Food Bioprocess Technol (2015) 8:548–557
DOI 10.1007/s11947-014-1412-9

ORIGINAL PAPER

Comparative Study on High-Intensity Ultrasound and Pressure Milk Homogenization: Effect on the Kinetics of Yogurt Fermentation Process

P. Sfakianakis · E. Topakas · C. Tzia

Received: 4 December 2013 / Accepted: 16 September 2014 / Published online: 17 October 2014

Abstract Ultrasound (US) application on milk fat homogenization was compared to conventional treatment by pressure in terms of milk fat globule (MFG) size, fermentation process kinetics, and viscosity of set type yogurt. Homogenization of milk by (i) US (frequency 20 kHz, amplitude 150–750 W) and (ii) two-stage pressure (10–30 MPa/5 MPa) was examined.

The more intense the homogenization was, the smaller the MFG became, regardless of the applied method; high-intensity US homogenization reduced the MFG size to 0.78 μm. The fermentation kinetics of ultrasonicated milk samples were significantly different to the samples homogenized by pressure in terms of pH and viscosity. The pH reduction rate and the duration of pH lag phase of US homogenized milk were significantly lower (42 % for μ_{pH} and 52 % for λ_{pH}) compared to those of milk homogenized by pressure. In terms of viscosity evolution, the US homogenization leads to increased rates of increase (by up to 64 %) and shorter lag phases (by up to 56 %), compared to pressure homogenization. Yogurt coagulum obtained at the end of the fermentation (pH=4.6) of milk homogenized by US had significantly higher viscosity values compared to those of milk homogenized with pressure. The difference in the evolution and the end values of yogurt's viscosity was attributed to the denaturation of milk proteins occurring during the US treatment of the milk. US treatment of milk leads to decrease of soluble protein content and composition, which is possibly connected with the formation of insoluble high molecular weight coaggregates that occurred due to whey protein denaturation during US treatment.

Keywords Milk fat globule size · Fermentation time · pH/viscosity evolution model · Yogurt apparent viscosity · Milk protein denaturation · Milk protein aggregates

P. Sfakianakis · C. Tzia (✉)
Laboratory of Food Chemistry and Technology, School of Chemical Engineering, National Technical University of Athens, 5 Iroon Polytechniou St.Polytechnioupoli 15780 Zografou, Greece
e-mail: tzia@chemeng.ntua.gr

E. Topakas
Biotechnology Laboratory, School of Chemical Engineering, National Technical University of Athens, 5 Iroon Polytechniou St.Polytechnioupoli 15780 Zografou, Greece

Introduction

Homogenization of milk is a standard process commonly applied in the dairy industry that is utilized in the processing technology of most dairy products, and in particular of yogurt. The aim of homogenization is to prevent the unsolicited phenomenon of phase separation (creaming) that occurs to the milk (Walstra et al. 2006). Milk is a known natural oil in water (o/w) emulsion with the milk fat globules (MFGs) acting as the dispersed phase. Due to interfacial tension and Brownian motion, the MFG collides and aggregates, thus rise to the surface of the milk volume creating two phases (Fox 2011). Milk homogenization subjects MFG to severe conditions causing shear stress gradient and cavitation phenomena; as a result, these formations are disrupted, and the new smaller globules are maintained in dispersion while a new membrane is formed at the fat serum interface Mather (2011). Such severe conditions can be achieved either by the application of pressure or by high-velocity flow of milk or by high-frequency vibrations (>10 kHz) (Fox 2011; Walstra et al. 2006; Wilbey 2011). The method considered as conventional and thoroughly studied is homogenization by pressure application; pressure values commonly applied in industrial dairy processes are in the range of 10–20 MPa (Walstra et al. 2006; Cano-Ruiz and Richter 1997). Main effects of homogenization include the reduction of the MFG diameter from 10–2 to 1–0.1 μm and the alteration of the MFG membrane, enriching it with protein

molecules, mostly caseins, absorbed from the milk serum (Cano-Ruiz and Richter 1997). The homogenization process further affects the formation and the quality of certain dairy products. In the case of yogurt, homogenization has a significant effect on the rheological, visual, and microstructural properties of the final gel Nguyen et al. (2013). In particular, the homogenized MFG, having more casein molecules on their membrane, shows structure-forming properties and becomes intergraded in the protein network of yogurt gel, thus increasing the latter's strength. (Aguilera and Kessler 1988; Cho et al. 1999; Tamime and Deeth 1980; Lucey et al. 1998). Heat treatment of milk has been proven to have significant effect on the yogurt viscosity and texture Tamime & Robinson (2007). In particular, temperature increase above 80 °C causes irreversible denaturation of whey proteins (β-lactoglobulin, serum albumin) and unfolding their peptide chains, exposing the thiol groups, thus allows the whey proteins to interact with other protein molecules via S–S bonds. Depending on the pH of the environment and the proximity of molecules available, whey proteins can bind with other whey proteins and caseins (κ- and αs1- mostly) and be incorporated at the MFG membrane. The denatured whey proteins, especially at pH values lower than 6.5, tend to be associated with casein micelles (Sfakianakis and Tzia 2014), and these whey/casein complexes contribute to the texture, viscosity, and strength of yogurt coagulum (Horne 1999; Horne and Davidson 1993). Therefore, increase of milk temperature above 80 °C will further facilitate the denaturation and result in yogurt with increased viscosity and texture characteristics (Guyomarc'h et al. 2009; Mandy et al. 2011).

