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Effect of casein to whey protein ratios on the protein interactions and coagulation properties of low-fat yogurt

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ABSTRACT

In this study, we investigated the effect of casein (CN) to whey protein (WP) ratios (4:1, 3:1, 2:1, and 1:1) on gelation properties and microstructure of low-fat yogurt made with reconstituted skim milk with or without addition of whey protein concentrate. The rheological properties (storage modulus, G′; yield stress; and yield strain) of the obtained low-fat yogurt were greatly enhanced, the fermentation period was shortened, and the microstructure became more compact with smaller pores as the CN:WP ratio decreased. When CN:WP was 2:1 or 1:1, the obtained yogurt coagulum showed higher G′ and greater yield stress, with more compact crosslinking and smaller pores. In addition, the more of skim milk powder was replaced by whey protein concentrate, the more disulfide bonds were formed and the greater the occurrence of hydrophobic interactions during heat treatment, which can improve the rheological properties and microstructure of low-fat yogurt.
Key words: casein to whey protein ratio, low-fat yogurt, rheological behavior, microstructure

INTRODUCTION

Low-fat yogurt has been attracting increasing attention from health-conscious consumers because of its nutritional values and health-promoting properties (Kücükcetin, 2008). However, milk fat has important roles in emulsification and in flavor and texture development in the formed gels. A reduced fat content may reduce viscosity and increase whey syneresis, which can affect the appearance, texture, and mouthfeel of low-fat yogurt (Lee and Lucey, 2004; Houzé et al., 2005). Therefore, improving the structure and flavor deficiencies of low-fat yogurt deserved close attention.

Several methods have been used to improve the properties of low-fat yogurt, including addition of whey protein, use of suitable starter cultures, enhanced total solids content, use of thickeners, and modification of processing parameters (Sodini et al., 2004). The viscoelasticity or apparent viscosity of yogurt can be increased 2 to 3 times by adjusting the total solids and protein contents or by adding thickeners or enzymes to the milk base. Addition of thickeners (polysaccharides or gelatin) or enzymes (lactoperoxidase, protease, and transglutaminase) allows for new cross-links in the network and enhances the rigidity of the gel and its water-holding capacity. Processing parameters, including heat treatment, shearing, homogenization, and storage period, affect texture and thickness-in-mouth of yogurt. Starter cultures help to improve the smoothness and water-holding capacity of yogurt, because they can produce exopolysaccharides (**EPS**) during fermentation and gel formation. The addition of increasing levels of β-LG causes marked increases in storage modulus (**G′**) compared with α-LA, and some differences in behavior exist among the different β-LG variants (Graveland-Bikker and Anema, 2003).

Heat treatment, one of the predominant processes of dairy product manufacturing, leads to denaturation of milk proteins and interaction among denatured milk proteins, which may dramatically affect the texture and consistency of yogurt (Mulvihill and Grufferty, 1995). Whey proteins are much more heat-sensitive than casein. The denatured whey proteins interact with each other to form soluble whey protein aggregates or interact with casein micelles to form whey protein-coated casein micelles (Krzeminski et al., 2011). Most whey proteins are denatured during normal heat treatment of yogurt manufacture, during which the formation of disulfide bonds and occurrence of hydrophobic interactions within denatured whey proteins and between denatured whey proteins and κ-CN on the surface of

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casein micelles leads to the formation of WP–κ-casein complexes (Smits and Brouwershaven, 1980; Haque and Kinsella, 1988; Singh and Myhr, 1998). Addition of whey protein has potential application in the manufacture of yogurt (Lucey et al., 1999; Graveland-Bikker and Anema 2003), cheese (Hinrichs, 2001; Kelly and O'Kennedy, 2001), and other coagulated dairy products; it not only improves physical properties and microstructure but also alters the functional properties of the obtained products. Therefore, heat treatment of milk fortified with whey protein is conducive to a high level of crosslinking within the gel network, which results in a denser yogurt structure and enhanced yogurt viscosity and water-holding capacity (Remeuf et al., 2003).

