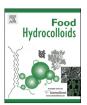
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Gelation of recombined soymilk and cow's milk gels: Effect of homogenization order and mode of gelation on microstructure and texture of the final matrix



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ABSTRACT

The present study examined the development of the gel network as well as the final structure obtained from mixed soymilk—cow's milk gels recombined with cream. An understanding of the structural changes occurring as a result of different process manipulations may aid with understanding the reported texture differences in these matrices. Results showed that changing the mode of homogenization, the composition of the fat globule surface changed. When both milk and soymilk were homogenized with the cream, the resulting gel had the highest gel stiffness at the end of fermentation. Confocal microscopy demonstrated that as long as milk was homogenized with cream (regardless of the presence of soymilk during homogenization), the resulting gel showed finer protein aggregates. On the other hand, without homogenization or when soymilk was homogenized with cream before milk addition, a coarser gel structure was noted. The composite gels where both casein micelles and soy proteins aggregated simultaneously appeared homogeneous compared to the gels formed by the gelation of caseins before soy proteins. This work clearly demonstrated that by modulating the timing of casein and soy protein aggregation, as well as the composition of the fat interface, it was possible to modify the gel structure and affect texture properties.

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1. Introduction

Previous studies have demonstrated that it is possible to obtain composite gels containing soy proteins and casein micelles (Lin, Hill, & Corredig, 2012; Roesch & Corredig, 2005). Protein gels made from a mixture of protein particles from soymilk and milk show great potential as platforms for a new category of food products, enriched in protein and providing additional health benefits. Indeed, there are demonstrated synergies in consuming milk and soy products. For example, it has been shown that addition of skim milk powder to soy yogurts can increase conversion of soy isoflavone glycosides to a more biologically active form (Pham & Shak, 2008). However, the presence of a significant amount of soy in mixed soymilk—dairy milk products presents a challenge to consumer acceptance in the western world as soybeans tend to impart beany and grassy flavors (Ankenman Granata & Morr, 1996;

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Yazici, Alvarez, & Hansen, 1997). In addition to flavor, texture is also a challenge, due to the different types of gels obtained with soymilk protein particles compared to casein micelles (Malaki Nik, Alexander, Poysa, Woodrow, & Corredig, 2011; Roefs, De Groot-Mostert, & Van Vliet, 1990).

Soymilk gels are typically prepared by heating soymilk to denature the proteins, followed by addition of ionic salts, such as MgCl₂, or by acidification using glucono-δ-lactone or bacterial cultures (Kohyama, Sano, & Doi, 1995; Wang, Yu, & Chou, 2002). Heating is a necessary step in soy protein gelation. Heating denatures the soy proteins and leads to formation of soluble aggregates of protein subunits linked by disulfide bonds. These covalently linked aggregates may also interact with each other via hydrophobic forces and hydrogen bridges (Ren, Tang, Zhang, & Guo, 2009). Addition of ionic salts or acidification induce charge shielding or charge neutralization on these heat-induced protein aggregates allowing for short-range interactions such as hydrogen bridging and van der Waals forces to take place and induce gel formation (Kohyama et al., 1995). Soymilk gels are not typically network gels but rather tend to be particulate in nature (Malaki Nik et al., 2011). Casein gels, on the other hand, are made up of a

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network of strands of aggregated caseins (Roefs et al., 1990). Such gels can be generated by destabilization of the $\kappa\text{-}casein$ hairy layer and subsequent aggregation of casein micelles by non-covalent interactions. This can be accomplished either by enzymatic cleavage of $\kappa\text{-}caseins$ using rennet or by causing the hairy layer to collapse by charge neutralization via acidification (Alexander & Dalgleish, 2004; Lucey, 2002). However, unlike the high gel point of acidified soymilk (around pH 6) (Malaki Nik et al., 2011), unheated dairy milk must be acidified further, to around pH 5.0 to produce an acid–gel (Alexander & Dalgleish, 2004). Such differences in protein properties can be capitalized upon when making mixed soymilk—skim milk gels.

It has previously been demonstrated that by simultaneous use of rennet and acidification, it is possible to make mixed soymilk and dairy milk gels in which both soy proteins and caseins contribute to the formation of the gel network (Lin et al., 2012). It is hypothesized that by careful manipulation of the gelation mechanism (i.e. aggregation of milk proteins before soy proteins or simultaneous aggregation) it may be possible to control the distribution of caseins and soy proteins within the gel network. If caseins are aggregated first, it is possible that when soy proteins begin to aggregate, they are incorporated in an already pre-formed network of casein micelles. Thus the resulting gel network might be less homogeneous. However, if caseins and soy proteins are aggregated simultaneously, they may be more evenly dispersed throughout the network. Previous work has demonstrated that the gelation mechanism may have an impact on the perception of fattiness in mixed soymilk—dairy milk gels with added cream (Grygorczyk. Lesschaeve, Corredig, & Duizer, 2013). However, the underlying causes of this effect have yet to be examined.

