International Dairy Journal 148 (2024) 105794

Contents lists available at ScienceDirect

International Dairy Journal

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

Use of thermal-treated or high-pressure treated liquid micellar casein concentrate as an ingredient to manufacture a high-protein content yoghurt

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ARTICLE INFO

Article history:
Received 25 April 2023
Received in revised form
29 August 2023
Accepted 29 August 2023
Available online 7 September 2023

ABSTRACT

Three types of yoghurts were elaborated from different milk mixtures and treatments: thermal-treated skim milk enriched with skim milk powder (CY); thermal-treated skim milk enriched with liquid micellar casein concentrate (TTY) and high-pressure treated skim milk enriched with liquid micellar casein concentrate (HPY). The effects of composition and treatments on the final yoghurt and the evolution over the storage time (28 d) at 4 °C were studied. According to the results, HPY showed low syneresis, high firmness, and moderate viscosity. These findings reveal the potential application of HP technology in the production of yoghurts with high-protein content.

1. Introduction

Nowadays consumers are demanding minimally processed and additive-free foods, with high nutritional value. In this context, the demand for low-fat and high-protein yoghurt is experiencing a growing consumer interest, which is expected to continue due to the improvements in taste and texture and also due to the clear evidence of dairy proteins' health benefits (Jørgensen et al., 2019; Küster & Vila, 2017).

Yoghurt is a semisolid dairy product elaborated with milk that is fermented by specific microorganisms, usually *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* cultures that convert lactose into lactic acid, reducing the pH of the milk and thus inducing the gel formation. Physical, textural, and sensory properties are important quality attributes in yoghurt that directly affect consumer preference and product acceptability (Guichard, 2002). For example, making yoghurt from a very low level of milk fat results in a product with impaired physicochemical and sensory properties, such as weak texture, increased syneresis (whey separation), and reduced smoothness and creaminess in mouth (Nguyen, Kravchuk, Bhandari, & Prakash, 2017).

Conventionally, syneresis is reduced by increasing the total solids of the yoghurt mixture with dry ingredients (Tamime & Deeth, 1980) such as skim milk powder (SMP), hydrocolloids (e.g., gelatin, pectin, carrageenan) or concentrated milk. Another dairy ingredient that presents a potential interest is micellar casein concentrate (MCC). It is because MCC can increase the total solids content, and at the same time, increase the amount of protein (especially caseins) with high nutritional value (Bong & Moraru, 2014) without adding large amounts of lactose, avoiding the formation of an unacceptable high post-acidity in high-protein yoghurts (Qi, Liu, Yuan, Regenstein, & Zhou, 2022).

MCC is a high-protein ingredient produced by membrane separation of different fractions of milk, which is based on the different sizes of milk constituents. Usually, skim milk is firstly heated to 50 °C and microfiltered (pore size of 1.4 μm) and, subsequently, the microfiltered fraction is again microfiltered using a membrane with a smaller pore size of 0.1 μm to separate caseins from whey proteins, lactose, minerals and water. During this process, bacteria, spores and somatic cells could also be reduced or removed from milk (Wang, Fritsch, & Moraru, 2019).

In addition, a diafiltration step completes the separation and concentration process. The final product is a retentate with high casein and low lactose content. This retentate can be spray-dried and converted into powder, which is the most common practice in the dairy industry, or kept in a liquid state (LMCC). However,

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https://doi.org/10.1016/j.idairyj.2023.105794

powder handling has certain disadvantages. MCCs have poor solubility and wettability, and a reconstitution process has to be done before yoghurt manufacture. Therefore, liquid micellar casein concentrates (LMCCs) are a good alternative to dry casein concentrates as no reconstitution process is needed. In addition, handling a liquid product saves the economic and energy cost of spray drying, and it can also improve taste and offer different functional properties (Amelia & Barbano, 2013).

In traditional yoghurt manufacture, milk is subjected to a thermal treatment, usually 90 °C for 10 min, and cooled to 41–45 °C before the addition of cultures, not only to kill spoilage microorganisms that can be naturally present in milk but also to denature whey proteins, increasing the viscosity of the final product and reducing the gelation time (Anema, Lowe, & Lee, 2004).

An alternative to traditional heat treatments that could be explored for yoghurt manufacture is high-pressure processing (HPP). HPP is a non-thermal emerging technology able to ensure the microbial safety of processed foods. Stratakos et al. (2019) demonstrated that HPP (600 MPa, 3 min) effectively achieved 5 log reductions for pathogenic *Escherichia coli*, *Salmonella* and *Listeria monocytogenes*; reductions similar to those obtained by pasteurisation of raw milk. The ability to control the quality and microbiological stability of LMCC by HP (600 MPa, 5 min) was also demonstrated by García, Iturmendi, Galarza, Maté, and Fernández-García (2022). However, HPP can alter the functionality and properties of the treated product. In the case of milk or milk protein concentrates, depending on the pressure applied, several changes in proteins occur, altering their structure mainly by disrupting the micelle and enhancing the supply of accessible nitrogen for bacteria (Huppertz, Fox, & Kelly, 2004a). Previously reported by other authors, HP-treated milk can produce acid gels with lower syneresis and greater rigidity and breaking strength compared with gels formed with untreated milk (Considine, Patel, Anema, Singh, & Creamer, 2007; Harte, Amonte, Luedecke, Swanson, & Barbosa-Cánovas, 2002; Harte, Luedecke, Swanson, & Barbosa-Cánovas, 2003).

Various studies have been focused on the physicochemical properties of enriched yoghurts with milk proteins (Bong & Moraru, 2014; Qi et al., 2022) or on high-pressure induced treatments for yoghurt elaboration (Harte et al., 2002, 2003; Lopes et al., 2019); however, to the best of our knowledge, there is no research focused on high-pressure-treated liquid micellar casein concentrates as an ingredient for yoghurt enrichment. Thus, the objective of this research was to compare the physical, chemical and rheological properties of three different yoghurts: a control yoghurt (CY) formulated with thermal-treated skim milk enriched with skim milk powder (SMP), a high-pressure treated yoghurt (HPY) formulated with high-pressure treated skim milk and LMCC, and thermally-treated yoghurt (TTY) manufactured with thermal-treated skim milk and LMCC, and their evolution over time during the standard shelf life of yoghurt (28 d at 4 °C).

