Uluslararası Tarım Araştırmalarında Yenilikçi Yaklaşımlar Dergisi
International Journal of Innovative Approaches in Agricultural Research 2023, Vol. 7 (4), 455-468
https://doi.org/10.29329/ijiaar.2023.630.7


Original article

# Effect of High-Pressure Homogenization and Fat Content on Yogurt Fermentation Process

Latife Betül Gül [a] & Abdullah Akgün [b,*]

[a] Department of Food Engineering, Faculty of Engineering, Giresun University, Giresun, Türkiye
[b] Department of Food Engineering, Faculty of Engineering, Trakya University, Edirne, Türkiye

**Abstract**

The application of the traditional homogenization process used in yogurt production under higher pressure, such as 50-200 MPa, is a new approach to improving yogurt structure and quality effectively. High-pressure homogenization (HPH) is considered a technology that changes the microstructure, water holding capacity, viscosity, and sensorial properties of yogurts by affecting fat globules and protein structures depending on fat content. In this study, the effects on bacterial growth, acidification kinetics and viscosity development were investigated in the production of yogurt from fatty and semi-skimmed milk with HPH. HPH treatment and fat content had a positive effect on the bacterial growth rate, and the maximum counts of *L. bulgaricus* and *S. thermophilus* were determined in the yogurt sample made from fatty milk treated with 100 MPa pressure as 8.65 and 9.16 log cfu/g, respectively. Also, the pH and viscosity change during incubation was affected and the $V_{max}$ and $\mu_{max}$ values for fatty milk treated with 100 MPa pressure reached maximum values of 1.67x10$^{-2}$ pH unit/min and 2.35x10$^{-2}$ Pa.s units/min, respectively. With the HPH treatment, the fermentation time in fatty yogurt was shortened by 60 min compared to the control sample.

**Keywords:** High-Pressure, Homogenization, Fat content, Acidification, Yogurt.

**Received:** 02 October 2023 * **Accepted:** 07 December 2023 * **DOI:** https://doi.org/10.29329/ijiaar.2023.630.7

* **Corresponding author:**

Abdullah Akgün is a instructor doctor in the Department of Food Engineering at Trakya University in Edirne, Türkiye. His research interests include the Dairy Products and Food Chemistry. He has lived, worked, and studied in Edirne, Türkiye.
Email: abdullahakgun@trakya.edu.tr

## INTRODUCTION

Fermented dairy products have been an important part of the human diet in many parts of the world since ancient times. One of these products, yogurt, is a nutritious, healthy and popular fermented milk product obtained by lactic acid coagulation of milk inoculated with *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (Rudra et al., 2017). Although milk from different mammals is used in yogurt production, cow's milk is the most preferred. Moreover, different types of yogurt are produced and classified in terms of fat content and recently, fat-free and low-fat yogurt varieties have become increasingly popular in the market due to consumer demands, especially due to the increased risk of cardiovascular diseases, obesity, cancer and diabetes (Rudra et al., 2017). However, fat reduction causes several quality problems in yogurt, including poor texture, low viscosity, serum separation and undesirable mouthfeel (Sodini et al., 2004; Torres et al., 2018). Therefore, the effects of different protein and fat ratios as well as processing, culture selection, incubation, and storage conditions on the elimination of defects in low-fat yogurts have been widely investigated (Nguyen et al., 2017; Sodini et al., 2004).

Homogenization is a standard process widely applied in the dairy industry and used in the production of most dairy products, especially yogurt, and is performed at 55-65 $^{o}$C at a pressure of 10-20 MPa (Ciron et al., 2010). Its main purpose is to prevent undesirable phase separation in milk and milk products, and to improve yogurt quality (Sfakianakis et al., 2014). After the milk homogenization, reduction of fat globule size and interaction mechanisms of homogenized fat with the protein matrix plays a role in the improvement of yogurt quality (Sodini et al., 2004). Reducing the size of the fat globule provides better binding of the fat to the protein network, while the increase in surface area during homogenization increases the interaction capacity of the fat with casein and denatured whey proteins during acidification and subsequent gel formation (Cho et al., 1999; Lucey & Singh, 1997; Massoud et al., 2016; Sodini et al., 2004). High-pressure homogenization (HPH) technology, which has recently attracted attention in food applications, is considered a promising new approach to improving the structure and stability of yogurt (Levy et al., 2022; Massoud et al., 2016; Serra et al., 2009b). This technology is based on the same principle as the traditional homogenization process but operates at significantly higher pressures (50-200 MPa) (Serra et al., 2009a; Serra et al., 2009b; Sevenich & Mathys, 2018). In the HPH process, the liquid material is forced through a narrow opening of the valve and is subjected to ultra-fast compression. The fluid is then subjected to a wide range of forces such as turbulence, shear, cavitation and temperature increases (Hayes & Kelly, 2003; Sert et al., 2023). In addition to reducing the fat globule size, the HPH process acts on food components, especially proteins, causing changes in their functional properties and activities (Lanciotti et al., 2007), leads to an increase casein-fat or casein-casein interactions and the water-holding capacity of denatured serum proteins, and consequently improve the viscosity index and gel strength of yogurt (Lanciotti et al., 2004; Patrignani et al., 2007). Moreover, due to the denaturation of serum proteins during the HPH process, insoluble high molecular weight co-aggregates are formed, resulting in a decrease in soluble protein content and a corresponding improvement in viscosity (Trujillo et al., 2002). Ciron et al. (2012) reported that the microstructure of low-fat yogurt changes with the application of HPH to milk, and gel particle size, gel strength and viscosity increase and sensory properties of low-fat yogurt are similar to that of full-fat yogurt without stabilizer. Moreover, it has been reported that the synthesis of some branched-chain amino acids and some beneficial compounds required for the proliferation of cultures used in yogurt production occurs with the HPH process, which shortens the incubation period (Sert et al., 2023) and a higher number of viabilities are reached after incubation (Lanciotti et al., 2007; Serra et al., 2007).