High-intensity ultrasound (US) has been thoroughly studied (power level higher than 10 W) and proved to generate conditions of immense pressure as well as temperature and shear gradient, thus cause cavitation, when propagate through a solution (Ashokkumar et al. 2010; Dolatowski et al. 2007). Ultrasonic treatment of 20 kHz has been referred to decrease size of whey protein aggregates (Koh et al. 2014) and the diameter of MFG to >1 μm (Wu et al. 2001; Sfakianakis and Tzia 2010) due to shear force induced by acoustic cavitation. Therefore, US can be considered as an alternative method for reducing MFG size that can be homogenization Mason (2003). Also, US treatment of milk can additionally reduce or even eliminate microbial content of milk (Cameron et al. 2009; Demirdöven and Baysal 2009). US treatment is referred to cause alteration on the composition and structure of MFG membrane, similar to those of conventional homogenization via pressure (Krešić et al. 2008; Villamiel and de Jong 2000). Furthermore, US may alter the secondary structure of milk proteins and cause aggregation as well as denaturation of the protein molecules (Chandrapala et al. 2011; Gülseren et al. 2007; Madadlou et al. 2009). US combined with heat treatment (thermosonication) (24 kHz, 120–400 W at 63 °C and 24 kHz, 400 W for 10 min at 45 °C) achieves similar effect on the MFG, reduction in size, and changes on the membrane, allowing interaction with casein micelles. Specifically, thermosonication treatment leads to average MFG diameter 0.6 μm and to enrich the MFG membrane in casein molecules (Bermúdez-Aguirre et al. 2008). Additionally, US treatment has been proven to cause denaturation of milk proteins leading in the unfolding of the peptide chains of whey proteins and subsequent formation of whey–whey and whey–casein aggregates (Shanmugam et al. 2012).

Milk gels and yogurt produced from milk treated by high-intensity US exhibited improved properties and particularly high texture characteristics. Increasing the amplitude level of a US treatment (20 kHz, 50–500 W for 1–10 min; Wu et al. 2001 20 kHz, 150–750 W for 10 min; Sfakianakis and Tzia 2010) significantly improved the water-holding capacity and viscosity of yogurt and reduced the syneresis. Moreover, higher US amplitude and higher US exposure time of milk resulted in yogurts with increased viscosity. Increased viscosity developed even in yogurts from skim milk treated with US (22 kHz, 50 W, 0–30 min) due to high degree of whey protein denaturation that occurred because of temperature increase during ultrasonication (Nguyen and Anema 2010). Thermosonication (25 kHz, 400 W and 45 or 75 °C for 10 min) also resulted in yogurts with greater viscosity and higher water-holding capacity compared to the US-untreated samples. The same treatment altered the microstructure of yogurt resulting in a honeycomb-like network and exhibiting a more porous nature with average structural size smaller (~2 μm) than those of conventionally manufactured (from homogenized by pressure milk) yogurt (Riener et al. 2009b). Furthermore, thermosonication treatment (40 °C and 20 kHz for 12 s) combined with moderate pressure (2 MPa) has proved to improve the rheological properties of yogurt and strengthen its structure (Vercet et al. 2002; Riener et al. 2009a).

The aims of the current work are to study US homogenization of full fat milk (3.5 % fat content) compared to the respective of pressure and examine the effect on the subsequent fermentation process kinetics (pH and viscosity evolution during fermentation) of milk into yogurt, in accordance to models described by De Brabandere and De Baerdemaeker (1999) and Soukoulis et al. (2007), as well as on the viscosity of the yogurt.

Materials and Methods

Materials

Skimmed bovine milk (fat content 0.1 % *w/w*, SNF 14 % *w/w*), milk cream (fat content 36–42 % *w/w*, SNF 11 % *w/w*), and an industrial symbiotic culture, *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* were obtained from a dairy industry (FAGE S.A.).

Methods

Milk Homogenization and Yogurt Production

Milk intended for homogenization was prepared using skimmed milk and untreated milk cream and was standardized at 3.5 % fat content. SNF content of the milk samples was standardized at 13 % *w/w*.

Milk samples of 500 mL were ultrasonicated using a VC750 Vibracell© (Sonics & Materials, Inc, Newtown, CT, USA) ultrasonic processor with a standard probe (Titanium alloy Ti-6Al-4 V, length 136 mm, tip diameter 13 mm). The ultrasonic processor's probe was immersed into the milk sample of approximately 2.5 cm. Ultrasonication was performed at a frequency of 20 kHz and output power of 150, 262, 375, 562, and 750 W for time of 10 min (corresponding to 20, 35, 50, 75, and 100 % of the processor's total power). During US homogenization, the temperature was monitored (RTD Thermometer HD2307, with a TP49AC sensor contact probe Delta OHM, Caselle di Selvazzano, Padova, Italy) and remained stable for 150- and 262-W treatments while reached 87 °C for 750-W treatment.

Pressure homogenization was performed at a two-stage pressure homogenizer APV 1000 (Albertslund, Denmark). The pressure applied to the milk samples at the first stage was 10, 15, 20, 25, and 30 MPa while on the second stage was 5 MPa for all samples. Each sample went through the homogenizer twice.

Homogenized milk samples (by US or pressure) and the nonhomogenized/untreated sample were heated at 80 °C for 20 min, then cooled at 46 °C, inoculated with 3.0 % *w/w* starter culture, and divided to aliquots of 200 mL. The mixtures were incubated at 45 °C until their pH reached the value 4.6±0.1 (Penna et al. 2007; Saint-Eve et al. 2008; Serra et al. 2009). Afterward, samples were stored at 4 °C for 24 h. Fermentation time of the entire acidification and yogurt curd formation process was considered as the time from the inoculation with the started culture till the pH reached the final value (4.6±0.1). All experiments were carried out three times.

Measurements

MFG Size and Distribution

After homogenization, MFG diameter and distribution were measured using a Mastersizer Micro (Malvern Instruments Ltd., Worcestershire, UK) equipped with an RF He–Ne laser (λ=300 nm). Before the measurement, a solution containing 8 M urea plus 50 mM EDTA, adjusted to pH 7.0, was added to the samples 10 % (*v/v*) and left for 1 h to disrupt clusters of fat globules and/or casein micelles (Thiebaud et al. 2003).

Monitoring of pH During Fermentation

Throughout the fermentation process, pH of the samples was monitored at 5-min intervals, using a WTW pHmeter 3310 set 3 (WTW, Weilheim in Oberbayern, Germany).

