins of the same class are incompatible when they differ in their conformations (i.e. native and denatured forms) (Polyakov, Grinberg, & Tolstoguzov, 1997). It has been reported that, even when incompatible, most mixed protein solutions in water have a rather high phase separation threshold, with the minimum total concentration of proteins at which phase separation of their mixed solution occurs ranging from 10% to 20% (Polyakov et al., 1997).

The objective of this study was to evaluate the effect of protein concentration and heat treatment temperature on the rheological properties of soy proteins–micellar casein mixtures, in a concentration range from 2% to 15%, and a weight ratio of 50:50. The knowledge gained from this study could then be used as a basis for the development of high protein foods with unique structure and functionality.

2. Materials and methods

2.1. Materials

The study was conducted on protein preparations obtained by membrane separation followed by spray drying, in order to ensure that the effects of the preparation methods on the native structure and properties of the two proteins are minimal. Soy protein isolate (SPI-6000; protein 90.9%, fat 3.3%, ash 5.8% on dry solids basis, moisture 5.4%) (Protient Inc., St. Paul, MN) was used as a source of soy proteins. Micellar casein powder (MCN-85, American Casein Company, Burlington, NJ) was used as a source of casein. The composition of the MCN powder is as follows: protein 84.93%, fat 2.1%, ash 9.5%, lactose 3.2% on dry solids basis, and moisture 4.8%. The following minerals were quantified in the MCN powder by testing at the Dairy One Forage Analysis Laboratory (Ithaca, NY), using the method described by Beliciu and Moraru (2009): calcium – 2.53% (d.b.), phosphorus – 1.47% (d.b.), magnesium – 0.10% (d.b.), potassium – 0.10% (d.b.), sodium – 0.07% (d.b.), and chloride 0.12% (d.b.).

2.2. Sample preparation

This study was performed in parallel on both individual (micellar casein and soy protein) and mixed protein systems; the latter contained the two proteins in a weight ratio of 50:50. Solutions[1] of micellar casein and soy protein of concentrations ranging from 2% to 15% (on a dry matter basis) were obtained by weighing the appropriate amount of protein powder and adding them to 100 mL of ultra-pure water. The method used for preparing the protein solutions (i.e. mixing with water) mimics the way in which dry ingredients are rehydrated for use in the food industry. The following preparation procedure was used for all protein solutions. To ensure that no clumping occurred, the protein powder was added slowly to the water in a beaker placed on a Fisher Thermix 310T stirring plate (American Instruments Exchange, Inc., Haverhill, MA), set at 500 rpm (dial speed 5). The water–protein mixture was kept on the stirring plate for 30 min at 25 °C under moderate agitation at 400 rpm (dial speed 4), in order to allow the powder to disperse well and the proteins to become hydrated. A second dispersion and hydration step consisted in pouring the solution in a Mojonnier bottle, in order to control foaming, and subjecting it to high shear agitation using an UltraTurrax Model T25 fitted with a S25N-18G dispersion tool (IKA Works Inc., Wilmington, NC), for 5 min at 21,500 rpm. The mixing time for the high-speed dispersion step was established by monitoring the evolution of particle size in the protein solution. The effect of the duration of the high-speed dispersion step on the effective diameter measured in micellar casein solutions is shown in Fig. 1. A mixing time of 5 min ensured that the particle sizes in solution reached the known particle size for casein micelles (Beliciu & Moraru, 2009). The soy protein powder dispersed and solubilized faster than casein, but the same mixing time was used for consistency. The procedure used for particle size analyses is described later in this manuscript. After the high-speed dispersion, the solutions were kept under continuous stirring at 300 rpm (dial speed 3) on the stirring plate until further

[1] Since casein is not water soluble, the casein–water mixtures are suspensions, not true solutions. However, in order to use a uniform terminology throughout the manuscript, the term "solution" will be used for all protein preparations, including casein.