J. Dairy Sci. 100:1–11
https://doi.org/10.3168/jds.2016-11704

Addition of sodium caseinate to skim milk increases nonsedimentable casein and causes significant changes in rennet-induced gelation, heat stability, and ethanol stability

Yingchen Lin,* Alan L. Kelly,† James A. O'Mahony,† and Timothy P. Guinee*[1]
*Teagasc Food Research Centre Moorepark, Fermoy, Co. Cork, Ireland
†School of Food and Nutritional Sciences, University College Cork, Ireland

ABSTRACT

The protein content of skim milk was increased from 3.3 to 4.1% (wt/wt) by the addition of a blend of skim milk powder and sodium caseinate (NaCas), in which the weight ratio of skim milk powder to NaCas was varied from 0.8:0.0 to 0.0:0.8. Addition of NaCas increased the levels of nonsedimentable casein (from ~6 to 18% of total casein) and calcium (from ~36 to 43% of total calcium) and reduced the turbidity of the fortified milk, to a degree depending on level of NaCas added. Rennet gelation was adversely affected by the addition of NaCas at 0.2% (wt/wt) and completely inhibited at NaCas ≥0.4% (wt/wt). Rennet-induced hydrolysis was not affected by added NaCas. The proportion of total casein that was nonsedimentable on centrifugation (3,000 × g, 1 h, 25°C) of the rennet-treated milk after incubation for 1 h at 31°C increased significantly on addition of NaCas at ≥0.4% (wt/wt). Heat stability in the pH range 6.7 to 7.2 and ethanol stability at pH 6.4 were enhanced by the addition of NaCas. It is suggested that the negative effect of NaCas on rennet gelation is due to the increase in nonsedimentable casein, which upon hydrolysis by chymosin forms into small nonsedimentable particles that physically come between, and impede the aggregation of, rennet-altered *para*-casein micelles, and thereby inhibit the development of a gel network.
Key words: milk, protein fortification, dairy ingredients, processing characteristics

INTRODUCTION

Milk protein powders are extensively used as ingredients because of their techno-functional and nutritional properties. Applications include their use as ingredients in high-protein beverages, nutritional beverages (e.g., for children), formulated and consumer foods, and recombined milks for the preparation of cheeses and fermented milk products (Gilles and Lawrence, 1982; McSweeney et al., 2013; Lagrange et al., 2015). The techno-functionalities required vary considerably according to application and may include water binding capacity, emulsification, heat stability, ability to undergo gelation (e.g., on heating, acidification, or rennet treatment), and structure formation. In many of these applications, milk proteins are exposed to various unit operations (including acidification, heating, rennet treatment, dehydration) and environments (e.g., food matrices differencing in solvent quality) that challenge their stability and functionality (Agarwal et al., 2015). The different functional requirements during food processing and formulation are met through the supply of a range of ingredients differing in protein type and content, extent of protein denaturation, degree of mineralization, and composition.

Skim milk powder (**SMP**) and sodium caseinate (**NaCas**) are widely used ingredients. They differ in method of manufacture, protein structure, and degree of mineralization. The manufacture of NaCas involves pH adjustment of the milk to the isoelectric point, precipitation of the casein and whey separation, washing and concentration, addition of sodium hydroxide to readjust the pH of the casein from ~6.8 to 7.0, and drying (Carr and Golding, 2016). During acidification, essentially all of the colloidal calcium phosphate, which contributes to the self-assembly of the casein into micelles, is dissolved, resulting in the dissociation of the casein micelles into smaller particles referred to as submicelles. Analysis of NaCas indicates significantly lower ratios of calcium- and phosphorus-to-casein compared with native casein in milk and the occurrence of the casein in the form of particles (~10 nm compared with ~150 to 200 nm in the native casein micelle; O'Connell and Fox, 2000). In contrast, the structure of the casein and its degree of mineralization in SMP is not affected by the method of manufacture, which involves evaporation and drying of the milk.