Studies in the literature show that the improvement in the structure of fermented products with high pressure applied to milk is mainly related to the fat content of the milk. The application of HPH to milk containing 3.5% fat increased the consistency of yogurt (Serra et al., 2007), while the opposite behavior was observed in milk containing 0.1% fat (Serra et al., 2008). Therefore, considering the product quality and cost of milk fat, it is essential to evaluate the effect of HPH depending on the fat content. This study aimed to determine the impact of fat content and HPH on the kinetics of the milk fermentation process, such as pH, cell viability, and viscosity change.

## MATERIALS and METHODS

### Materials

The milk used for yogurt production was obtained from the local producer of Kastamonu province as low fat (1.72%) and fat (3.1%), brought to the laboratory under a cold chain (at 4 °C) and processed immediately. The fatty milk used in yogurt production had a pH value of 6.48 with 3.54% protein and 3.1% fat, while the low-fat milk had a pH value of 6.46 with 3.49% protein and 1.72% fat. In yogurt production, YC-X11 (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) starter culture (Peyma Chr. Hansen, İstanbul, Türkiye) was used.

### Methods

#### *Yogurt production*

The non-fat dry matter content of the milk was standardized to 13% using skimmed milk powder (Pınarsüt A.Ş., İzmir, Türkiye), and homogenization process was applied by passing through a high-pressure homogenizer (Panda PLUS, 2000; GEA Niro Soavi, Parma, Italy) at 50 and 100 MPa pressure for one time. The milk considered as the control group was subjected to 25 MPa pressure. Homogenized milk was heat treated in a water bath (WBN 22, Memmert, Schwabach, Germany) at 90 ºC for 5 min and then cooled to fermentation temperature (~ 45 ºC). Culture (2%) was added to the milk and incubated at 43 °C until pH 4.5. At the end of incubation, yogurt samples were capped and allowed to cool under refrigerator conditions (+4 °C).

***Chemical analysis of milk***

The dry matter, fat, and protein contents of milk samples were determined based on standard methods (AOAC, 2000). Dry matter content was determined as using oven at 105 °C until constant weight. Fat content was determined by Gerber methods. Protein content was measured using Kjeldahl's method.

***pH and acidification kinetics***

The pH values of the samples were measured at room temperature using a pH meter (Seven2Go, Mettler Toledo, Greifensee, Switzerland) calibrated with standardized buffer solutions (pH 4.0, 7.0, and 10.0). For acidification kinetics, the change in pH during milk fermentation was continuously monitored with the pH meter until the pH reached 4.5. The maximum acidification rate ($V_{max}$) was calculated as the time-dependent change of pH value ($dpH/dt$) and given as an absolute value. The time to reach the maximum acidification rate ($T_{max}$) and the time to complete fermentation ($t_f$) were determined as responses characterizing the fermentation kinetics (Shiby et al., 2008).

***Viscosity***

The change in the samples' viscosity during fermentation was determined with a digital viscometer (Fungilab Expert L viscometer, USA) using a spindle coded R6. The measurement was performed at 12.00 rpm, and the measurement parameters were recorded between 10-90% torque values.

***Microbiological analysis***

*S. thermophilus* and *L. bulgaricus* were counted to monitor microbial growth during fermentation. For this purpose, serial dilutions were prepared using sterile physiological saline, and the appropriate dilutions were inoculated onto petri dishes. Streptococcal colony counts were performed on M17 agar (Merck) medium after incubation at 37 °C for 48 h. Lactobacilli colonies were counted after incubation at 45 °C for 72 h in anaerobic conditions on MRS (Merck) medium adjusted to pH 5.2 with acetic acid (Gardini et al., 1999). After incubation, counting was performed in petri dishes containing 30-300 colonies and the results were given as log cfu/g.

***Statistical analysis***

The study was conducted in two replicates and two parallels, and the data obtained were given as mean and standard deviation (SD). Statistical evaluation of the analyzed data was performed with SPSS Statistics version 21.0 (SPSS Inc., Chicago, Illinois, USA). The data were subjected to one-way analysis of variance to determine significant differences between the samples and Duncan's multiple comparison test was applied to significant differences ($p<0.05$).

## RESULTS and DISCUSSION

### Acidification kinetics

After HPH of milk with different fat ratios, pH change during milk fermentation was monitored and the data obtained are given in Figure 1. In addition, $V_{max}$, $T_{max}$ and $T_f$ values used to describe the acidification kinetics are shown in Table 1. The initial pH values of the milks after homogenization were between 6.43 and 6.45 and there was no difference between the samples ($p>0.05$). After the incubation period, the pH values ranged between 4.44 and 4.61 ($p<0.05$), and the lowest pH value was determined in the yogurt obtained from fatty milk homogenized with 100 MPa pressure, while the highest value was measured in the yogurt obtained from semi skimmed milk homogenized with 50 MPa pressure.

