Food Chemistry 141 (2013) 1716–1721

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Acid induced gelation of soymilk, comparison between gels prepared with lactic acid bacteria and glucono-δ-lactone

A. Grygorczyk, M. Corredig *

Department of Food Science, University of Guelph, Guelph, Ontario, Canada N1K 2W5

ARTICLE INFO

Article history:
Received 16 November 2012
Received in revised form 24 March 2013
Accepted 25 March 2013
Available online 25 April 2013

Keywords:
Soy protein
Lactic acid bacteria
Glucono-δ-lactone
Soymilk
Acid induced gel

ABSTRACT

The objective of this work was to compare the gelation of soymilk particles induced by the acidification of a commercial starter culture with that resulting by addition of glucono-δ-lactone (GDL). Structure formation was followed using rheology, and the microstructure was observed by confocal microscopy. Acidification of lactic acid bacteria resulted in a higher gelation pH (pH 6.29 ± 0.05) compared to that of a gel induced by GDL (pH 5.9 ± 0.04). This difference was attributed to the longer time available for rearrangements of the soymilk particles in soymilk with starter cultures compared to the fast acidification by GDL. In spite of the earlier gelation pH, there were no observed differences in the final gel stiffness measured at pH 5.1, the value of tan δ, the frequency dependence and the linear viscoelastic range of the gels measured at the final pH. Microstructural observations also showed a similar protein network structure.

1. Introduction

In addition to the use of soymilk as a base for traditional soy based products such as soy beverages and tofu, soymilk is increasingly employed as a protein matrix to design novel food products. For this reason, a better understanding of the details of its colloidal stability and gelation behaviour are needed. Soymilk is a beverage produced by grinding soaked soybeans with water, followed by cooking around boiling temperatures for around 15 min and removal of insoluble fibre (okara) by filtration or centrifugation (Canabady-Rochelle, Sanchez, Mellema, & Banon, 2009; Prabhakaran, Perera, & Valiyaveettil, 2006). Heating of soymilk is an essential step to denature anti-nutritional compounds, and to modify the structure of soymilk particles to improve their colloidal stability and decrease their size distribution to a few micrometre in diameter (Malaki Nik, Tosh, Woodrow, Poysa, & Corredig, 2009). It has also been shown that soy protein denaturation induced by heating is a necessary step for gel formation (Renkema & Van Vliet, 2002). During heating, soluble protein aggregates, composed of acidic and basic polypeptides of glycinin linked *via* disulfide bonds form, as well as a small amount of α and α′ subunits of β-conglycinin (Ren, Tang, Zhang, & Guo, 2009). These aggregates, of submicron sizes, interact by hydrophobic interactions and hydrogen bonding to make protein particles with basic subunits of glycinin in the interior and the acidic glycinin subunits and α and α′ subunits of β-conglycinin on the exterior (Ren et al., 2009). When acid curds are prepared, acidification is usually carried out using glucono-δ-lactone (GDL), as the pH decreases gradually, creating a homogeneous gel (Malaki Nik, Alexander, Poysa, Woodrow, & Corredig, 2011). Unlike in heat-induced gelation of soy protein isolates (Renkema & van Vliet, 2002), except for the bonds that develop during heating, covalent bonds do not play a major role in the network formation of acid-induced gels. The driving forces behind acid gelation of soy proteins are non-covalent in nature (Kohyama, Sano, & Doi, 1995), and include salt bridging (Zhang, Liang, Tian, Chen, & Subirade, 2012) and short range interactions such as hydrogen bonding and Van der Waals forces (Ringgenberg, Alexander, & Corredig, 2013).

As the pH of soy protein suspensions decreases to values around 6, it has been observed that the basic subunits of glycinin and the β subunit of β-conglycinin are the first to destabilise. As the system approaches a net neutral charge, the acidic subunits of glycinin and the α and α′ subunits of β-conglycinin then also begin to participate in gel network formation (Ringgenberg et al., 2013).

