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Effect of dynamic high pressure on milk fermentation kinetics and rheological properties of probiotic fermented milk

Miguel Meirelles de Oliveira [a], Pedro Esteves Duarte Augusto [b], Adriano Gomes da Cruz [a], Marcelo Cristianini [a,*]

[a] Department of Food Technology, School of Food Engineering, University of Campinas — UNICAMP, Campinas, SP, Brazil
[b] Department of Agri-food Industry, Food and Nutrition (LAN), Luiz de Queiroz College of Agriculture (ESALQ), University of São Paulo (USP), Piracicaba, SP, Brazil

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ABSTRACT

This work studied the fermentation kinetics and the rheological behavior of gel formation during fermentation and storage. The milk (2% v/v fat) was subjected to conventional homogenization (15/5 MPa, control treatment) and dynamic high pressure (50/5 MPa, 100/5 MPa, 150/5 MPa and 180/5 MPa), and fermented by *Streptococcus thermophilus* in co-culture with *Lactobacillus acidophilus*. The results showed that dynamic high pressure (DHP) did not alter the fermentation kinetics ($p > 0.05$). However, the rheological behavior during fermentation showed that higher pressures increased the consistency. During storage, an increase of 15% in the consistency index (K′) and a reduction of 31% in the gel syneresis were observed for samples processed at 180/5 MPa, as compared to the control ones (124.67 $Pa \cdot s^{n'}$; 13.5%). After the 28th day, these differences increased up to 27% and 40%, respectively. It is concluded that DHP promotes an increase in consistency and reduction syneresis of fermented milk without changing the fermentation.

Industrial relevance: The use of DHP allows to increase the consistency of probiotic fermented milk without the addition of any additive. Thus, the product would attend to the probiotic's consumer market, which demands high quality products, with a reduced amount of fat (2%), high consistency and without addition of other ingredients. Furthermore, the DHP does not affect the product fermentation, which would facilitate its direct implementation in the industry.

1. Introduction

The intake of probiotic fermented milks has been associated with several human health benefits (Awaisheh et al., 2013; Tabasco et al., 2012; Wang et al., 2012), and the technological parameters involved in their processing are of great interest to be investigated (Cruz, Faria, Saad, Sant' Ana, & Cristianini, 2010; Cruz et al., 2010, 2013). Moreover, the impact of the use of unconventional technologies on the intrinsic quality attributes of probiotic fermented milks should also be assessed (Shiby & Mishra, 2013).

Dynamic high pressure (DHP) is an emerging technology based on the continuous pumping of a fluid through a narrow gap. Due to the mass and energy conservation laws, the fluid drastically increases its velocity and temperature, resulting in high shear stress, high turbulence and cavitation, also result of a great pressure drop to atmospheric pressure. Consequently, the product constituents are subjected to high shear stress, leading to structural changes (Dumay et al., 2013; Floury, Desrumaux, Axelos, & Legrand, 2002; Hayes & Kelly, 2003; Pinho, Franchi, Augusto, & Cristianini, 2011). This technology has been widely studied in food preservation (Tribst, Sant'ana, & De Massaguer, 2009) once it inactivates vegetative bacterial cells (Campos & Cristianini, 2007; Tribst, Franchi, & Cristianini, 2008), yeasts and molds (Tahiri, Makhlouf, Paquin, & Fliss, 2006; Tribst, Franchi, Cristianini, & De Massaguer, 2009; Tribst, Franchi, Cristianini, & Massaguer, 2011; Tribst, Sant'ana, & De Massaguer, 2009). Additionally, some studies have reported the use of DHP in the alteration of some milk constituents, including: the size of fat globules (Hayes, Fox, & Kelly, 2005; Thiebaud, Dumay, Picart, Guiraud, & Cheftel, 2003), functionality of proteins by increasing the level of denaturation and aggregation (Grácia-Juliá et al., 2008; Sandra & Dalgleish, 2005) and inactivation of enzymes (Datta, Hayes, Deeth, & Kelly, 2005; Hayes & Kelly, 2003).