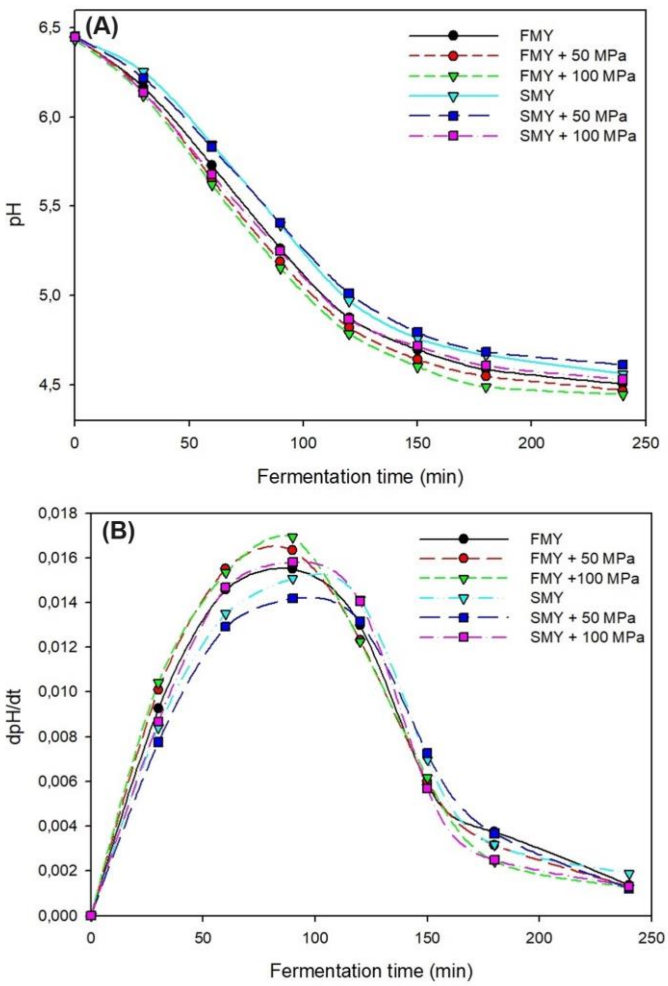


**Figure 1.** pH change and fermentation kinetics during the incubation period in yogurt production from fatty and semi skimmed milk treated with HPH (SMY: Yogurt from semi skimmed milk; SMY + 50 MPa: Yogurt from semi skimmed milk treated with 50 MPa pressure; SMY + 100 MPa: Yogurt from semi skimmed milk treated with 100 MPa pressure; FMY: Yogurt from fatty milk; FMY + 50 MPa: Yogurt from fatty milk treated with 50 MPa pressure; FMY + 100 MPa: Yogurt from fatty milk treated with 100 MPa pressure)

As seen in Figure 1(A), no significant difference was observed between the samples at the beginning of the fermentation period (first 30 min) ($p>0.05$). However, afterward, differences in pH change were detected, and the decrease in pH was faster in the fatty milk sample treated at 100 MPa pressure. On the other hand, a slower pH change was observed in the semi-skimmed milk sample treated at 50 MPa pressure. Similarly, Lanciotti et al. (2004) reported that homogenization pressure and milk fat had a significant effect on the change in pH during incubation and that the decrease in pH improved up to 4% fat content and 45 MPa homogenization pressure. In the same study, it was stated that the maximum reduction in pH was obtained at the level of fat content and pressure that maximized starter culture development. Serra et al. (2007) reported that in control milk, the change in *dpH/dt* was slow and continuous during the first 100 min, followed by a higher rate of decrease until $V_{max}$ was reached, but on the contrary, two different phases occurred in the *dpH/dt* change during fermentation in HPH-treated milk.

**Table 1.** Acidification kinetic parameters during fermentation of fatty and semi skimmed milk treated with HPH

| **Parameters** | **SMY** | **SMY + 50 MPa** | **SMY + 100 MPa** | **FMY** | **FMY + 50 MPa** | **FMY + 100 MPa** |
|---|---|---|---|---|---|---|
| $V_{max}$ (pH units/min) | $1.44 \times 10^{-2} \pm 0.02 \times 10^{-3}$ | $1.41 \times 10^{-2} \pm 0.03 \times 10^{-3}$ | $1.51 \times 10^{-2} \pm 0.12 \times 10^{-3}$ | $1.53 \times 10^{-2} \pm 0.08 \times 10^{-3}$ | $1.58 \times 10^{-2} \pm 0.04 \times 10^{-3}$ | $1.67 \times 10^{-2} \pm 0.25 \times 10^{-3}$ |
| $T_{max}$ (min) | 90 | 90 | 90 | 90 | 90 | 90 |
| $T_f$ (min) | 240 | 240 | 240 | 240 | 180 | 180 |

$V_{max}$: Maximum acidification rate; $T_{max}$: Time to reach maximum acidification rate; $T_f$: Completion time of fermentation. SMY: Yogurt from semi skimmed milk; SMY + 50 MPa: Yogurt from semi skimmed milk treated with 50 MPa pressure; SMY + 100 MPa: Yogurt from semi skimmed milk treated with 100 MPa pressure; FMY: Yogurt from fatty milk; FMY + 50 MPa: Yogurt from fatty milk treated with 50 MPa pressure; FMY + 100 MPa: Yogurt from fatty milk treated with 100 MPa pressure.