2. Materials and methods

2.1. Ingredients

A single batch of 300 L of raw skim milk (RSM) was used as ingredient and also to obtain LMCC. For LMCC production, RSM was heated to 50 °C and microfiltered through a ceramic membrane of 1.4 μm and then microfiltered again through a spiral-wound membrane with a nominal pore size of 0.1 μm. The complete LMCC production method is detailed by Galarza, Iturmendi, García, Fernández, and Maté (2022). Commercial skim milk powder (SMP) was purchased from a local grocery. The average compositions of the three ingredients (RSM, LMCC and SMP) are shown in Table 1. A freeze-dried starter culture (YO-MIX 300 LYO 10 DCU) containing a mixed strain culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Danisco; Copenhagen, Denmark) was used, and the culture was stored at 4 °C until use.

2.2. High-pressure treatment

Raw skim milk (~3 L) or LMCC (~3 L) were transferred to polyethylene bags that were filled without headspace and sealed. Further, the bags were vacuum-packed and sealed in polyethylene bags (El Carmen, Spain). High-pressure treatments were carried out with a Stansted FOOD-LAB 9000 unit (Stansted Fluid Power, Stansted, Essex UK). The selected pressure treatment was 600 MPa for 5 min with a decompression rate of 8.3 MPa s^{-1}. Two products were obtained: HP-treated milk (HP-M) and HP-treated LMCC (HP-LMCC).

2.3. Heat treatment

Raw skim milk (~8 L) or LMCC (~3 L) were transferred to glass jars and introduced in the autoclave (Marrodan; Lodosa, Spain) and treated at 90 °C for 10 min and then cooled to 42 °C in 2 different runs. The equivalent thermal treatment applied was calculated considering the kinetic factor of whey protein denaturation (Agrawala & Reuter, 1979), being F (T_{ref} 90 °C, z-value = 14.4) = 19.4 ± 0.6 min. A control jar was used to register the internal temperature reached in the treated product. Two different products were obtained: thermal-treated milk (TT-M) and thermal-treated LMCC (TT-LMCC).

2.4. Yoghurt manufacture

Three different mixtures of treated dairy fractions were done standardised with a total solids content of 10% to obtain 3 different types of yoghurts, i.e., high-pressure yoghurt (HPY; a mixture of 55% HP-M and 45% HP-LMCC), thermal-treated yoghurt (TTY; a mixture of 55% TT-M and 45% TT-LMCC) and control yoghurt (CY; composed of 98.6% TT-M and 1.4% SMP).

For HPY and TTY the fractions were mixed and warmed at 38 °C before the addition of the starter culture. For CY, SMP was added to TT-M and mixed for 15 min at 38 °C to reach the complete dissolution of the powder.

Then, the starter culture (0.02%, w/w) was added to the milk mixtures and mixed at low speed for 30 min at 38 °C. Subsequently, individual sterilised glass jars (37 for each type of yoghurt; 111 in total) were filled with the corresponding mixtures (125 ± 2 g) and introduced in a water bath at 43 °C. The fermentation rate was controlled by measuring the evolution of pH and temperature of yoghurts. For this purpose, one yoghurt of each type (CY, TTY and HPY) was monitored by introducing a pH meter and a temperature probe. Once the pH reached 4.60 the yoghurts were cooled down and stored at 4 °C. The analyses were carried out on day 1 (at least

Table 1
Composition of the fractions used for yoghurt formulation.[a]

Fraction	Component (g 100 g^{-1})				
	Total solids	Total protein	Lactose	Fat	Ash
RSM	9.09 ± 0.01	3.35 ± 0.00	5.01 ± 0.01	0.00 ± 0.00	0.73 ± 0.00
LMCC	11.76 ± 0.50	9.40 ± 0.85	1.79 ± 0.19	0.00 ± 0.00	1.00 ± 0.06
SMP	95.99 ± 0.03	32.54 ± 0.05	54.53 ± 0.03	1.00 ± 0.00	7.92 ± 0.02

[a] Abbreviations are: RSM, raw skim milk; LMCC, liquid micellar casein concentrate; SMP, skim milk powder.

18 h since they were produced and stored at 4 °C), day 14 and day 28.

The acid lactic bacteria load of yoghurts ad day was measured to check the accomplishment with the national regulations (lactic acid bacteria load >10^7 cfu g^{-1}) as well as the enterobacteria load (<10 cfu g^{-1}) (data not shown).

2.5. Physicochemical analyses

The total solid content (TS) was determined in triplicate by oven drying the samples at 105 °C for 24 h, and the ash content was determined by drying the samples at 550 °C for 4 h. Total nitrogen (TN) and non-casein nitrogen (NCN) were determined in triplicate by the Kjeldahl method (AOAC, 2000); methods 991.20 and 998.05, respectively. Total protein (TP) and the non-casein protein (NCP) were calculated as TN or NCN multiplied by factor 6.38. The casein protein content (CP) was calculated by subtracting NCN from TN and multiplying by the same factor.

Lactose content was determined in triplicate by the official method AOAC 2006.06 and the results are expressed in g lactose 100 g^{-1} yoghurt.

The pH was measured in triplicate with a Basic 20 pH meter (Crison, Spain) at 20 °C. Titratable acidity (TA) was determined in triplicate: the yoghurts were diluted in distilled water (1:2) and the acidity was determined by titration with 0.1 M NaOH to pH 8.4. The results are expressed as TA (%).

Colour evaluations were carried out in a CM-2500d colorimeter (Konica Minolta, Japan) and were expressed in the CIELAB scale L*a*b*. To exclude variable cover surface conditions, the specular interference was included in the colour measurement. The following settings were used: 100% UV; illuminant: D65; observer angle: 10° and measurement area: 8 mm. For each sample, three measurements were performed at 20 °C.

2.6. Water holding capacity and texture determination

The water-holding capacity was determined following the method described by Bong and Moraru (2014). For each sample, 10 g of yoghurt were centrifuged at 1.250 × *g* for 10 min at 5 °C in a refrigerated centrifuge (Sigma; Osterode am Harz, Germany). The amount of whey after centrifugation was weighed in triplicate and the results are expressed as % (sample weight after removing whey in total sample).