Viscosity Measurements During Fermentation and Yogurt

The apparent viscosity of the samples was monitored during fermentation, and the viscosity of the final yogurts was measured using the method described by Soukoulis et al. (2007). During fermentation, a different sample was taken from the incubator per 30 min, and its viscosity was measured by using a Brookfield viscometer model LV (Brookfield Engineering Laboratories Inc., Stoughton, MA) using a helipath stand at 50 rpm with T-bar spindles A, B, C, and F. For each sample, three dial readings were taken at 30-s intervals, and their mean value was reported. Viscosity measurements were performed in yogurts at the incubation temperature, which was maintained by a circulating water bath (Lauda ecoline RE 312, Lauda-Königshofen, Germany).

Fermentation Kinetics of pH and Viscosity Development

Milk fermentation process into yogurt can be described adequately by the evolution of pH and viscosity versus time; the model that expresses the evolution of pH during fermentation time is the modified Gompertz models of De Brabandere and De Baerdemaeker (1999) (Eq. 1).

$$pH = pH_0 + (pH_0 - pH_\infty) - \left\{-exp\left[\frac{e \cdot \mu_{pH}}{(pH_0 - pH_\infty)} \cdot (\lambda_{pH} - t) + 1\right]\right\} \quad (1)$$

pHo, pH∞=initial and end values of pH, respectively

μ_{pH} (min^{-1})=maximum rate of pH decrease

λ_{pH} (min)=duration of pH lag phase, and the model that describes the evolution of viscosity during fermentation is the modified Gomperz model of Soukoulis et al. (2007) (Eq. 2).

$$\mu_\alpha = \mu_{\alpha 0} + (\mu_{\alpha 0} - \mu_{\alpha\infty}) - \left\{-exp\left[\frac{e \cdot \mu_v}{(\mu_{\alpha 0} - pH_{\alpha\infty})} \cdot (\lambda_v - t) + 1\right]\right\} \quad (2)$$

$\mu_{\alpha o}$, $\mu_{\alpha\infty}$ (Pa*s)=initial and end values of viscosity, respectively

μ_v (min^{-1})=maximum rate of viscosity decrease

λ_v (min)=duration of viscosity lag phase

The parameters μ_{pH}, λ_{pH}, μ_v, and λ_v would be calculated based on the experimental results of pH and viscosity measured during fermentation.

Milk Protein Behavior After Homogenization

Milk protein content was determined by the Bradford assay (Bradford 1976), using bovine serum protein (BSA, Sigma, USA) as standard, and the denaturation degree was estimated. The soluble protein composition of the US-treated milk samples was detected by the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (1970), using a 12.5 % polyacrylamide gel. 2-Mercaptoethanol was used in SDS-PAGE loading buffer under denaturing conditions ensuring the degradation of coaggregates possibly formed by disulfide interactions. For the visualization of milk whey proteins and caseins, the gel was stained with Coomasie Blue R-250. The homogenized, either by pressure or US, milk samples as well as the raw milk, prior to the Bradford assay and the SDS-PAGE electrophoresis, were centrifuged at 10,000 rpm (18,000*g*) for 10 min to monitor the soluble protein fraction.

Statistical Processing of Experimental Data

Data of measurements were statistically analyzed by factorial analysis of variance with categorical predictors the method (US and pressure) and the US amplitude and pressure value while comparisons between the groups of data were performed using Statistica® (Version 10, Statsoft, Tulsa, OK, USA). For the graphical representation, Sigmaplot® (Version 10 SYSTAT, Point Richmond, CA, USA) was used. All measurements were carried out in duplicate, and the mean values are presented.

Results and Discussion

Size and Distribution of MFG

The effectiveness of the two homogenization methods of concern was evaluated by the reduction of the MFG particle size. Both methods proved efficient resulting in reduction of the MFG diameter according to data displayed in Table 1. Both high US intensity (>300 W) and pressure (15/5 MPa) homogenization lead to acceptable MFG size (d<1 μm), while equivalent homogenization treatments can be suggested.

Yogurt Fermentation Process

Figures 1 and 2 illustrate the representative evolution of pH and viscosity with time of the fermentation experiments until the pH reached the value of 4.6±0.1 using milk homogenized by US or pressure; sigmoidal functions of pH and viscosity versus time were verified for both homogenization methods. The modified Gompertz model of De Brabandere and De Baerdemaeker (1999) was verified for the evolution of pH for all samples, treated by US or pressure, with very good regression (R^2>0,999), and the evolution of viscosity verified the sigmoidal modified Gompertz model of Soukoulis et al. (2007) with very

Table 1 Effect of homogenization method on the size diameter (μm) of the MFG

Homogenization method	Dv 0.1	Dv 0.5	Dv 0.9	$d_{3.2}$	$d_{4.3}$	
	None (*Raw milk*)	0.69±0.016	2.21±0.024	5.33±0.172	2.73±0.082	1.53±0.024
Ultrasound (W)	*150*	0.62 ± 0.041^{c}	1.73 ± 0.082^{e}	5.07 ± 1.125^{b}	1.31 ± 0.025^{d}	2.05 ± 0.021^{d}
	262	0.45 ± 0^{a}	1.04 ± 0.008^{d}	2.55 ± 0.033^{a}	$0.95\pm0.021^{b.c}$	$1.41\pm0.008^{b.c}$
	375	0.41 ± 0.008^{a}	$0.68\pm0.016^{a.b}$	1.25 ± 0.042^{a}	$0.89\pm0.041^{a.b}$	$0.75\pm0.041^{a.b}$
	562	0.41 ± 0.005^{a}	0.64 ± 0^{a}	1.15 ± 0.017^{a}	0.86 ± 0.021^{a}	0.62 ± 0^{a}
	750	0.40 ± 0.005^{a}	0.60 ± 0.005^{a}	0.99 ± 0.033^{a}	0.78 ± 0.005^{a}	0.58 ± 0.016^{a}
Pressure (Mpa)	*10*	0.51 ± 0^{b}	1.13 ± 0.024^{d}	2.31 ± 0.072^{a}	1.34 ± 0.082^{c}	1.27 ± 0.024^{c}
	15	0.45 ± 0.015^{a}	0.85 ± 0.08^{c}	1.77 ± 0.025^{a}	$1.16\pm0.015^{a.b}$	$0.90\pm0.095^{a.b.c}$
	20	0.44 ± 0.016^{a}	$0.81\pm0.057^{b.c}$	1.82 ± 0.041^{a}	1.07 ± 0.074^{a}	$0.77\pm0.016^{a.b}$
	25	0.41 ± 0.009^{a}	0.64±0.021	1.18 ± 0.017^{a}	0.95 ± 0.018^{a}	0.62 ± 0.025^{a}
	30	0.40 ± 0^{a}	0.62±0.014	1.04 ± 0.026^{a}	0.78 ± 0.017^{a}	0.61 ± 0.012^{a}