The difference in pH between the samples was also observed in acidification kinetic parameters ($p<0.05$). $V_{max}$ values of the samples were determined between $1.41 \times 10^{-2}$ and $1.67 \times 10^{-2}$ pH units/min and the highest $V_{max}$ value was obtained in the incubation of fatty milk with 100 MPa pressure ($p<0.05$). Similarly, 100 MPa pressure application to semi skimmed milk caused a significant increase in $V_{max}$ value. On the other hand, a partial decrease in $V_{max}$ values was observed in semi skimmed milk pressurized at 50 MPa compared to the control sample. This is due to the longer initial adaptation period of the starter cultures in the 50 MPa pressurized semi skimmed milk sample and thus lower acidification rate as observed in the control sample. After the adaptation period, the acidification rate tended to increase rapidly between pH 6.25 and 5.15 and reached $1.67 \times 10^{-2}$ pH units/min in 100 MPa pressurized fatty milk. After about pH 5.1, the acidification rate decreased in all samples, probably due to the low metabolic activity of *S. thermophilus* (Galia et al., 2009).

As seen in Figure 1(B), the $T_{max}$ value was similar for all samples and was approximately 90 min. However, when the time to reach pH 4.5 ($T_f$) was taken into consideration, it was observed that the pH reached approximately pH 4.5 at 180 min for the fatty milk treated with HPH, while the pH value was

>4.6 in the other samples. Similarly, Sert et al. (2023) reported that the $T_f$ value of yogurt samples produced from sheep's milk treated with HPH was shorter compared to the control sample, thus HPH treatment significantly shortened the fermentation time. Patrignani et al. (2016) reported that high-pressure treatment significantly reduced the time required to reach pH 4.6 in fermented products, probably because the balance between soluble and insoluble forms of calcium, phosphorus, and nitrogen changes with the pressure applied to the milk. Nguyen et al. (2015) reported that the fermentation time of homogenized buffalo milk was shorter compared to the control sample and this was due to the breakdown of milk fat globules with homogenization and the release of nutrients that positively affect the activity and metabolism of lactic acid bacteria. In our current study, it was observed that the high fat content had a positive effect on acidification kinetics and was found to be compatible with the results of Nguyen et al. (2015). In another study, it was found that the time to reach pH 4.6 was similar in milk homogenized at pressures higher than 200 MPa, but it was lower than the $T_f$ value of control milk or samples with 100 MPa pressure (Serra et al., 2007). Due to the breakdown of fat globules, the HPH process causes a redistribution of proteins on the surface, increases protein solubility and denaturation, changes the properties of casein micelles and activates milk enzymes (Patrignani et al., 2007). Together with these changes, the increased availability of nutrients necessary for the metabolism and proliferation of starter cultures and the promotion of proteolysis may indirectly affect starter activity and acidification kinetics (Sert et al., 2023). Contrary to our findings, Oliveira et al. (2014) reported that the $T_f$ value did not change with the pressure applied to the milk. This difference may be due to the difference in milk composition, starter culture type and concentration, and fermentation temperature used in the production of fermented milks (Kristo et al., 2003).

**Microbial development**

The change in the number of microorganisms during fermentation in milk with different fat content and homogenization pressures is given in Figure 2. In the first stage of the fermentation period, the rate of change in the number of microorganisms was similar in almost all samples. Especially after 60 min, the rate of microbial growth increased in all samples and the rate of microbial growth varied depending on the applied pressure and fat ratio. The rate of increase in viability reached the highest value at 120 min in 100 MPa pressurized fatty milk compared to the other milks and then slowed down. Similarly, the bacterial growth rate showed a tendency to slow down after 120 min in fatty and semi skimmed milk, which were pressurized at 50 MPa and 100 MPa, respectively. In the other kinds of milk, a decrease in the growth rate was detected after 150 min.