A texture test was conducted using a TA-XT plus (Stable Micro Systems; Surrey, UK), equipped with a 5-kg loading cell. The selected parameters were: cylinder probe diameter 36 mm (model P/36R), test force 0.05 N, pre-test speed 5 mm s^{-1}, test speed 1 mm s^{-1}, post-test speed 3 mm s^{-1} and penetration distance 75%. The results are expressed as firmness (N) (maximum force developed within penetration). For each sample, three measurements were performed at 20 °C.

2.7. Rheological properties

The rheological properties of yoghurts were measured at 10 °C using a cup and bob geometry (Z34DIN) in a Haake Rotovisco RV1 viscosimeter (Mess-TechnikGmbH U., Go, Germany). The analysis was carried out in 2 steps. First, samples were placed in the cup for thixotropy test: a shear rate sweep from 1 to 100 s^{-1} (30 s), followed by a steady shear rate at 100 s^{-1} (840 s) and 100 to 1 s^{-1} (30 s). The hysteresis area was used to express the time-dependent flow behaviour. The analysis was performed in triplicate and the results were expressed in Pa s^{-1}.

In the second step, the samples were stirred gently with a spatula and allowed to equilibrate before placing 20 mL of the sample into the cup. A shear rate sweep from 1 to 300 s^{-1} (300 s), followed by a steady shear rate at 300 s^{-1} (60 s), and 300 to 1 s^{-1} (300 s) was used. The results were fitted to the Ostwald de Waele rheological model (Eq. (1)):

$$\sigma = K\,\gamma^n \tag{1}$$

where σ is the shear stress (Pa), γ is the shear rate (s^{-1}), K is the consistency coefficient (Pa s^n) and n is the flow behaviour index.

The analysis was performed in triplicate.

2.8. Statistical analysis

The results are expressed as means ± standard deviation. Data were analysed by one-way ANOVA followed by Tukey's HSD test with a 95% of significance level. All statistical analyses were performed with SPSS (IBM, USA).

3. Results and discussion

3.1. Characterisation and fermentation rate of yoghurts

The initial composition of the yoghurts and their fermentation rate are shown in Table 2 and Fig. 1, respectively.

Yoghurts were elaborated maintaining an average total solids content of ~10.3 g 100 g^{-1} (Table 2). Due to the different composition of the dairy mixtures, the protein content varied between yoghurts; thus, yoghurts with ~6 g 100 g^{-1} of protein content (TTY and HPY) had lower lactose levels (3.2 g 100 g^{-1}), while CY had ~4 g 100 g^{-1} protein with 5.4 g 100 g^{-1} lactose.

The fermentation rates (Fig. 1) of the yoghurts were mainly affected by the composition of the milk mixtures, whose initial pH was 6.55 (CY), 6.60 (HPY) and 6.63 (TTY), and the time to reach 4.60 was 90 min for CY, and 252 and 260 min for TTY and HPY, respectively. The differences between the fermentation rates of CY and the casein enriched yoghurts (HPY and TTY) can be attributed to the protein content: milk formulations with high protein content have higher buffering capacities, resulting in an increased fermentation time to obtain a predetermined pH value (Salaün, Mietton, & Gaucheron, 2005).

3.2. Physicochemical properties of yoghurts

In Fig. 2 the evolution over time of pH and titrable acidity of yoghurts is shown. Although the fermentation was stopped when the pH of the three yoghurts reached 4.60, pH values showed significant differences after 1 d of storage between the samples: HPY showed a slight pH increase (4.64 ± 0.02), TTY practically did not vary (4.58 ± 0.03) and CY likely showed a post-acidification process (4.51 ± 0.02).

Regarding the evolution of pH over time, the pH of the three types of yoghurts continued dropping during the storage at 4 °C, especially during the first 14 d. Post-acidification during cold storage occurs mainly as a result of the continued conversion of lactose to lactic acid by *Lactobacillus bulgaricus* (Wang, Kristo, & LaPointe, 2020). In the second part of storage (14–28 d), TTY showed a slightly faster rate of decline than the others, but lower than that of the previous 14 d. At the end of storage (d 28) CY and HPY reached similar pH.

These results are in line with the evolution of the acidity (Fig. 2). Acidity was influenced by the composition and also by the treatment. CY showed the lowest values of acidity, even during storage. In contrast, HPY and TTY did not show significant differences in acidity between them. These differences between CY and TTY-HPY are likely attributable to composition; on the one hand, the high

Table 2
Composition of control yoghurt (CY), heat-treated yoghurt (TTY) and high-pressure yoghurt (HPY).[a]

Yoghurt	Component (g 100 g^{-1})				
	Total solids	Total protein	Casein	Lactose	Ash
CY	10.34 ± 0.05[c]	4.12 ± 0.12[a]	3.90 ± 0.12	5.42 ± 0.09[b]	0.93 ± 0.00[c]
TTY	10.22 ± 0.04[a]	6.07 ± 0.03[b]	5.95 ± 0.04	3.16 ± 0.02[a]	0.88 ± 0.00[a]
HPY	10.29 ± 0.06[ab]	6.47 ± 0.04[c]	6.12 ± 0.06	3.21 ± 0.04[a]	0.90 ± 0.00[b]

[a] Values are presented as the mean ± standard deviation; means followed by different lowercase superscript letters in the same column differ significantly (P <0.05). Total protein calculated as total nitrogen × 6.38; casein content calculated as (total nitrogen − non-casein protein) × 6.38.

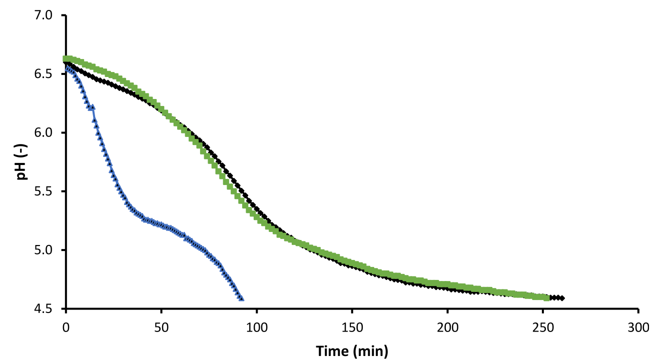

Fig. 1. Fermentation rate of control yoghurt (CY; ▲), heat-treated yoghurt (TTY; ■) and high-pressure yoghurt (HPY; ◆).

buffering capacity provided by the dairy blend with higher protein content, and on the other hand, their bound minerals (García et al., 2022).