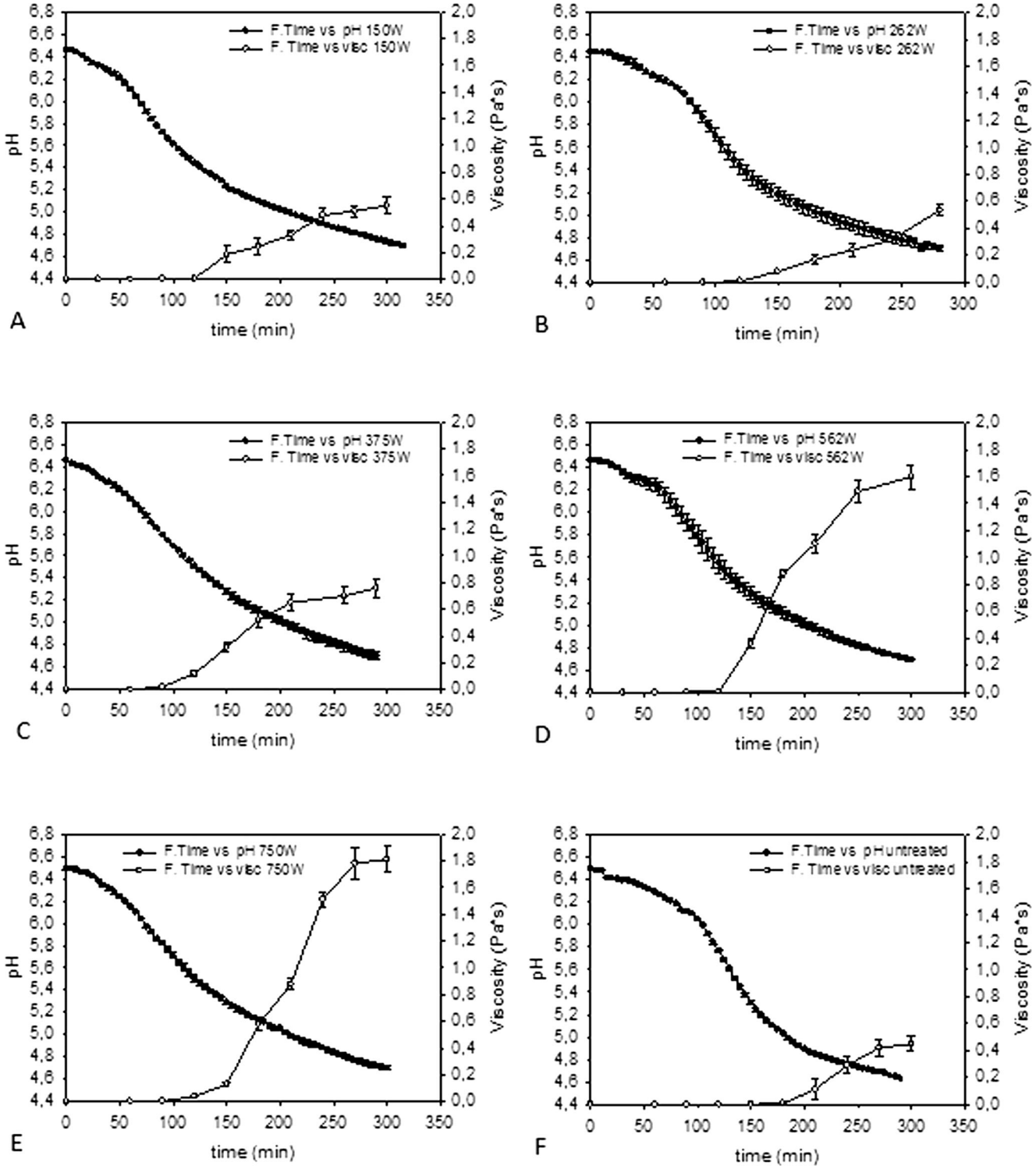

Fig. 1 pH and viscosity evolution versus time during fermentation of milk homogenized by US. **1a** corresponds to 150 W, **1b** to 262 W, **1c** to 375 W, **1d** to 562 W, **1e** to 750 W, and **1f** to untreated/nonhomogenized

good regression (R^2>0,997) as well. However, the curves describing the pH during fermentation of US-homogenized milk were steeper, without the three phases being distinguishable (Fig. 1), unlike the respective curves of milk homogenized by pressure (Fig. 2).

As far as the viscosity evolution during fermentation is concerned, a significant increase was noticed at the final viscosity values for samples homogenized by high-amplitude US (562 and 750 W, Fig. 1d, e) compared to those from conventionally homogenized milk samples.