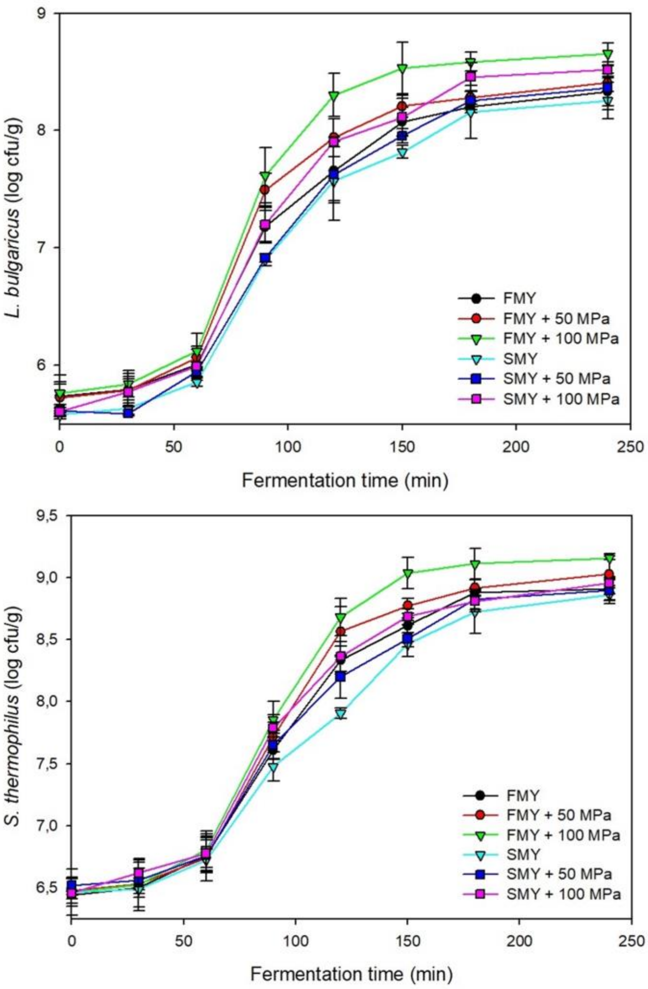


**Figure 2.** Changes in *L. bulgaricus* and *S. thermophilus* counts during incubation in yogurt made from fatty and semi skimmed milk treated with HPH (SMY: Yogurt from semi skimmed milk; SMY + 50 MPa: Yogurt from semi skimmed milk treated with 50 MPa pressure; SMY + 100 MPa: Yogurt from semi skimmed milk treated with 100 MPa pressure; FMY: Yogurt from fatty milk; FMY + 50 MPa: Yogurt from fatty milk treated with 50 MPa pressure; FMY + 100 MPa: Yogurt from fatty milk treated with 100 MPa pressure)

Fat content and applied pressure were also found to be effective on the cell viability at the end of fermentation ($p<0.05$). The counts of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* in yogurt samples were determined in the range of 8.25 - 8.65 log cfu/g and 8.85 - 9.15 log cfu/g, respectively. The HPH treatment of the milk increased the cell viability during fermentation compared to the control sample. The higher microbial population during fermentation may explain the decrease in the fermentation time of yogurts obtained from the HPH-treated milk. The HPH treatment of milk proved to be an important process in achieving shorter fermentation time due to higher microbiological quality. A similar result was found in various studies on fermented milk products in the literature (Patrignani et al., 2009; Gül et al., 2023; Sert et al., 2023). The essential amino acids required for the growth of LAB in milk are either absent or considerably lower than the concentration required to support optimum growth (Law and Haandrikman, 1997). However, a symbiotic relationship between LAB used as starter cultures during fermentation is known, with *L. bulgaricus* producing essential amino acids due to its proteolytic property and promoting the growth of *S. thermophilus* that has relatively weaker proteolytic capacity (Neviani et al., 1995; Fira et al., 2001; Lanciotti et al., 2004; Serra et al., 2009b). The HPH treatment probably causes the dissolution of casein-casein micelles, which has been reported to increase the soluble nitrogen fraction at pH 4.6 (Lanciotti et al., 2004). It has also been reported that homogenization after pasteurization of skim milk has the effect of promoting proteolysis of *k*-casein, which is subject to high degradation (García-Risco et al., 2002). On the other hand, it was also observed that HPH treatment positively affects the proteolytic activity of some LAB strains (Lanciotti et al., 2007). Therefore, the increase in the rate of microbial growth by HPH is associated with the increase in the amount of soluble $\beta$-lactoglobulin and *k*-casein with the high pressure and the development of sensitivity to proteolysis and consequently the increase in the amount of amino acids (Lanciotti et al., 2004).

**Viscosity development**

The viscosity development of high pressure homogenized fatty and semi skimmed milk during fermentation until pH 4.5 is given in Figure 3. Except for 100 MPa pressurized fatty milk, significant viscosity development starts after 60 min in all samples. However, it was observed that the viscosity development started earlier (30 min) in 100 MPa pressurized fatty milk and it can be said that the duration of the lag phase of the viscosity is significantly affected by the HPH (Table 2). A similar result was found in the yogurt study conducted by Sfakianakis et al. (2014). Up to 180 min of the fermentation period, viscosity development was observed at an increasing rate in all samples, while the rate of viscosity development tended to slow down after 180 min. The maximum rate of viscosity increase ($\mu_{max}$) was significantly affected by the homogenization pressure ($p<0.05$) and $\mu_{max}$ value increased in parallel with the increase in pressure.

The highest $\mu_{max}$ values were determined as $1.82\times10^{-2}$ and $2.35\times10^{-2}$ Pa.s units/min in semi skimmed and fatty milks treated with 100 MPa pressure, respectively. At the same time, the application of HPH caused a decrease in the time to reach the maximum rate of viscosity increase ($T_{\mu max}$), and while this time was 150 min in the control sample, the $T\mu_{max}$ value decreased to 120 min as a result of 100 MPa pressure application. Oliveira et al. (2014) reported that the increase in the viscoelastic behavior of the product during fermentation starts when the pH value of the milk reaches 5.0 and accordingly, the gel formation of the samples subjected to HPH treatment (120 min) started earlier than the control sample (140 min). They stated that this behavior is related to the partial disintegration of casein micelles as a result of the application of the HPH treatment to milk, which promotes the dissolution of colloidal calcium phosphate and the precipitation of micelles (Roach and Harte, 2008).