In Table 3 the colour parameters (L*, a*, b*) of the yoghurts and their evolution over time are shown. In general terms, the three yoghurts showed high lightness (L*) due to the opacity of the gel, and a low chromaticity (a*, b*), adopting typical values of this fermented milk product. However, there are small differences between yoghurts. The lightness value lightly decreased (ΔL*~2,7) for yoghurts elaborated with the pressurised milk mixture (HPY), although these differences diminished during storage time. This difference may lie in the HP-induced effects: during pressurisation the casein micelles are disrupted losing their ability to scatter the light, leading to a decline in the L* value (Yang et al., 2020). However, Harte et al. (2003), did not observe differences in L* values after lactic fermentation between yoghurts made with skim milk subjected to HPP (300, 400, 500 and 676 MPa for 5 min) or heat treatment (85 °C for 30 min). This was probably due to casein micelle aggregation induced by both treatments, giving rise to a matrix of large aggregates. To a lesser extent, differences in composition and treatments also generated variations in the a* coordinate, decreasing its greenness chromaticity due to the greater protein concentration in the enriched yoghurts (HPY and TTY). In part, this may be because the compounds in the permeate portion of the milk provide a green colour that has been removed, i.e., green riboflavin (Hurt & Barbano, 2010). Concerning the b* coordinate, it was observed that CY (lower CN:WP ratio) showed higher yellow chromaticity than those containing LMCC. This can be attributed to an increase in Maillard reaction products due to the higher lactose content of CY.

3.3. Water-holding capacity and firmness

The ability of yoghurt to retain the serum within the gel structure is commonly known as water-holding capacity (WHC). This parameter is inversely related to syneresis, which is an undesirable phenomenon; it consists of an accumulation of whey on the surface of the yoghurt as a consequence of gel contraction. This complex phenomenon can be accelerated by several factors such as total solids content (Lee & Lucey, 2010), high incubation temperature (Lee & Lucey, 2003), gel porosity, etc.

The WHC and the firmness of yoghurts and their evolution over time are shown in Fig. 3. On day 1, yoghurts elaborated with SMP (CY) showed the lowest WHC (P <0.05). Syneresis is a time-dependent phenomenon and the aforementioned differences were observed during the 28 d of storage. This may be attributed to the different composition of this yoghurt: lower TP content and higher lactose content. In addition, the treatments applied to the milk mixtures (TT or HP) also influenced the WHC: on day 1, yoghurts elaborated with thermal-treated milk and LMCC (TTY) showed the highest value, but after 14 d of storage it was surpassed by HPY, ending up (28 d) with no differences between them.

During the acidification process by lactic fermentation of a thermal-treated yoghurt milk base, whey protein-coated casein micelles begin to aggregate and form a network (Lucey, 2004). Lower WHC is related to an unstable gel network and excessive rearrangements of a weak gel network (Lucey, 2001). Harwalkar and Kalab (1986) found that when casein concentration is increased in a formulation of yoghurt with skim milk powder, a rise in the interparticle interactions occur, the casein chains become shorter, the pore dimensions decrease, and the density of the matrix increases. In addition, higher amounts of lactose should lower the hydration of casein, which may result in smaller sizes of casein particles. These two mechanisms together result in an overall reduction of the interstitial space in the gel, increasing the WHC. Meletharayil, Patel, Metzger, and Huppertz (2016) investigated the effect of lactose level on acid gels elaborated with reconstituted milk protein concentrate (MPC) thermal-treated at 90 °C for 10 min, and acidified with glucono-delta-lactone (GDL). These authors observed that increasing the lactose content of the MPC dispersions to 5.6 or 11.2%, increased the WHC and decreased the microstructural porosity of the acid gels at pH 4.6. In our study, the experimental data (Fig. 3) showed that the effect of LMCC on WHC was much greater than the potential increase due to the differential lactose content of CY.

Treatments and composition also affected the firmness of the yoghurts (Fig. 3). Yoghurts elaborated with LMCC (TTY and HPY) showed higher firmness values than CY. Also, higher records for HPY were observed compared with TTY. During storage, the firmness of the three yoghurts increased, but with a steeper slope for those with higher protein content.

Differences in firmness (Fig. 3) may be attributed to the different compositions of total solids and also to the treatments applied to the milk mixture. In general, an increase in protein content of a yoghurt formulation, yields a yoghurt with greater firmness, mainly due to the increased amount of proteins participating in the gel network (Mistry & Hassan, 1992). Regarding the effect of the treatments, microscopic observations of yoghurt samples elaborated with unpressurised milk versus milk treated at 600 MPa for 30 min carried out by Johnston, Austin, and Murphy (1993), noticed an increase in the number of network strands in pressurised milk