Several studies have been conducted to investigate the influence of DHP on milk constituents used to manufacture fermented milks, especially in yogurts. These studies have focused on the extension of shelf life and improvement of water-holding capacity, texture, reduction of post-acidification and development of dairy cultures (Grácia-Juliá et al., 2008; Hayes et al., 2005; Patrignani et al., 2007; Serra, Trujillo, Quevedo, Guamis, & Ferragut, 2007).

However, these studies have shown that the promotion of texture in fermented milk by the DHP is dependent upon the amount of fat in milk. In milk containing 3.5% of fat (Serra et al., 2007), it showed an increase

* Corresponding author at: Monteiro Lobato, 80, School of Food Engineering, University of Campinas, Postal Code 13083-862 Campinas, SP, Brazil. Tel.: +55 19 3521 7592; fax: +55 19 3521 0202.
E-mail address: olecram@fea.unicamp.br (M. Cristianini).

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in consistency due to DHP, while the opposite behavior was shown on low fat milk, with 0.1% of fat content (Serra, Trujillo, Jaramillo, Guamis, & Ferragut, 2008). Further, when milk with 1.5% of fat was subjected to the microfluidization process, no changes in the viscoelastic properties between different treated samples were observed (Ciron, Gee, Kelly, & Auty, 2011). Thus, due to the milk fat high importance and cost to the industry, it becomes important to assess the effect of DHP on the reduced fat milk (2%) consistency. Furthermore, no studies have evaluated the effect of DHP processed milk fermented by *Streptococcus thermophilus* with *Lactobacillus acidophilus* on the pH reduction during fermentation in comparison with the kinetics of gel formation, as well as the evaluation of rheological behavior of fermented milk during storage in correlation with the microstructure and syneresis.

However, the present study aimed to evaluate the influence of dynamic high pressure of milk (2% v/v fat) on its fermentation kinetics by *S. thermophilus* in co-culture with *L. acidophilus* and the rheological behavior of gel formation during fermentation. During refrigerated storage, both the rheological behavior and the spontaneous syneresis of the probiotic fermented milk were evaluated.

2. Material and methods

2.1. Inoculum preparation

Lyophilized commercial cultures of *S. thermophilus* TA-40 and *L. acidophilus* NFCM® (Danisco, São Paulo, Brazil) were used in the experiments. DVS (Direct Vat Set) cultures were diluted separately in sterile reconstituted skimmed milk (10% w/v) (Tangará Foods, Espírito Santo, Brazil). Subsequently, the inoculums of *S. thermophilus* (10^{11} CFU·mL^{-1}) and *L. acidophilus* (10^{10} CFU·mL^{-1}) were fractionated separately in Eppendorf tubes (1 mL) and frozen at −20 °C until use. Thus, each 1 mL of inoculum added to 1 L of milk had 10^8 CFU·mL^{-1} of *S. thermophilus* and 10^7 CFU·mL^{-1} of *L. acidophilus*. This procedure was performed to obtain inoculums with the same concentration of microorganisms.

The culture of *L. acidophilus* was thawed and pre-activated in a proportion of 5% (v/v) in 20 mL of sterile reconstituted skimmed milk (10% w/v) (Tangara Foods, Espírito Santo, Brazil), supplemented with 2% w/v glucose, and incubated at 37 °C for 16 h. The inoculum of *S. thermophilus* was not pre-activated.

To confirm the amount of culture added to the milk at the beginning of the fermentation, enumeration of *S. thermophilus* in M17 Agar (OXOID®, UK) incubated aerobically at 37 °C for 48 h was performed and culture of *L. acidophilus* was enumerated in Man Rogosa agar Sharpe (MRS) (HIMEDIA®, India) incubated aerobically at 37 °C for 72 h (Lima et al., 2009). All steps were performed under aseptic conditions.

2.2. Treatment and processing of fermented milks

This experiment used ultra-high temperature cow's milk (2.0% v/v fat, 3.0% w/v protein, and 10.4% w/w total solids) (Shefa, São Paulo, Brazil) that was processed at 90 °C/5 min in a water bath (model AV-30 autoclave Phoenix, São Paulo, Brazil), and then cooled to 60 °C (the total time took 15 min: 10 min to reach 90 °C, maintained for 5 min and immediately cooled at 60 °C in 30 s). After the thermal process the milk was subjected to a dynamic high pressure process using a homogenizer (GEA-Niro-Soavi, Parma, Italy). The sequence of this process was chosen based in the study conducted by Hernández and Harte (2008) that showed more consistences in the product when the heat treatment was conducted before DHP and the smaller consistency was observed when the DHP was conducted before heat treatment.