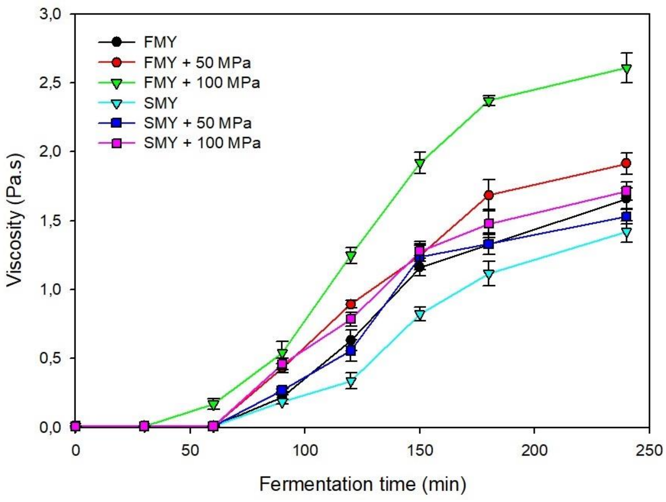


**Figure 3.** Viscosity development during incubation in yogurt obtained fatty and semi skimmed milk treated with HPH (SMY: Yogurt from semi skimmed milk; SMY + 50 MPa: Yogurt from semi skimmed milk treated with 50 MPa pressure; SMY + 100 MPa: Yogurt from semi skimmed milk treated with 100 MPa pressure; FMY: Yogurt from fatty milk; FMY + 50 MPa: Yogurt from fatty milk treated with 50 MPa pressure; FMY + 100 MPa: Yogurt from fatty milk treated with 100 MPa pressure)

**Table 2.** Viscosity kinetic parameters during fermentation of fatty and semi skimmed milk treated with HPH

| Parameters | SMY | SMY + 50 MPa | SMY + 100 MPa | FMY | FMY + 50 MPa | FMY + 100 MPa |
|---|---|---|---|---|---|---|
| $\mu_{max}$ (*Pa.s* units/min) | $1.62x10^{-2} \pm 0.72x10^{-4}$ | $1.94x10^{-2} \pm 0.46x10^{-4}$ | $1.82x10^{-2} \pm 0.68x10^{-3}$ | $1.78x10^{-2} \pm 0.75x10^{-3}$ | $1.81x10^{-2} \pm 0.67x10^{-4}$ | $2.35x10^{-2} \pm 0.38x10^{-4}$ |
| $T\mu_{max}$ (min) | 150 | 150 | 120 | 150 | 120 | 120 |

$\mu_{max}$: Maximum rate of viscosity increase; $T\mu_{max}$: Time to reach the maximum rate of viscosity increase; (SMY: Yogurt from semi skimmed milk; SMY + 50 MPa: Yogurt from semi skimmed milk treated with 50 MPa pressure; SMY + 100 MPa: Yogurt from semi skimmed milk treated with 100 MPa pressure; FMY: Yogurt from fatty milk; FMY + 50 MPa: Yogurt from fatty milk treated with 50 MPa pressure; FMY + 100 MPa: Yogurt from fatty milk treated with 100 MPa pressure)

Rheological changes during fermentation can be explained by the physical and chemical changes occurring in milk. During fermentation, a decrease in pH to 6.0 leads to a decrease in the negative charges on the casein micelles, reducing the electrostatic repulsion and thus destabilizing the micelles. The continuous decrease in pH from 6.0 to 5.0 reduces the negative charges on the casein micelles more strongly, which causes the micelles to bond better to form the gel. In addition, the dissolution of colloidal calcium phosphate in the casein micelle starts at this point and contributes to its precipitation by weakening the internal structure of the casein. When the pH value reaches 5.0, the solubility of colloidal calcium phosphate increases, the micelle structure is disrupted, and gel formation starts with the increase in the viscoelastic behavior of the milk. As the pH value decreases below 5.0, casein micelles get closer to each other and at the end of fermentation when the isoelectric point is reached, a three-dimensional network is formed by the interactions between casein and casein due to increased hydrophobic and plus-minus (electrostatic) charge interactions (Lee and Lucey, 2010; Oliveira et al, 2014).

The viscosity value at the end of fermentation was determined between 1.42 and 2.61 Pa.s depending on the applied pressure and fat content. In general, it was determined that the viscosity of yogurt increased significantly with the increase in homogenization pressure and the difference was more significant in yogurt obtained from fat milk ($p<0.05$). Swelam (2018) reported that the viscosity of yogurt made from milk pressurized at 700 MPa increased sharply compared to the control sample. According to Massoud et al. (2016), the formation of insoluble co-aggregates with high molecular weight due to the denaturation of serum proteins during HPH can lead to increase in viscosity values. Furthermore, casein micelles that are partially disintegrated during HPH cause an increase in milk particles and may contribute more to the formation of networks, resulting in a finer network of smaller casein micelles in a three-dimensional network structure (Serra et al., 2008). Moreover, denatured serum proteins form bonds with water and fat, and casein-fat or casein-casein interactions caused by the HPH process may play a role in increasing the viscosity index (Lanciotti et al., 2004; Shah, 2007).

**Conclusion**

This study demonstrated the effects of HPH on acidification kinetics, bacterial growth and viscosity development during yogurt production from fatty and semi skimmed milk. Bacterial growth during incubation was positively affected with the increase in the applied pressure and this effect was reflected in the pH change and thus viscosity development. The number of *L. bulgaricus* and *S. thermophilus* increased by 0.26 to 0.32 log units after fermentation with the applied pressure. Similarly, the pH change in the milk during the incubation process was accelerated with the application of HPH and the highest $V_{max}$ value was obtained in the incubation of fat milk with 100 MPa pressure and the fermentation time was shortened by about 30 min. On the other hand, rapid pH change during the fermentation process led to a shortening viscosity development time. However, fat content and applied pressure were effective on the viscosity value after fermentation and the highest viscosity value was determined in the yogurt sample obtained from 100 MPa pressure applied milk due to the effects of high pressure on milk components and the change in microbial population. As a result, HPH, one of the new processing techniques, has an important potential for the dairy industry and is an important tool for shortening the fermentation time and improving the structure in yogurt production. However, new studies should be focused on the effect of HPH application on volatile components such as flavors and aromas occurring during the fermentation process and sensory properties during the storage process.