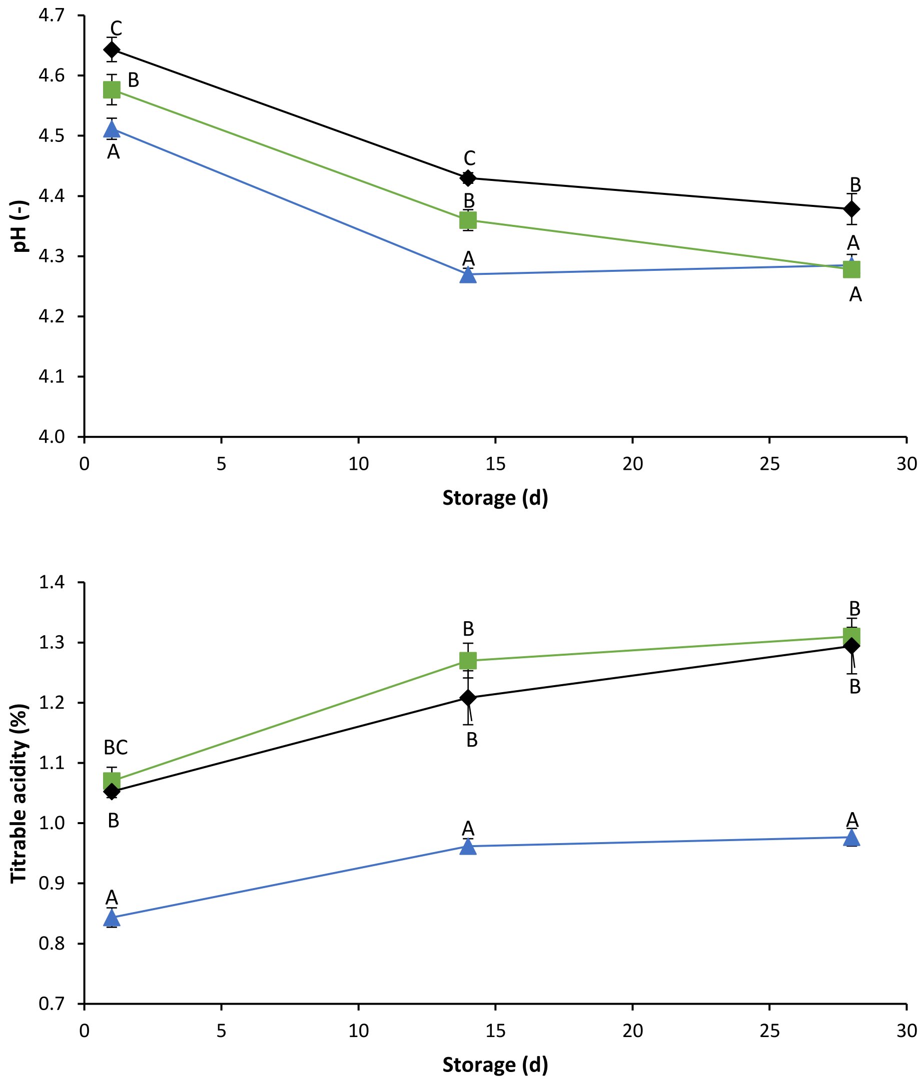

Fig. 2. Evolution of pH and titrable acidity (%) for 28 days at 4 °C of control yoghurt (CY; ▲), heat-treated yoghurt (TTY; ■) and high-pressure yoghurt (HPY; ◆). Different lowercase letters (a,b,c) indicate differences (P <0.05) between days of storage. Different capital letters (A,B,C) indicate differences (P <0.05) between yoghurts on the same day of storage.

Table 3
Evolution of colour parameters of control yoghurt (CY), heat-treated yoghurt (TTY) and high-pressure yoghurt (HPY) stored for 28 days at 4 °C.[a]

Yoghurt	Colour parameter								
	L			a			b		
	D1	D14	D28	D1	D14	D28	D1	D14	D28
CY	83.09 ± 0.05^{bB}	82.84 ± 0.17^{abB}	82.73 ± 0.03^{aAB}	-2.00 ± 0.03^{bA}	-2.09 ± 0.01^{aA}	-2.09 ± 0.03^{aA}	6.97 ± 0.08^{aA}	7.30 ± 0.01^{bB}	7.35 ± 0.11^{bC}
TTY	84.00 ± 0.49^{bB}	82.77 ± 0.27^{aB}	83.65 ± 0.11^{bC}	-1.51 ± 0.11^{aB}	-1.58 ± 0.10^{aB}	-1.51 ± 0.02^{aB}	6.29 ± 0.36^{aA}	6.21 ± 0.50^{aA}	6.26 ± 0.13^{aB}
HPY	81.26 ± 0.94^{aA}	81.54 ± 0.19^{aA}	82.04 ± 0.40^{aA}	-1.30 ± 0.10^{aB}	-1.29 ± 0.00^{aC}	-1.26 ± 0.03^{aC}	6.00 ± 0.61^{aA}	5.78 ± 0.22^{aA}	5.88 ± 0.05^{aA}

[a] Values are presented as the mean ± standard deviation; means followed by different lowercase superscript letters in the same row for each parameter (L, a, b) and by different uppercase superscript letters in the same column for each day of storage (1, 14 and 28) differ significantly (P <0.05).

gels, which may explain the gel strength increase and the improvement of the WHC. In addition, in transmission electron microscopy (TEM) micrographs, Penna, Subbarao-Gurram, and Barbosa-Cánovas (2007) observed that yoghurts made with thermal-treated enriched skim milk showed micelles less interconnected with irregular shapes and large pores when compared with yoghurt made by HP. These authors attributed the lower syneresis of the HP-yoghurts to their larger pores when compared with TT-yoghurts.

3.4. Rheological properties of yoghurts

Thixotropy is associated with isothermal gel–sol transitions. In the case of yoghurts, the fragile structure formed by a three-dimensional network is destroyed by shear stress. Since the energy required to break the yoghurt structure is proportional to the hysteresis area, the thixotropy can be estimated by the difference between the areas under the shear stress-shear rate curves (ramp up and ramp down).