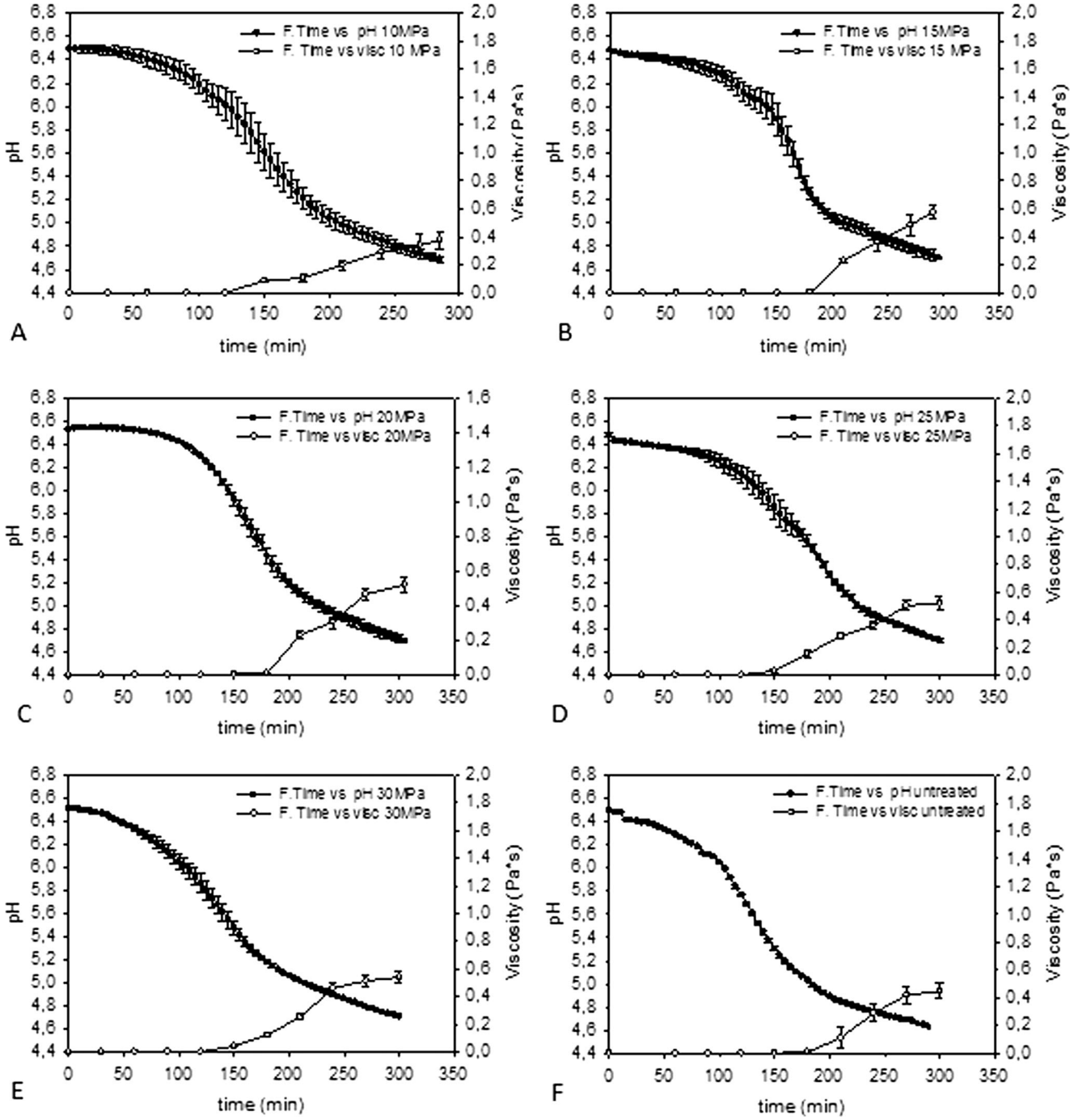

Fig. 2 pH and viscosity evolution versus time during fermentation of milk homogenized by pressure. 2A corresponds to 10 MPa, 2B to 15 MPa, 2C to 20 MPa, 2D to 25 MPa, 2E to 30 MPa and 2 F to untreated/non-homogenized

Fermentation Time

Actual fermentation time was t_{Ferm}=295±15 min and statistically not affected by the homogenization method neither by the intensity of the method. Therefore, fermentation time of yogurt may depend mostly on the milk heating, the fermentation temperature, and the starter culture (De Brabandere and De Baerdemaeker 1999; Sodini et al. 2004), though not by the homogenization method used.

Kinetic Parameters of pH Evolution During Fermentation (μ_{pH} and λ_{pH})

The duration of lag phase of pH (λ_{pH}) was significantly affected by the homogenization method and by the US

Table 2 Fermentation kinetics parameters for milk samples homogenized by pressure and US

	μ_{pH}	λ_{pH} (min)	μ_{visc}	λ_{visc} (min)
Untreated	12.8e-3±6.51e–4[b]	127±9.18[c]	4.29e–3±2.58e–4[b]	205±8.15[d]
150 W	8.27e–3±7.9e–4[a]	36±6.47[a]	4.00e–3±3.25e–4[a]	115±23.18[a,b]
262 W	8.40e–3±5.05–4[a]	83±8.79[b]	5.54e–3±4.17e–4[a]	135±12.25 [a,b,c]
375 W	8.14e–3±2.72e–4[a]	66±5.63[b]	7.36e–3±3.28e–3[b]	106±18.49[a,b]
562 W	8.53e–3±4.61e–4[a]	76±4.23[b]	15.52e–3±2.32e–3[b,c]	126±14.32 [a,b]
750 W	8.72e–3±3.01e–4[a]	24±4.13[a]	17.17e–3±5.68e–4[c]	141±5.88[a]
10/5 MPa	14.2e–3±5.13e–4[b,c]	116. ± 0.92[c]	2.69e–3±8.06e–4[b]	134±23.64[c]
15/5 MPa	13.6e–3±1.49e–3[b]	117±4.92[c]	6.62e–3±4.24e–5[b]	180±9.32[c,d]
20/5 MPa	15.1e–3±4.93e–4[c]	117±3.54[c]	5.76e–3±1.38e–4[b]	177±7.14[c,d]
25/5 MPa	16.2e–3±9.39e–4[c]	129±4.85[c]	4.47e–3±6.42e–4[b]	148±4.86[c]
30/5 MPa	15.7e–3±5.62e–4[c]	124±4.97[c]	5.88e–3±2.08e–3[b]	162±5.32[c]

amplitude ($P<0.05$) as well (Table 2). In general, samples derived from homogenized by US milk had shorter lag phase than those from homogenized by pressure milk. The samples from milk homogenized by US at 150- and 750-W amplitude presented shorter lag phase from those from milk homogenized by US at 262- and 562-W amplitude. The duration of lag phase did not differ in samples from milk homogenized by pressure, while it was generally found longer than the respective durations of the samples from milk homogenized by US. This difference occurred at the λ_{pH} in which according to the homogenization method may be attributed possibly to the sterilization effect of US on milk (Cameron et al. 2009), thus providing a more hospitable environment for the starter culture to inhabit and grow, therefore facilitating the initiation of the acidification process.