**Conflict of Interest**

There is no conflict of interest between the authors of the article.

## REFERENCES

AOAC. (2000). (Association of official chemists). In Official methods of analysis (17th ed.) (Washington, DC, USA).

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*(8), 537-545. https://doi.org/10.1016/S0958-6946(99)00123-5

Ciron, C. I. E., Gee, V. L., Kelly, A. L., & Auty, M. A. E. (2010). Comparison of the effects of high-pressure microfluidization and conventional homogenization of milk on particle size, water retention and texture of non-fat and low-fat yoghurts. *International Dairy Journal, 20*(5), 314-320. https://doi.org/10.1016/j.idairyj.2009.11.018

Ciron, C. I. E., Gee, V. L., Kelly, A. L., & Auty, M. A. E. (2012). Modifying the microstructure of low-fat yoghurt by microfluidisation of milk at different pressures to enhance rheological and sensory properties. *Food Chemistry, 130*(3), 510-519. https://doi.org/10.1016/j.foodchem.2011.07.056

Fira, D., Kojic, M., Banina, A., Spasojevic, I., Strahinic, I., & Topisirovic, L. (2001). Characterization of cell envelope-associated proteinases of thermophilic lactobacilli. *Journal of Applied Microbiology, 90*(1):123-130. https://doi.org/10.1046/j.1365-2672.2001.01226.x

Galia, W., Perrin, C., Genay, M., & Dary, A. (2009). Variability and molecular typing of Streptococcus thermophilus strains displaying different proteolytic and acidifying properties. *International Dairy Journal, 19*(2):89-95. https://doi.org/10.1016/j.idairyj.2008.08.004

García-Risco, M. R., Ramos, M., & López-Fandiño, R. (2002). Modifications in milk proteins induced by heat treatment and homogenization and their influence on susceptibility to proteolysis. *International Dairy Journal, 12*(8):679-688. https://doi.org/10.1016/S0958-6946(02)00060-2

Gardini, F., Lanciotti, R., Guerzoni, M. E., & Torriani, S. (1999). Evaluation of aroma production and survival of Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus and Lactobacillus acidophilus in fermented milks. *International Dairy Journal*, *9*(2):125-134. https://doi.org/10.1016/S0958-6946(99)00033-3

Gül, L. B., Bekbay, S., Akgün, A., & Gül, O. (2023). Effect of oleaster (Elaeagnus angustifolia L.) flour addition combined with high-pressure homogenization on the acidification kinetics, physicochemical, functional, and rheological properties of kefir. *Food Science & Nutrition, 11* 5325-5337. https://doi.org/10.1002/fsn3.3491

Hayes, M. G., & Kelly, A. L. (2003). High pressure homogenisation of raw whole bovine milk (a) effects on fat globule size and other properties. *Journal of Dairy Research*, *70*(3), 297-305. https://doi.org/10.1017/s0022029903006320

Kristo, E., Biliaderis, C. G., & Tzanetakis, N. (2003). Modelling of the acidification process and rheological properties of milk fermented with a yogurt starter culture using response surface methodology. *Food Chemistry, 83*(3):437-446. https://doi.org/10.1016/S0308-8146(03)00126-2

Lanciotti, R., Vannini, L., Pittia, P., & Guerzoni, M. E. (2004). Suitability of high-dynamic-pressure-treated milk for the production of yogurt. *Food Microbiology*, *21*(6), 753-760. https://doi.org/10.1016/j.fm.2004.01.014

Lanciotti, R., Patrignani, F., Iucci, L., Saracino, P., & Guerzoni, M. (2007). Potential of high pressure homogenization in the control and enhancement of proteolytic and fermentative activities of some

Lactobacillus species. *Food Chemistry* *102*(2):542-550. https://doi.org/10.1016/j.foodchem.2006.06.043

Law, J. & Haandrikman, A. (1997). Proteolytic enzymes of lactic acid bacteria. *International Dairy Journal, 7*(1):1-11. https://doi.org/10.1016/0958-6946(95)00073-9

Lee, W. J. & Lucey, J. A. (2010). Formation and physical properties of yogurt. *Asian-Australasian Journal of Animal Sciences, 23*(9):1127-1136. https://doi.org/10.5713/ajas.2010.r.05

Levy, R., Okun, Z., & Shpigelman, A. (2022). Utilizing high-pressure homogenization for the production of fermented plant-protein yogurt alternatives with low and high oil content using potato protein isolate as a model. *Innovative Food Science & Emerging Technologies*, *75*. https://doi.org/10.1016/j.ifset.2021.102909

Lucey, J. A., & Singh, H. (1997). Formation and physical properties of acid milk gels: a review. *Food Research International*, *30*(7), 529-542. https://doi.org/10.1016/S0963-9969(98)00015-5

Massoud, R., Belgheisi, S., & Massoud, A. (2016). Effect of high pressure homogenization on improving the quality of milk and sensory properties of yogurt: A review. *International Journal of Chemical Engineering and Applications*, *7*(1), 66-70. https://doi.org/10.7763/ijcea.2016.v7.544