In addition to changing how proteins come together to form a gel network, the sensory properties of protein gels are significantly modified in the presence of fat globules. Recently, some strides have been made in understanding perception of fatty attributes and creaminess in semi-solid gels through studies of oil droplet release and fat globules—matrix interactions (Dresselhuis, de Hoog, Cohen Stuart, Vingerhoeds, & van Aken, 2008; Sala, van de Valde, Cohen Stuart, & van Aken, 2007; Sala, de Wijk, van de Velde, & van Aken, 2008). It has been shown that it is possible to alter perception of fat-related attributes by modifying the extent of the interactions between fat globules and the gel matrix or by selecting gel matrices with differing melting properties, to control release of fat globules (Sala et al., 2007, 2008). In the case of dairy yogurt, addition of fat globules is known to increase gel thickness and perception of creaminess (De Wijk, Terpstra, Janssen, & Prinz, 2006; Lucey, 2004).

While simple addition of fat globules to a gel is sufficient to make a substantial impact on sensory properties, these properties can also be modified by homogenization of the fat globules (Lucey, 2004). Intact, non homogenized milk fat globules are enwrapped by the milk fat globule membrane. When this milk is gelled, for example in the case of yogurt-making, the gel network forms around the fat globules and these large globules become entrapped in the pores of the gel network (Van Vliet, 1988). However, when cow's milk is homogenized with fat, caseins are the main component present on the fat globule membrane (Cano-Ruiz & Richter, 1997), and the fat globules become an integral part of the gel network, and contribute positively to an increase in the gel modulus (Van Vliet, 1988). In such gels, the fat globules are referred to as "interacting fillers". The majority of the work on interacting and non-interacting fillers has been carried out on dairy protein platforms. To date, there is no such published work carried out on mixed soymilk-dairy milk gels.

It has been previously demonstrated (Grygorczyk, Lesschaeve et al., 2013) that modifying the homogenization order and

gelation mechanism had a significant impact on the thickness, mouthcoating and perceived fattiness of the samples. The present study followed from the texture perception work reported above, and examined further the physico-chemical details underlying these changes in texture of mixed protein networks. The purpose of this study was to examine the differences in gel network development and microstructure between soymilk—dairy milk gels produced using different homogenization orders and gelation mechanisms. The results were compared to the previous texture perception work (Grygorczyk, Lesschaeve et al., 2013) and provided increased understanding of the structure—texture relationships and principles for the design of functional structures in protein matrices.

2. Materials and methods

2.1. Preparation of materials

Two sets of treatments were employed to rearrange the localization of the matrix components: the order of homogenization (designated by letters A–D) and the gelation mechanism (designated by numbers 1 and 2). Table 1 lists the 8 treatments that were examined. Four homogenization treatments were applied including: (A) no homogenization, (B) soymilk homogenized with cream followed by addition of skim milk (homogenized separately), (C) skim milk homogenized with cream followed by addition of soymilk (homogenized separately), and (D) skim milk, soymilk and cream homogenized together. The use of both renneting and bacterial acidification allowed for improved control of the timing of protein gelation. Rennet was added either at pH 6.6 to induce casein aggregation before soy protein aggregation (gelation mode 1), or at pH 6.4 to induce simultaneous casein and soy protein aggregation (gelation mode 2).

2.1.1. Soymilk preparation

Soymilk was prepared with food grade practices, to be able to better relate this research to previous literature (Grygorczyk, Lesschaeve et al., 2013). Soybeans were purchased from a local grocery store (Guelph, ON) and characterized as reported in a previous study (Grygorczyk, Lesschaeve et al., 2013). Soybeans (240 g) were rinsed and soaked overnight in filtered water (Brita® Faucet Filtration System (Model FF-100), Brita Canada Corp., Brampton, ON, Canada). Once hydrated, the soybeans were rinsed once more with water and placed into a household soymilk maker with 855 mL of filtered water (Soyquick™ Premier Milk Maker Model SQ930P, Kitchen's Best Manufacturing Group Ltd., Nanaimo, BC, Canada). After the soymilk maker cycle was completed, okara (soymilk insoluble fiber) was removed by filtering the hot soymilk

Table 1 Treatments examined in the study.

Treatment identifier	Homogenization treatment	Gelation mode
A1 B1 C1 D1	Unhomogenized Homogenized soymilk + milk fat Homogenized skim milk + milk fat Homogenized skim milk + soymilk + milk fat	Caseins aggregated first Caseins aggregated first Caseins aggregated first Caseins aggregated first
A2	Unhomogenized	Simultaneous casein and soy protein aggregation
B2	Homogenized soymilk + milk fat	Simultaneous casein and soy protein aggregation
C2	$Homogenized\ skim\ milk+milk\ fat$	Simultaneous casein and soy protein aggregation
D2	$\label{eq:homogenized} \mbox{Homogenized skim milk} + \mbox{soymilk} + \\ \mbox{milk fat}$	Simultaneous casein and soy protein aggregation

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