The maximum rate of pH decrease (μ_{pH}) was significantly affected by the method of the homogenization ($P<0,05$), not by the intensity of the homogenization method used (Table 2). Specifically, pressure-homogenized samples showed mean μ_{pH} $1.50*10^{-2}\pm1.05*10^{-3}$ min^{-1} and US-homogenized samples μ_{pH} $8.6*10^{-3}\pm5.5*10^{-4}$ min^{-1}, respectively.

Kinetic Parameters of Viscosity Evolution During Fermentation (μ_v and λ_v)

The duration of the lag phase of viscosity (λ_v) was significantly affected only by the homogenization method ($P<0.05$) (Table 2). Moreover, lag phase was found shorter in samples from homogenized by US milk compared to samples from homogenized by pressure milk.

The maximum rate of viscosity increase (μ_v) was significantly affected by the homogenization intensity ($P<0.05$) (Table 2). High μ_v values resulted in samples from milk homogenized by high US amplitude (562 and 750 W), followed by US amplitude US (375 W) or pressure (10, 15, 20, 25, and 30 MPa), while the lowest μ_v values found in samples from milk homogenized by US amplitude (150 and 262 W).

In US treatment, specifically of very high amplitude, the increased μ_v values and decreased λ_v values can be attributed to the denaturation of milk proteins occurring during treatment, either due to temperature increase or due to cavitation phenomena Shaker et al. (2000). During acidification, the denatured whey proteins, being more susceptible to association with casein micelles, aggregate due to the reduction of their repulsive charge, acting as bridging material between casein micelles; as a result, the bonds of the casein matrix are formed more easily, thus facilitating the yogurt coagulation (Horne and Davidson 1993; Morand et al. 2011). Additional denaturation of whey proteins, occuring during ultrasonication due to temperature increase and acoustic cavitation, contributes to the matrix formation and the strength of the coagulum (Shanmugam et al. 2012). Viscosity lag phase of US samples ended at the pH value of 5.1, which corresponds to the pI of β-lactoglobulin, so the coagulation started at the pI of β-lactoglobulin rather than the pH value where the caseins aggregate. The above claims about protein denaturation are strengthened by the results of Bradford assay (Table 3) and SDS-PAGE electrophoresis (Figs. 3a, b). According to

Table 3 Soluble proteins in milk samples homogenized by US and pressure

	Protein content (*mg/100 mL*)	Protein content reduction %
Untreated	36.36±0.90[a]	0
150 W	33.97±0.32[a]	7.08
262 W	32.74±0.58[a,b]	10.42
375 W	21.69±0.63[b]	40.67
562 W	16.35±0.91[c]	55.26
750 W	11,41±0.31[c]	68.78
10/5 MPa	33.97±0.72[a]	0.52
15/5 MPa	32.74±0.27[a]	0.55
20/5 MPa	21.69±0.77[a]	1.31
25/5 MPa	16.35±0.44[a]	2.22
30/5 MPa	11,41±0.96[a]	2.83

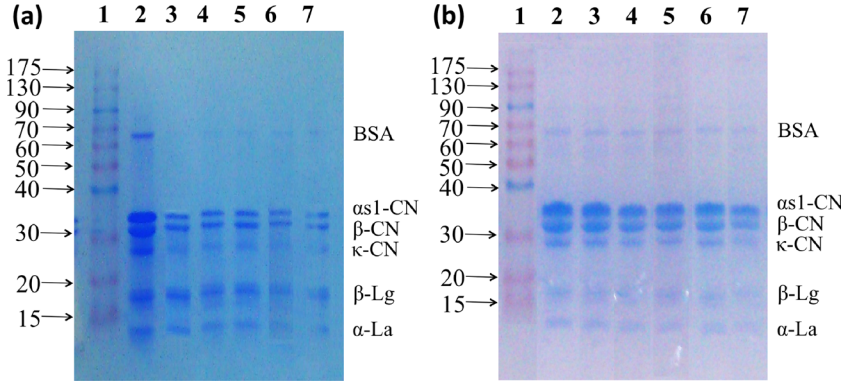

Fig. 3 **a** SDS-PAGE analysis of milk proteins on 12.5 % gel. *Lanes*: *1* standard protein markers (PINK, Nippon Genetics); *2* untreated milk; *3, 4, 5, 6, and 7* milk homogenized by US at 150, 262, 375, 562, and 750 W, respectively. **b** SDS-PAGE analysis of milk proteins on 12.5 % gel. *Lanes*: *1* standard protein markers (PINK, Nippon Genetics); *2* untreated milk; *3, 4, 5, 6, and 7* milk homogenized by pressure at 10/5, 15/5, 20/5, 25/5, and30/5 MPa, respectively

Table 3, the soluble protein content of milk is reduced after ultrasonication, while after pressure homogenization, no significant change was observed. In particular, the higher the US amplitude, the lower the soluble protein content. SDS-PAGE showed the major milk protein bands, such as BSA, α_{s1}- β- and κ- caseins, as well as β-lactoglobulin (β-Lg) and α-lactalbumin (α-La) (Fig. 3a, b) Jovanovic et al. (2007). All major protein bands were more strongly visible and thicker in untreated and pressure (Fig. 3b) treated milk compared with those from the US-treated milk samples (Fig. 3a); the band corresponding to BSA is barely visible on the US-treated samples. The above observations may confirm the formation of high molecular weight coaggregates due to US treatment. The temperature increase combined with the high shear stress during milk ultrasonication causes denaturation of several whey proteins (β-lactoglobulin, serum albumin), unfolding their peptide chain and leading to the subsequent formation of whey–whey and whey–casein aggregates via disulfide bonds (Shanmugam et al. 2012). After separating these insoluble high molecular weight aggregates from milk volume by centrifugation, the US-treated samples displayed lower density bands in the electrophoresis gel and lower absorbance in the Bradford assay measurements, compared with the untreated and pressure-treated samples.