The use of lactic acid bacteria to produce soymilk curds has been evaluated in the past (Liu et al., 2009; Mital & Steinkraus, 1975). Lactic acid bacteria are known to primarily ferment sucrose in soy products. However, some lactic acid cultures are also capable of fermenting other low molecular weight carbohydrates found in soybeans, such as raffinose and stachyose (Mital & Steinkraus, 1975). Although several studies have focused on lactic acid bacteria metabolism of soy products (Mital & Steinkraus, 1975; Mital, Steinkraus, & Naylor, 1974), and on large deformation rheological properties and microstructure of final gel products (Donkor, Henriksson, Vasiljevic, & Shah, 2007; Ghosh, Chattoraj,

* Corresponding author. Tel.: +1 (519) 824 4120x56101; fax: +1 (519) 824 6631.
E-mail address: milena.corredig@uoguelph.ca (M. Corredig).

0308-8146/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2013.03.096

& Chattopadhyay, 2011; Yang, Fu, & Li, 2012), no data exist in the literature regarding the formation of the structure of soymilk acid-gels produced using lactic acid bacteria.

Several studies exist on the acid-induced aggregation of soymilk particles acidified with GDL (Kohyama et al., 1995; Malaki Nik et al., 2011; Tay & Perera, 2006). The gel pH ranges between 5.6 and 5.8 depending on the variety of soybean used, and different concentrations of GDL do not result in differences in the gelation pH (Malaki Nik et al., 2011). However, acidification using lactic acid bacteria is known to be far slower than GDL acidification (Lucey, Tamehana, Singh, & Munro, 1998).

In consideration of the complex composition of soymilk particles, it may be hypothesised that allowing more time for protein rearrangements to occur may influence the gelation behaviour of soymilk. Although studies exist comparing the acidification of cow's milk using GDL or lactic acid cultures (Lucey et al., 1998), no such studies are reported for soymilk. Thus the purpose of this study was to evaluate the difference in the gelation behaviour of soymilk particles during acidification induced with GDL or bacterial culture.

2. Materials and methods

2.1. Soymilk preparation

Soymilk was prepared using food grade materials with a procedure that would resemble that of a household process. Soybeans were obtained from a local grocery store and characterised as described in the following section. A portion of 175 g of beans was washed with filtered water (Brita® Faucet Filtration System (Model FF-100), Brita Canada Corp., Brampton, ON, Canada) and soaked overnight in water. The hydrated soybeans were rinsed once again with water and placed into a household soymilk maker (Soyquick™ Premier Milk Maker Model SQ930P, Kitchen's Best Manufacturing Group Ltd., Nanaimo, BC, Canada) with 933 mL of filtered water to produce 4% protein soymilk. The soymilk maker cycles involved the following steps: soybeans and water were heated to 80 °C (in approximately 5 min) and once the temperature was reached, the soybeans were ground for 5 s. After this short grinding cycle, the soymilk temperature was brought up to just below boiling temperature (1 min) and then four grinding cycles were performed, each cycle lasting 40 s with a 5-s pause between cycles. After grinding, the soymilk maker continued to hold the soymilk just below boiling temperature for 10 min. The hot soymilk was then immediately poured through a strainer (Kitchen's Best Manufacturing Group Ltd., Vancouver, BC, Canada) to remove the okara, and then passed twice through a cheese cloth (Kitchen's Best Manufacturing Group Ltd, Vancouver, BC, Canada). After filtration the soymilk was cooled to refrigeration temperatures.