Received July 6, 2016.
Accepted November 1, 2016.
[1]Corresponding author: tim.guinee@teagasc.ie

These differences in casein structure and degree of mineralization are likely to affect rennet gelation, a critical parameter in the manufacture of cheese. Gaygadzhiev et al. (2012) found that the rennet-induced gelation of milk was impaired by the addition of 0.05% (wt/wt) NaCas and completely inhibited at a level ≥0.2% (wt/wt). The authors suggested the inhibitory effect of NaCas was likely due to the adsorption of the rennet-hydrolyzed NaCas to the surface of the *para*-casein micelle and the concomitant increase in steric and electrostatic repulsion, which impeded aggregation of the latter. Subsequently, Nair and Corredig (2015) found that the addition of 0.6% (wt/wt) NaCas to milk concentrated 3-fold had no effect on rennet gelation when the milk had been concentrated by ultrafiltration, but severely impeded gelation when the milk had been concentrated quiescently by osmotic concentration using polyethylene glycol. The dependence on the method of concentration was attributed to potential differences in the extent of rearrangement of the native micelles during concentration, which affected their interaction with the added NaCas and the degrees to which it became adsorbed at the surface of, or incorporated into, the micelle. Thomar and Nicolai (2015) reported that the addition of NaCas to an aqueous dispersion of native phosphocasein powder (**NPC**, 1.5%, wt/wt, protein) promoted dissociation of casein, Ca, and P from the micelle to a degree that increased with weight fraction of added NaCas.

The heat stability of dairy proteins is important in products such as UHT milk, infant milk formula, and coffee whiteners. Consequently, heat stability of milk and the factors affecting it have been extensively studied (Huppertz, 2016). Comparatively, little information is available on the effect of adding NaCas to milk on the heat stability. Cho and Singh (1999) observed an increase in the heat stability (140°C) of recombined milk, formulated by blending an aqueous milk fat emulsion and reconstituted SMP, over the pH range 6.4 to 7.1, when the emulsion was stabilized using NaCas instead of SMP or whey protein concentrate.

Cream liqueurs are formulated mainly from cream (33–40%, wt/wt), ethanol (~12–15% vol/vol), sucrose (~18.5%, wt/wt), milk protein (typically ~3.5%, wt/wt, NaCas), and water (~25–30%, wt/wt; Muir, 1988). The ethanol stability of NaCas is of particular relevance in emulsion stabilization and control of storage-related flocculation, thickening, or gelation. O'Kennedy et al. (2001) reported that the ethanol stability of a 3% (wt/wt) aqueous dispersion of NaCas depended on pH and ionic strength.

The principal objective of the current study was to investigate the effect of incrementally increasing NaCas from 0 to 0.8% (wt/wt) on the rennet gelation, heat stability, and ethanol stability of fortified milk (4.1%, wt/wt, protein) prepared by adding a blend of SMP and NaCas to skim milk (3.3%, wt/wt, protein) in which the weight ratio of protein from SMP-to-NaCas was varied from 0.8:0.0 to 0.0:0.8. A secondary objective was to relate the effects of NaCas on the above properties to changes in the partition of protein and minerals between the sedimentable and nonsedimentable phases obtained on ultracentrifugation of the fortified milk at 100,000 × *g*.

Table 1. Composition of dairy ingredients used for fortifying the protein content of skim milk[1]

Composition[2]	Ingredient	
	SMP	NaCas
Total protein (%, wt/wt)	36.4	87.9
Casein (% TP)	82.8	97.0
Whey protein (% TP)	11.2	1.2
Lactose (%, wt/wt)	46.35	1.27
Ca (mg/100 g)	1,295	193
P (mg/100 g)	913	687

[1]SMP = low heat skim milk powder; NaCas = sodium caseinate.
[2]TP = total protein.

MATERIALS AND METHODS

Milk Protein Ingredients

Milk protein ingredients used included extra low-heat SMP (<4% of total whey protein denatured) and NaCas. The respective levels of total protein, casein, whey protein, lactose, Ca, and P contents of the SMP and NaCas are shown in Table 1.

Preparation of Milk

A skim milk base (3.3%, wt/wt, protein) was prepared from reconstituted SMP, rather than using fresh skim milk, to ensure a compositionally consistent starting material and to avoid the potential confounding effects of seasonal changes in milk quality and composition on the measured characteristics, during replicate trials. The skim milk base was prepared by dispersing the SMP in distilled water at 50°C while shearing at 6,300 rpm for 5 min using a high-shear mixer (Silverson model L4RT, Silverson, Chesham, UK) until the powder was visually dispersed. The sample was then placed at 4°C for 22 h to allow for hydration of the protein. The preparation of the fortified milk (4.1%, wt/wt, protein) involved adding the blend of SMP and NaCas powders to the base skim milk in sufficient quantities to increase the protein content from 3.3 to 4.1% (wt/wt). The ratio of protein derived from the SMP to protein