Conclusions

High-intensity US homogenization proved equally efficient in reduction of the MFG diameter with pressure homogenization in addition to cause denaturation of whey proteins and the formation of protein molecule aggregates. Fermentation kinetics of ultrasonicated milk into yogurt verified the modified Gompertz model (pH vs time and viscosity vs time) and displayed significantly different parameters in comparison with milk homogenized by pressure. The μ_{pH} (pH decrease rate) and the λ_{pH} (lag phase duration) of ultrasonicated milk samples were lower than the respective of pressure-homogenized milk samples. The viscosity evolution parameters were also affected by the method and the intensity of homogenization. Ultrasonicated samples displayed short λ_v and high μ_v compared to pressure-homogenized samples. Especially, the samples homogenized with very high intensity US (562 and 750 W) had significantly lower λ_v and higher μ_v. Noticeable is the higher viscosity values at the end of the fermentation process (pH=4.6) of the samples derived from milk homogenized by high-amplitude US, compared to samples homogenized by pressure. The impact of US treatment on viscosity and viscosity evolution is attributed to the whey protein denaturation and the subsequent aggregate formation between whey and casein molecules caused by high shear stress and temperature increase during ultrasonication.

References

Aguilera, J. M., & Kessler, H. G. (1988). Physicochemical and rheological properties of milk-fat globules with modified membranes. *Milchwissenschaft, 43*, 411–415.

Ashokkumar, M., Bhaskaracharya, R., Kentish, S., Lee, J., Palmer, M., & Zisu, B. (2010). The ultrasonic processing of dairy products, an overview. *Dairy Science & Technology, 90*(2–3), 147–168.

Bermúdez-Aguirre, D., Corradini, M. G., Mawson, R., & Barbosa-Canova, G. V. (2008). *Innovative Food Science & Emerging Technologies, 10*(2), 172–178.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*, 248–254.

Cameron, M., McMaster, L. D., & Britz, T. J. (2009). Impact of ultrasound on dairy spoilage microbes and milk components. *Dairy Science & Technology, 89*(1), 83–98.

Cano-Ruiz, M. E., & Richter, R. L. (1997). Effect of homogenization pressure on the milk Fat globule membrane proteins. *Journal of Dairy Science, 11*, 2732–2739.

Chandrapala, J., Zisu, B., Palmer, M., Kentish, S., & Ashokkumar, M. (2011). Effects of ultrasound on the thermal and structural characteristics of proteins in reconstituted whey protein concentrate. *Ultrasonics Sonochemistry, 18*, 951–957.

Cho, Y. H., Lucey, J. A., & Singh, H. (1999). Rheological properties of acid milk gels as affected by the nature of the fat globule surface material and heat treatment of milk. *International Dairy Journal, 9*, 537–545.

De Brabandere, A. G., & De Baerdemaeker, J. G. (1999). Effects of process conditions on the pH development during yogurt fermentation. *Journal of Food Engineering, 41*, 221–227.

Demirdöven, A., & Baysal, T. (2009). The use of ultrasound and combined technologies in food preservation. *Food Reviews International, 25*, 1–11.

Dolatowski, Z. J., Stadnik, J., & Stasiak, D. (2007). Applications of ultrasound in food technology. ACTA Scientiarum Polonorum. *Technologia Alimentaria, 6*, 89–99.

Fox, P. P. (2011). Fat globules in milk. In J. W. Fuquay (Ed.), *Encyclopedia of Dairy Sciences* (Second Editionth ed., pp. 1564–1548). London: Elsevier Ltd, Academic Press.

Gülseren, I., Güzey, D., Bruce, B. D., & Weiss, J. (2007). Structural and functional changes in ultrasonicated bovine serum albumin solutions. *Ultrasonics Sonochemistry, 14*, 173–183.

Guyomarc'h, F., Jemin, M., Tilly, V. L., Madec, M. N., & Famelart, M. H. (2009). Role of the heat-induced whey protein/κ-casein complexes in the formation of acid milk gels: a kinetic study using rheology and confocal microscopy. *Journal of Agricultural and Food Chemistry, 57*, 5910–5917.

Horne, D. S. (1999). Formation and structure of acidified milk gels. *International Dairy Journal, 9*(3–6), 261–268.

Horne, D. S. & Davidson, C. M. (1993). Influence of heat treatment on gel formation in acidified milks. In Protein and fat globule modifications by heat treatment, homogenization and other technological means for high quality dairy products. *IDF Special Issue* 9303, Brussels: International Dairy Federation. 267–276

Jovanovic, S., Barac, M., Macej, O., Vucic, T., & Lacnjevac, M. (2007). SDS-PAGE analysis of soluble proteins in reconstituted milk exposed to different heat treatments. *Sensors, 7*, 371–389.

Krešić, G., Lelas, L., Jambrak, A. R., Herceg, Z., & Brnčić, S. R. (2008). Influence of novel food processing technologies on the rheological and thermophysical properties of whey proteins. *Journal of Food Engineering, 1*, 64–73.

Koh, L. L. A., Chandrapala, J., Zisu, B., Martin, G. J. O., Kentish, S. E., & Ashokkumar, M. (2014). A comparison of the effectiveness of sonication, high shear mixing and homogenization on improving the heat stability of whey protein solutions. *Food and Bioprocess Technology, 7*(2), 556–566.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, 227*, 680–685.