2.2. Soybean and soymilk characterisation

To determine the amount of protein as well as the polypeptide distribution of the soybeans, the beans were milled with a grain mill (IKA® Works Inc., Wilmington, NC) and the ground flour was analysed using the Dumas combustion method in a LECO FP-528 (Leco Corp., St. Joseph, MI) with 6.25 as a conversion factor for % protein from % N (AACC method 46–30.01, 1999). The protein concentration in soymilk was also measured with Dumas, while solids were determined by weighing 1 mL soymilk in dry aluminium pans containing dry sand (Fisher Sci., Mississauga, ON, Canada) as a dispersing agent. The pans were placed into an IsoTemp forced air oven (Fisher Sci., Mississauga, ON, Canada) at 105 °C for 24 h. Particle size distribution of the soymilk was determined using laser light scattering (Mastersizer S; Malvern Instruments Inc., Southborough, MA), using 1.46 as the refractive index of the soymilk particles and 1.333 as the refractive index of the dispersant (water) as previously reported (Malaki Nik et al., 2009). The ratio of 11S:7S protein in the soy flour and soymilk was determined using SDS–PAGE, using conditions published in the literature (Keerati-u-rai & Corredig, 2009). Gel analysis was carried out using a Gel Doc™ EZ Imager (Bio-Rad, Mississauga, ON, Canada). Crude oil content of the soybeans was determined using the soxhlet oil extraction method with petroleum ether (AOAC method 945.16) with a Labline multi-unit extraction heater (Barnstead Labline; Thermo Scientific, Asheville, NC). The fat content of soymilk was determined using the Babcock method (AOAC method 989.04, 2000) with a Babcock System for Fat Analysis (Cole-Parmer, Montreal, QC, Canada) as previously reported in the literature (Buono, Erickson, Fung, & Jeon, 1990). Mineral analysis was determined using inductively coupled plasma optical emission spectroscopy by the advanced analytical laboratories at the University of Guelph (Guelph, Ontario, Canada).

2.3. Gelation experiments

Soymilk was acidified at 40 °C with either 0.6% glucono-δ-lactone (GDL) (Sigma–Aldrich Co., St. Louis, MO) or YO-MIX™ 511 LYO 375 DCU (Danisco Canada Inc., Scarborough, ON, Canada). The starter culture was pre-diluted by adding 0.2 g of the freeze-dried starter culture to 30 mL of warm soymilk (40 °C) and stirring for 30 s before adding 157 μL of the diluted culture to 30 mL of soymilk sample for a final concentration of 0.00349% starter culture. Yo-mix is a starter culture commonly employed in the fermentation of milk for yoghourt production, containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus*. Soymilk was pre-warmed at 40 °C for 5 min. After addition of GDL or bacterial culture, the soymilk was mixed for 30 s and then immediately placed in the rheometer.

Aliquots of the same samples were kept at 40 °C in a circulating water bath to measure pH in parallel to the rheology experiments. The pH was recorded on line using an Accumet AR15 pH metre (Fisher Sci., Mississauga, ON, Canada) connected to a computer, using AR15 pH recorder software (Mediavention Engineering Inc., Guelph, ON, Canada). Gel formation was followed using an Advanced Rheometer AR 1000 with Rheology Advantage Instrument Control AR software v5.4.0 (TA Instruments Ltd., New Castle, DE) at a constant strain of 0.01, and a frequency of 1 Hz. The temperature was controlled with an external water bath and kept at 40 °C. Once the samples reached a pH of 5.1, a frequency sweep was performed, using a constant strain of 0.01 and frequencies of 0.01–10 Hz. Finally, a strain sweep was carried out at a frequency of 1 Hz and oscillation stress of 0.5–150 Pa, to determine a yield strain, defined as the value of strain at which the elastic modulus deviated by 10% from its value in the linear viscoelastic range.

2.4. SDS–PAGE of fermented soymilk

Samples of fermented soymilk were collected at pH 5.8 and 5.5 and analysed by SDS–PAGE to determine if there was any protein hydrolysis occurring during fermentation. Fermented soymilk was analysed for proteolysis at pH values soon after the gel point, since if proteolysis was occurring at an extent sufficient to alter the gel point, it is expected that proteolysis would have been detectable by such pH values. The method employed has been previously described others (Keerati-u-rai & Corredig, 2009). The gels were scanned using a Bio-Rad Gel Doc™ EZ Imager (Bio-Rad, Mississauga, ON, Canada). The protein composition of fermented soymilk was compared to that of unfermented soymilk and of unfermented soymilk incubated at 40 °C for 2.5 h.