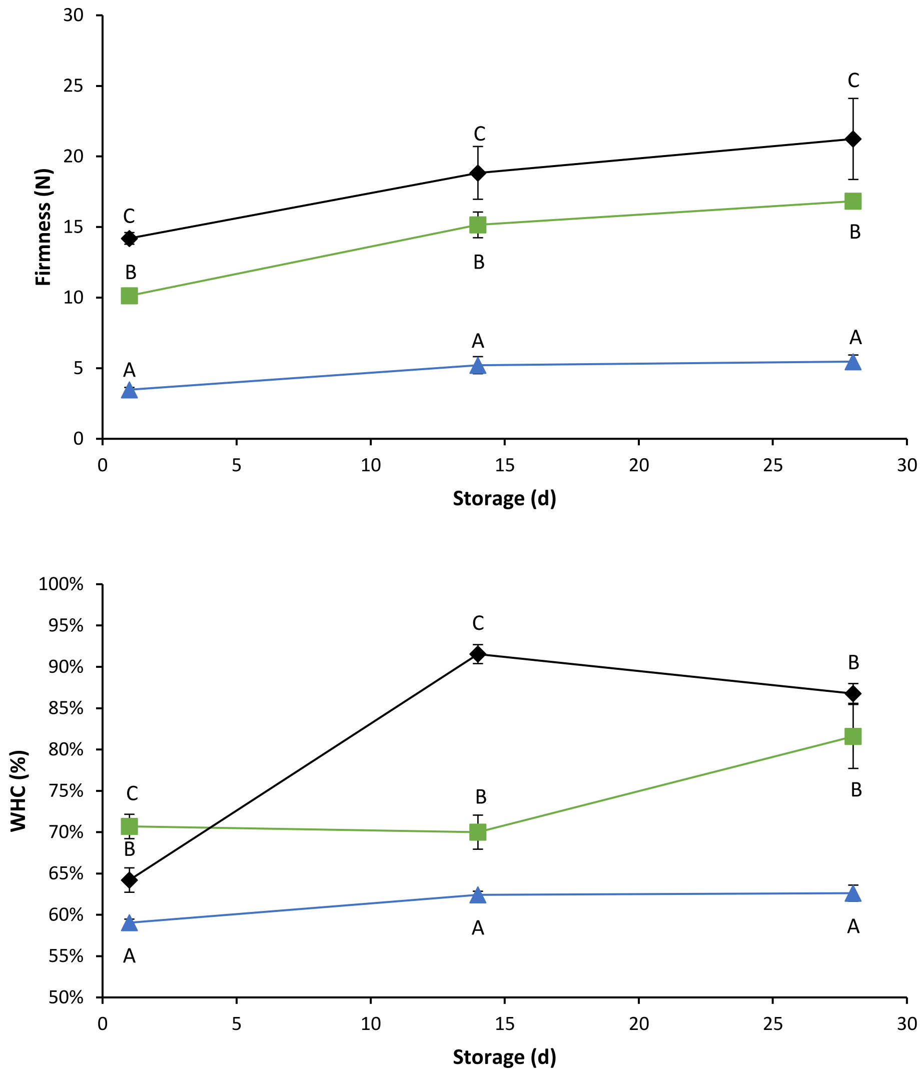

Fig. 3. Evolution of firmness (N) and water holding capacity (WHC; %) for 28 days at 4 °C of control yoghurt (CY; ▲), heat-treated yoghurt (TTY; ■) and high-pressure yoghurt (HPY; ◆). Different lowercase letters (a,b,c) indicate differences (*P* <0.05) between days of storage. Different capital letters (A,B,C) indicate differences (*P* <0.05) between yoghurts on the same day of storage.

In concordance with the rheological values presented in Table 4, the three types of yoghurts analysed can be characterised according to a time-dependent behaviour (partial thixotropy), which is coincident with other studies (Debon, Prudêncio, & Cunha Petrus, 2010). The composition of the milk mixture had a statistically significant influence on thixotropy during all storage time; in addition, the values were different between the three yoghurts at the end of storage indicating the influence of the treatments. Yoghurts enriched with LMCC showed values ~3 times higher for thixotropy than those made with SMP. These findings are in line with the results obtained for WHC and firmness (Fig. 3), and coincident with Marafon, Sumi, Alcântara, Tamime, and Nogueira de Oliveira (2011), who attributed their results to the high-protein composition, allowing a greater number of proteins involved in the protein network. No variation of thixotropy during storage time was observed, except for HPY at d 28 that showed a slight increase.

In Table 4 the data of the other rheological properties are presented. Yoghurts exhibited a shear-thinning behaviour. The Power law was selected to describe the rheological behaviour of (stirred) yoghurts. Viscosity decreased steadily with increasing shear rates, due to the gradual disruption of protein—protein interactions and hence, the gel network. On day 1, yoghurts with similar composition (TTY and HPY) showed differences that can be attributed to the treatment applied: TTY had a higher consistency index and apparent viscosity than HPY and CY.

Thus, pressure and temperature can have different effects on the interactions that maintain protein and colloidal structures, such as hydrophobic and other noncovalent interactions, mineral and ion equilibria, etc. (Considine et al., 2007).

During heat treatment (95 °C for 5 min), close to 100% denaturation of β-lactoglobulin (β-Lg) (Anema, 2000) and approximately 75% denaturation of α-lactalbumin (α-La) occur (Anema, 2001). Consequently, the reactive thiol group of β-Lg is exposed and can form disulphide bonds with other β-Lg molecules or other proteins (α-La, κ-CN or αS2-CN). According to Vasbinder, Alting, and De Kruif (2003), 65% of β-Lg and 50% of α-La are associated with the casein micelle after the heat treatment of skim milk at 90 °C for 10 min. During acidification of TTY, the whey protein-coated casein micelles start to aggregate and form a network (Lucey, 2004).

During HP treatments, serum proteins are denatured and also participate in the formation of new intermolecular associations. A significant denaturation of β-Lg (about 41—59%) was observed,

Table 4
Evolution of rheological parameters of control yoghurt (CY) heat-treated yoghurt (TTY) and high-pressure yoghurt (HPY) stored for 28 days at 4 °C.[a]

Parameter	Storage time (days)		
	D1	D14	D28
Thixotropy (Pa s^{-1})			
CY	2873 ± 147^{aA}	2815 ± 59^{aA}	2779 ± 139^{aA}
TTY	8912 ± 745^{aB}	7886 ± 902^{aB}	8933 ± 183^{aB}
HPY	9418 ± 503^{aB}	9797 ± 372^{aB}	10875 ± 332^{aC}
Apparent viscosity (mPa)			
CY	47.8 ± 10.6^{aA}	65.1 ± 6.0^{abA}	88.1 ± 5.9^{bA}
TTY	230.1 ± 15.5^{aB}	242.5 ± 6.9^{aC}	269.7 ± 4.4^{aB}
HPY	88.8 ± 12.5^{aA}	137.1 ± 15.7^{aB}	100.3 ± 9.3^{aA}
Flow index, n			
CY	0.603 ± 0.141^{aA}	0.511 ± 0.006^{aA}	0.436 ± 0.011^{aA}
TTY	0.358 ± 0.004^{aA}	0.399 ± 0.027^{aA}	0.401 ± 0.006^{aA}
HPY	0.479 ± 0.026^{aA}	0.466 ± 0.050^{aA}	0.510 ± 0.059^{aA}
Consistency index, K (Pa s^n)			
CY	0.358 ± 0.280^{aA}	0.627 ± 0.074^{abA}	1.204 ± 0.151^{bA}
TTY	4.449 ± 0.391^{aB}	3.799 ± 0.532^{aB}	4.099 ± 0.113^{aB}
HPY	0.984 ± 0.036^{aA}	1.599 ± 0.145^{aA}	0.977 ± 0.321^{aA}
r^2			
CY	0.988 ± 0.014	0.999 ± 0.001	0.998 ± 0.001
TTY	0.993 ± 0.004	0.995 ± 0.003	0.992 ± 0.000
HPY	0.992 ± 0.002	0.997 ± 0.000	0.997 ± 0.001