Lucey, J. A., Munro, P. A., & Singh, H. (1998). Rheological properties and microstructure of acid milk gels as affected by fat content and heat treatment. *Journal of Food Science, 63*, 660–664.

Madadlou, A., Mousavi, M. E., & Emam-Djomeh, Z. (2009). Comparison of pH-dependent sonodisruption of re-assembled casein micelles by 35 and 130 kHz ultrasounds. *Journal of Food Engineering, 95*, 505–509.

Mandy, J., Stefan, N., Doris, J., & Harald, R. (2011). Physical properties of acid milk gels: acidification rate significantly interacts with cross-linking and heat treatment of milk. *Food Hydrocolloids, 25*, 928–934.

Mason, T. J. (2003). Sonochemistry and sonoprocessing: the link, the trends and (probably) the future. *Ultrasonics Sonochemistry, 10*, 175–179.

Mather, I. H. (2011). Milk lipids, milk fat globule membrane. In J. W. Fuquay (Ed.), *Encyclopedia of Dairy Sciences* (Second Editionth ed., pp. 680–690). London: Elsevier Ltd., Academic Press.

Morand, M., Guyomarc'h, F., & Famelart, M. H. (2011). How to tailor heat-induced whey protein/κ-casein complexes as a means to investigate the acid gelation of milk—a review. *Dairy Science & Technology, 91*, 97–126.

Nguyen, H. A., & Anema, S. G. (2010). Effect of ultrasonication on the properties of skim milk used in the formation of acid gels. *Innovative Food Science and Emerging Technologies, 11*, 616–622.

Nguyen, H. T. H., Ong, L., Lefèvre, C., Kentish, S. E., & Gras, S. L. (2013). The Microstructure and physicochemical properties of probiotic buffalo yogurt during fermentation and storage: a comparison with bovine yogurt. *Food and Bioprocess Technology, 4*(7), 937–953.

Penna, A. L. B., Gurram, S. R., & Barbosa-Cánovas, G. V. (2007). High hydrostatic pressure processing on microstructure of probiotic low-fat yogurt. *Food Research International, 40*, 510–519.

Riener, J., Noci, F., Cronin, D. A., Morgan, D., & Lyng, J. G. (2009a). Characterisation of volatile compounds generated in milk by high intensity ultrasound. *International Dairy Journal, 19*, 269–272.

Riener, J., Noci, F., & Cronin, D. A. (2009b). The effect of thermosonication of milk on selected physicochemical and microstructural properties of yoghurt gels during fermentation. *Food Chemistry, 3*(1), 905–911.

Saint-Eve, A., Lévy, C., Le Moigne, M., Ducruet, V., & Souchon, I. (2008). Quality changes in yogurt during storage in different packaging materials. *Food Chemistry, 110*, 285–293.

Shaker, R. R., Jumah, R. Y., & Abu-Jdayil, B. (2000). Rheological properties of plain yogurt during coagulation process: impact of fat content and preheat treatment of milk. *Journal of Food Engineering, 44*, 175–180.

Shanmugam, A., Chandrapala, J., & Ashokkumar, M. (2012). The effect of ultrasound on the physical and functional properties of skim milk. *Innovative Food Science and Emerging Technologies, 16*, 251–258.

Serra, M., Trujillo, A. J., Guamis, B., & Ferragut, V. (2009). Evaluation of physical properties during storage of set and stirred yogurts made from ultra-high pressure homogenization-treated milk. *Food Hydrocolloids, 23*, 82–91.

Sodini, I., Remeuf, F., Haddad, S., & Corrieu, G. (2004). The relative effect of milk base, starter, and process on yogurt texture: a review. *Critical Reviews in Food Science and Nutrition, 44*, 113–137.

Soukoulis, C., Panagiotidis, P., Koureli, R., & Tzia, C. (2007). Industrial yogurt manufacture: monitoring of fermentation process and improvement of final product quality. *Journal of Dairy Science, 90*(6), 2641–2654.

Sfakianakis P. & Tzia C. (2010). Yogurt from ultrasound treated milk: monitoring of fermentation process and evaluation of product quality characteristics. *Food Process Engineering in a Changing World, ICEF11 proceedings*, (3), 1649–1654

Sfakianakis, P., & Tzia, C. (2014). Conventional and innovative processing of milk for yogurt manufacture; development of texture and flavor: a review. *Foods, 3*(1), 176–193.

Tamime, A. Y., & Deeth, H. C. (1980). Yogurt–technology and biochemistry. *Journal of Food Protection, 43*, 939–977.

Tamime, A. Y., & Robinson, R. K. (2007). *Tamime and Robinson's Yoghurt: Science and Technology* (3dth ed.). Cambridge, UK: Woodhead Publishing Ltd.

Thiebaud, M., Dumay, E., Picart, L., Guiraud, J. P., & Cheftel, J. C. (2003). High-pressure homogenization of raw bovine milk. Effects on fat globule size distribution and microbial inactivation. *International Dairy Journal, 13*, 427–439.

Vercet, A., Oria, R., Marquina, P., Crelier, S., & López-Buesa, P. (2002). Rheological properties of yoghurt made with milk submitted to Manothermosonication. *Journal of Agricultural and Food Chemistry, 50*, 6165–6171.

Villamiel, M., & de Jong, P. (2000). Influence of high-intensity ultrasound and heat treatment in continuous flow on fat, proteins, and native enzymes of milk. *Journal of Agricultural and Food Chemistry, 48*, 472–478.

Walstra, P., Wouters, J. T. M., & Geurts, T. J. (2006). *Dairy Science and Technology*. LLC, FL, USA: Taylor & Francis Group.

Wilbey, R. A. (2011). Homogenization of milk. In J. W. Fuquay (Ed.), *Encyclopedia of Dairy Sciences* (Second Editionth ed., pp. 750–754). London: Elsevier Ltd, Academic Press.

Wu, H., Hulbert, G. J., & Mount, J. R. (2001). Effects of ultrasound on milk homogenization and fermentation with yogurt starter. *Innovative Food Science & Emerging Technologies, 3*, 211–218.