Two stages were applied to all treatments, in which the control sample was processed at 15/5 MPa (homogenization conditions conventionally applied in the dairy industry), and the other treatments were processed under dynamic high pressure at 50/5 MPa, 100/5 MPa, 150/5 MPa and 180/5 MPa. A stainless steel coil (305 cm long and 0.5 cm in diameter) was coupled to the output of the DHP device and immersed in a water bath at 5 °C which reduced the milk temperature to approximately 10 °C in 5 s. A total of 2 L of milk was collected for each treatment.

After 20 min, samples were heated in a water bath (FANEM MOD.116®, São Paulo, Brazil) to 43 °C and *S. thermophilus* and *L. acidophilus* were added to the milk to obtain concentrations of approximately 10^8 CFU·mL^{-1} and $7.5 \cdot 10^7$ CFU·mL^{-1}, respectively. Each treatment was fractionated into their respective sterile glass jars (121 °C/15 min) up to 150 mL (50 mm diameter and 85 mm height). Fermentation was carried out at 43 °C and the pH was monitored using a potentiometer (BEL Engineering W3B, Italy) until pH 4.6 was reached. Then the samples were cooled to 5 °C and stored at this temperature until further analysis.

2.3. Fermentation kinetics

Cow's milk previously processed by DHP and inoculated with the cultures (see Section 2.2) was incubated at 43 °C and its pH was monitored with an electrode inserted in the sample during the entire fermentation period. The pH data were collected every 20 min during fermentation using a potentiometer (BEL Engineering W3B, Italy) coupled to a computer. The acidification rate was calculated as the pH variation in 1 min (dpH / dt), and expressed as pH units·min^{-1}. Then, the maximum acidification rate was defined as the maximum value of dpH / dt.

2.4. Rheological analysis

Two types of small amplitude oscillatory rheological tests (SAOR) were performed. By conducting small amplitude tests, it retains the integrity of the gel, evaluating the interactions between proteins and fat globules. A controlled-stress rheometer (AR2000ex, TA Instruments, USA), equipped with a *Vaned Quarter* geometry (28 mm diameter and 42 mm length) and a Peltier temperature control system were used.

The first analysis was performed to evaluate the kinetics of gel formation by time sweep procedures, based on a study conducted by Lee and Lucey (2003). For this purpose, milk previously processed by DHP, heated to 43 °C and inoculated with both bacterial cultures (as described in Section 2.2) was transferred to the rheometer's cup (30 mm diameter and 80 mm height) and the geometry was adjusted to 4 mm gap. The strain and oscillatory frequency values were within the minimum detectable sensitivity of the equipment to minimize interference in the gel formation, being set up to 0.1 Pa and 0.1 Hz, respectively. Storage modulus (G′) values were recorded every 20 min for 6 h of fermentation at 43 °C. The rate of gel formation was calculated as the G′ variation in 1 min (dG′ / dt), and expressed as Pa·min^{-1}. The G′ describes the product elastic (solid) behavior and, consequently, the energy stored and released at each oscillatory cycle. Therefore, it directly reflects the gel formation during fermentation.

The second analysis consisted in evaluating the gels during storage at 5 °C, using the same rheometer geometry, but through frequency sweep procedures. The rheometer's cup was replaced by the jar containing the fermented milk in order to maintain the gel structure unchanged. Once this analysis preserves the original structure of the gel (Lee & Lucey, 2010), it is necessary to choose a strain within the linear viscoelastic region. For this purpose, a strain sweep (0.01–10 Pa) procedure, under a fixed frequency of 1.0 Hz, was performed and the stress within the linear viscoelastic region of 1 Pa was determined. Finally, a frequency sweep (0.01 to 100 Hz) procedure under controlled strain of 1 Pa was carried out, and the viscoelastic parameters G′ (storage modulus, elastic behavior) and G″ (loss module, viscous behavior) were attained (Ciron et al., 2011). For the evaluation of the experimental results, values obtained before the breaking of the gel were used according to the