[a] Values are presented as the mean ± standard deviation; means followed by different lowercase superscript letters in the same row for each parameter and by different uppercase superscript letters in the same column for each day of storage (1, 14 and 28) differ significantly (P <0.05).

whereas only a slight denaturation of α-La (6%) was found in pressurised milk (600 MPa for 5 min) (Liu et al., 2020), and the majority of denatured β-Lg of HP-treated milk is associated with casein micelles (Huppertz, Fox, & Kelly, 2004b).

Penna et al. (2007) carried out a study with SMP enriched skim milk and subjected to two treatments before inoculation: heat treatment (85 °C for 30 min) and HP (676 MPa for 5 min). The microstructure of the thermal-treated dairy yoghurt showed fewer interconnected chains of irregularly shaped casein micelles. On the other hand, the microstructure of the HP yoghurt had more interconnected clusters of densely aggregated protein of reduced particle size, appearing more spherical in shape. A lower polydispersity index of unpressurised LMCC (García et al., 2022), differences in protein denaturation and distinct intermolecular associations in yoghurts preceded by heat treatment, as well as a lower sphericity of microgel of stirred set type yoghurt (Penna et al., 2007), could explain the higher consistency index and apparent viscosity of thermal-treated yoghurts compared with those obtained by HP.

4. Conclusions

The protein enrichment with LMCC of yoghurts increased the fermentation time mainly due to the buffering capacity of caseins. The treatments applied to the milk mixtures also affected the final product. Thus, yoghurts made from pressurised dairy mix formulation showed high values for firmness and good water-holding capacity with low viscosity during the shearing process, which was close to that of the yoghurt manufactured from SMP-fortified milk base (CY). This suggests that HP treatment of the dairy mix formulation may be potentially interesting to produce a high-protein stirred yoghurt with a smooth texture without increasing its coarseness, lumpiness and graininess, and avoiding major changes in pumping capacity, stirring, and physical handling during distribution compared with yoghurts with lower protein content.

Although HPP have minimal impact on food quality from a sensory and nutritional point of view, one of the barriers of this technology is the cost of the processing unit. Furthermore, the additional time for compression-decompression increases the overall processing time and makes it difficult to convert HPP into a continuous process.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank Bioder Technology S.L. company for its valuable advice, discussion and technical support.

This work was supported by the Government of Navarra (Servicio de Innovación del Departamento de Desarrollo Económico del Gobierno de Navarra) through the project OPTICASEINAPPLY (expedient number 0011-1365-2017-000173).

The authors would like to thank the Public University of Navarra (Predoctoral grant Res. 1385/2017), Government of Navarra (Mobility grant nº: 0011-35642021-000015) and Erasmus+ for the financial support granted to Arantza Garcia. Open access funding provided by Public University of Navarra.

References

Agrawala, S. P., & Reuter, H. (1979). Effects of different temperatures and holding-times on whey protein denaturation in a UHT pilot-plant. *Milchwissenschaft, 34*, 735–737.

Amelia, I., & Barbano, D. M. (2013). Production of an 18% protein liquid micellar casein concentrate with a long refrigerated shelf life. *Journal of Dairy Science, 96*, 3340–3349.

Anema, S. G. (2000). Effect of milk concentration on the irreversible thermal denaturation and disulfide aggregation of beta-lactoglobulin. *Journal of Agricultural and Food Chemistry, 48*, 4168–4175.

Anema, S. G. (2001). Kinetics of the irreversible thermal denaturation and disulfide aggregation of α-lactalbumin in milk samples of various concentrations. *Journal of Food Science, 66*, 2–9.

Anema, S. G., Lowe, E. K., & Lee, S. K. (2004). Effect of pH at heating on the acid-induced aggregation of casein micelles in reconstituted skim milk. *LWT - Food Science and Technology, 37*, 779–787.

AOAC. (2000). *Official methods of analysis*. Gaithersburg, MD, USA: AOAC International.

Bong, D. D., & Moraru, C. I. (2014). Use of micellar casein concentrate for Greek-style yogurt manufacturing: Effects on processing and product properties. *Journal of Dairy Science, 97*, 1259–1269.

Considine, T., Patel, H. A., Anema, S. G., Singh, H., & Creamer, L. K. (2007). Interactions of milk proteins during heat and high hydrostatic pressure treatments—A review. *Innovative Food Science and Emerging Technologies, 8*, 1–23.

Debon, J., Prudêncio, E. S., & Cunha Petrus, J. C. (2010). Rheological and physico-chemical characterization of prebiotic microfiltered fermented milk. *Journal of Food Engineering, 99*, 128–135.

Galarza, U., Iturmendi, N., García, A., Fernández, T., & Maté, J. I. (2022). Evolution of microbial and protein qualities of fractions of milk protein processed by microfiltration. *LWT - Food Science and Technology, 157*, Article 113064.

García, A., Iturmendi, N., Galarza, U., Maté, J. I., & Fernández-García, T. (2022). Physical, chemical and microbiological changes in liquid micellar casein concentrates induced by high-pressure treatments. *LWT - Food Science and Technology, 172*, Article 114228.

Guichard, E. (2002). Interactions between flavor compounds and food ingredients and their influence on flavor perception. *Food Reviews International, 18*, 49–70.

Harte, F., Amonte, M., Luedecke, L., Swanson, B. G., & Barbosa-Cánovas, G. V. (2002). Yield stress and microstructure of set yogurt made from high hydrostatic pressure-treated full fat milk. *Journal of Food Science, 67*, 2245–2250.