Although whey protein fortification has been investigated in the processing of low-fat yogurt, it is difficult to independently evaluate the effect of whey proteins on yogurt properties because the addition of WP also changes the protein and TS contents (Damin et al., 2009). Including a high proportion of whey protein might impart an undesirable whey flavor as well as a grainy texture under some conditions (Lucey and Singh, 1997; González-Martınez et al., 2002). In addition, a relatively low content of casein is believed to result in a more open gel structure, making the coagulum network more sensitive to syneresis (González-Martınez et al., 2002). Therefore, determining the proper ratio of CN to whey protein (**WP**) is critically important. Whey protein concentrate (**WPC**) had been used to partially substitute for skim milk powder (**SMP**) to alter the CN:WP ratio in yogurt manufacture (Bhullar et al., 2002; Akalin et al., 2008). By using a decreased CN:WP ratios, increased maximum gel strength, reduced whey drainage, and a denser network could be obtained (Puvanenthiran et al., 2002; Kücükcetin, 2008), and lower viscosity and higher intensities of graininess and yellow color would be also exhibited (Tomaschunas et al., 2012). Although some research has been conducted on CN:WP ratios in yogurt, the effects on the fermentation process and the interaction between CN and WP have not yet been investigated.

The objective of this research was to investigate the effect of CN:WP ratios with constant protein content on the gelation properties and microstructure of low-fat yogurt. The interactions within denatured whey protein and casein micelles was also evaluated by determination of disulfide bonds and surface hydrophobicity. Samples with CN:WP ratios of 3:1, 2:1, and 1:1 were compared with a reference yogurt manufactured with skim milk powder with a CN:WP ratio of 4:1.

Table 1. The formula of milk base with different casein to whey protein ratios

Item[1]	Casein to whey protein ratio 4:1	3:1	2:1	1:1
SMP (g/100 mL)	12.94	12.13	10.78	8.08
WPC (g/100 mL)	0	0.33	0.87	1.96
Total protein (%)	4.0	4.0	4.0	4.0
Total solids (%)	12.94	12.46	11.65	10.04

[1]SMP = skim milk protein; WPC = whey protein concentrate.

MATERIALS AND METHODS

Materials and Chemicals

Skim milk powder (by weight, 32.5% protein, 0.43% fat, 52.1% carbohydrates; CN:WP = 4:1) and whey protein concentrate 80 (**WPC80**; by weight, 81.23% protein, 0.11% fat, 13.30% carbohydrates) were supplied by Hilmar Ingredients (Hilmar, CA). 8-Anilino-1-naphthalenesulfonic acid (**ANS**) and 5,5′-dithio-bis2-nitrobenzoic acid (DTNB) were obtained from Sigma Chemical Co. (St. Louis, MO). Other reagents used in the present study were of analytical grade.

Preparation of Low-Fat Yogurt

Milk Base Preparation. Control milk base was prepared as follows: 12.94 g of SMP was added to 87.06 g of distilled water at 25°C (CN:WP ratio = 4:1). Experimental trials were prepared by mixing SMP with WPC80 quantitatively at CN:WP ratios of 3:1, 2:1, and 1:1, respectively, as shown in Table 1. The total milk base content was 100 g and the protein content of all samples was 4% (wt/wt). The milk bases were stirred for 3 h at 25°C and stored at 4°C overnight to ensure complete hydration.

Fermentation. One hundred grams of each sample was prepared. After homogenization at 20 MPa and 55°C, each milk base was treated in a thermostatically controlled boiling water bath (DK-8B, Jinghong Laboratory Equipment Co. Ltd., Shanghai, China) until the center temperature of sample reached 95°C and was then held for 5 min. The heated samples were immediately cooled to 42°C for analysis and further fermentation. Direct Vat Set starter culture (ABY-8, Chr. Hansen, Milwaukee, WI) containing *Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, and *Bifidobacterium* was added at the recommended concentration of 0.005% (wt/wt). Milk samples were incubated at 42°C, and the fermentation process was immediately stopped by rapidly cooling to