Harte, F., Luedecke, L., Swanson, B., & Barbosa-Cánovas, G. V. (2003). Low-fat set yogurt made from milk subjected to combinations of high hydrostatic pressure and thermal processing. *Journal of Dairy Science, 86*, 1074–1082.

Harwalkar, V. R., & Kalab, M. (1986). Relationship between microstructure and susceptibility to syneresis in yoghurt made from reconstituted non-fat dry milk. *Food Microstructure, 5*, 287–294.

Huppertz, T., Fox, P. F., & Kelly, A. L. (2004a). Influence of high pressure treatment on the acidification of bovine milk by lactic acid bacteria. *Milchwissenschaft, 59*, 246–249.

Huppertz, T., Fox, P. F., & Kelly, A. L. (2004b). High pressure treatment of bovine milk: Effects on casein micelles and whey proteins. *Journal of Dairy Research, 71*, 97–106.

Hurt, E., & Barbano, D. M. (2010). Processing factors that influence casein and serum protein separation by microfiltration. *Journal of Dairy Science, 93*, 4928–4941.
Johnston, D. E., Austin, B. A., & Murphy, R. J. (1993). Properties of acid-set gels prepared from high pressure treated skim milk. *Milchwissenschaft, 48*, 206–209.
Jørgensen, C. E., Abrahamsen, R. K., Rukke, E. O., Hoffmann, T. K., Johansen, A. G., & Skeie, S. B. (2019). Processing of high-protein yoghurt – A review. *International Dairy Journal, 88*, 42–59.
Küster, I., & Vila, N. (2017). Healthy lifestyle and eating perceptions: Correlations with weight and low-fat and low-sugar food consumption in adolescence. *Frontiers in Life Science, 10*, 48–62.
Lee, W. J., & Lucey, J. A. (2003). Rheological properties, whey separation, and microstructure in set-style yogurt: Effects of heating temperature and incubation temperature. *Journal of Texture Studies, 34*, 515–536.
Lee, W. J., & Lucey, J. A. (2010). Formation and physical properties of yogurt. *Asian-Australasian Journal of Animal Sciences, 23*, 1127–1136.
Liu, G., Carøe, C., Qin, Z., Munk, D. M. E., Crafack, M., Petersen, M. A., et al. (2020). Comparative study on quality of whole milk processed by high hydrostatic pressure or thermal pasteurization treatment. *LWT - Food Science and Technology, 127*, Article 109370.
Lopes, R. P., Mota, M. J., Pinto, C. A., Sousa, S., Lopes da Silva, J. A., Gomes, A. M., et al. (2019). Physicochemical and microbial changes in yogurts produced under different pressure and temperature conditions. *LWT - Food Science and Technology, 99*, 423–430.
Lucey, J. A. (2001). The relationship between rheological parameters and whey separation in milk gels. *Food Hydrocolloids, 15*, 603–608.
Lucey, J. A. (2004). Cultured dairy products: An overview of their gelation and texture properties. *International Journal of Dairy Technology, 57*, 77–84.
Marafon, A. P., Sumi, A., Alcântara, M. R., Tamime, A. Y., & Nogueira de Oliveira, M. (2011). Optimization of the rheological properties of probiotic yoghurts supplemented with milk proteins. *LWT - Food Science and Technology, 44*, 511–519.
Meletharayil, G. H., Patel, H. A., Metzger, L. E., & Huppertz, T. (2016). Acid gelation of reconstituted milk protein concentrate suspensions: Influence of lactose addition. *International Dairy Journal, 61*, 107–113.
Mistry, V. V., & Hassan, H. N. (1992). Manufacture of nonfat yogurt from a high milk protein powder. *Journal of Dairy Science, 75*, 947–957.
Nguyen, P. T. M., Kravchuk, O., Bhandari, B., & Prakash, S. (2017). Effect of different hydrocolloids on texture, rheology, tribology and sensory perception of texture and mouthfeel of low-fat pot-set yoghurt. *Food Hydrocolloids, 72*, 90–104.
Penna, A. L. B., Subbarao-Gurram, & Barbosa-Cánovas, G. V. (2007). High hydrostatic pressure processing on microstructure of probiotic low-fat yogurt. *Food Research International, 40*, 510–519.
Qi, X., Liu, D., Yuan, J., Regenstein, J. M., & Zhou, P. (2022). Effects of heating temperatures and pH of skim milk fortified with milk protein concentrate on the texture and microstructure of high-protein yoghurts. *International Dairy Journal, 131*, Article 105395.
Salaün, F., Mietton, B., & Gaucheron, F. (2005). Buffering capacity of dairy products. *International Dairy Journal, 15*, 95–109.
Stratakos, A. C., Inguglia, E. S., Linton, M., Tollerton, J., Murphy, L., Corcionivoschi, N., et al. (2019). Effect of high pressure processing on the safety, shelf life and quality of raw milk. *Innovative Food Science and Emerging Technologies, 52*, 325–333.
Tamime, A. Y., & Deeth, H. C. (1980). Yogurt: Technology and biochemistry (1). *Journal of Food Protection, 43*, 939–977.
Vasbinder, A. J., Alting, A. C., & De Kruif, K. G. (2003). Quantification of heat-induced casein-whey protein interactions in milk and its relation to gelation kinetics. *Colloids and Surfaces B: Biointerfaces, 31*, 115–123.
Wang, D., Fritsch, J., & Moraru, C. I. (2019). Shelf life and quality of skim milk processed by cold microfiltration with a 1.4-μm pore size membrane, with or without heat treatment. *Journal of Dairy Science, 102*, 8798–8806.
Wang, X., Kristo, E., & LaPointe, G. (2020). Adding apple pomace as a functional ingredient in stirred-type yogurt and yogurt drinks. *Food Hydrocolloids, 100*, Article 105453.
Yang, B., Zhang, S., Pang, X., Lu, J., Wu, Z., Yue, Y., et al. (2020). Separation of serum proteins and micellar casein from skim goat milk by pilot-scale 0.05-μm pore-sized ceramic membrane at 50 °C. *Journal of Food Process Engineering, 43*, Article e13334.