Neviani, E., Giraffa, G., Brizzi, A., & Carminati, D. (1995). Amino acid requirements and peptidase activities of Streptococcus salivarius subsp. thermophilus. Journal of Applied Bacteriology 79(3):302-307. https://doi.org/10.1111/j.1365-2672.1995.tb03141.x

Nguyen, H. T. H., Ong, L., Kentish, S. E., & Gras, S. L. (2015). Homogenisation improves the microstructure, syneresis and rheological properties of buffalo yoghurt. *International Dairy Journal, 46*:78-87. https://doi.org/10.1016/j.idairyj.2014.08.003

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. https://doi.org/10.1016/j.foodhyd.2017.05.035

Oliveira, M. M. d., Augusto, P. E. D., Cruz, A. G. d., & Cristianini, M. (2014). Effect of dynamic high pressure on milk fermentation kinetics and rheological properties of probiotic fermented milk. *Innovative Food Science & Emerging Technologies, 26*:67-75. https://doi.org/10.1016/j.ifset.2014.05.013

Patrignani, F., Iucci, L., Lanciotti, R., Vallicelli, M., Mathara, J. M., Holzapfel, W. H., & Guerzoni, M. E. (2007). Effect of high-pressure homogenization, nonfat milk solids, and milkfat on the technological performance of a functional strain for the production of probiotic fermented milks. *Journal of Dairy Science*, *90*(10), 4513-4523. https://doi.org/10.3168/jds.2007-0373

Patrignani, F., Serrazanetti, D. I., Mathara, J. M., Siroli, L., Gardini, F., Holzapfel, W. H., & Lanciotti, R. (2016). Use of homogenisation pressure to improve quality and functionality of probiotic fermented milks containing Lactobacillus rhamnosus BFE 5264. *International Journal of Dairy Technology, 69*(2):262-271. https://doi.org/10.1111/1471-0307.12251

Roach, A. & Harte, F. (2008). Disruption and sedimentation of casein micelles and casein micelle isolates under high-pressure homogenization. *Innovative Food Science & Emerging Technologies, 9*(1):1-8. https://doi.org/10.1016/j.ifset.2007.03.027

Rudra, S. G., Nath, P., Kaur, C., & Basu, S. (2017). Rheological, storage stability and sensory profiling of low-fat yoghurt fortified with red capsicum carotenoids and inulin. *Journal of Food Processing and Preservation*, *41*(4). https://doi.org/10.1111/jfpp.13067

Serra, M., Trujillo, A. J., Quevedo, J. M., Guamis, B., & Ferragut, V. (2007). Acid coagulation properties and suitability for yogurt production of cows' milk treated by high-pressure homogenisation. *International Dairy Journal, 17*(7):782-790. https://doi.org/10.1016/j.idairyj.2006.10.001

Serra, M., Trujillo, A. J., Jaramillo, P. D., Guamis, B., & Ferragut, V. (2008). Ultra-high pressure homogenization-induced changes in skim milk: impact on acid coagulation properties. *Journal of Dairy Research, 75*(1):69-75. https://doi.org/10.1017/S0022029907003032

Serra, M., Trujillo, A. J., Guamis, B., & Ferragut, V. (2009a). Evaluation of physical properties during storage of set and stirred yogurts made from ultra-high pressure homogenization-treated milk. *Food Hydrocolloids*, *23*(1), 82-91. https://doi.org/10.1016/j.foodhyd.2007.11.015

Serra, M., Trujillo, A. J., Guamis, B., & Ferragut, V. (2009b). Flavour profiles and survival of starter cultures of yoghurt produced from high-pressure homogenized milk. *International Dairy Journal*, *19*(2), 100-106. https://doi.org/10.1016/j.idairyj.2008.08.002

Sert, D., Mercan, E., & Tanrıkulu, M. (2023). Impact of high-pressure homogenisation of milk on physicochemical, microbiological, and textural characteristics of sheep milk yoghurt. *International Dairy Journal*, *144*. https://doi.org/10.1016/j.idairyj.2023.105704

Sevenich, R., & Mathys, A. (2018). Continuous versus discontinuous ultra-high-pressure systems for food sterilization with focus on ultra-high-pressure homogenization and high-pressure thermal sterilization: A review. *Comprehensive Reviews in Food Science and Food Safety*, *17*(3), 646-662. https://doi.org/10.1111/1541-4337.12348

Sfakianakis, P., Topakas, E., & Tzia, C. (2014). Comparative study on high-intensity ultrasound and pressure milk homogenization: Effect on the kinetics of yogurt fermentation process. *Food and Bioprocess Technology*, *8*(3), 548-557. https://doi.org/10.1007/s11947-014-1412-9

Shah, N. P. (2007). Functional cultures and health benefits. *International Dairy Journal*, *17*(11):1262-1277. https://doi.org/10.1016/j.idairyj.2007.01.014

Shiby Varghese K., & Mishra, H. N. (2008). Modelling of acidification kinetics and textural properties in dahi (Indian yogurt) made from buffalo milk using response surface methodology. *International Journal of Dairy Technology*, *61*(3):284-289. https://doi.org/10.1111/j.1471-0307.2008.00411.x

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*(2), 113-137. https://doi.org/10.1080/10408690490424793

Swelam, S. (2018). Impact of high hydrostatic pressure on composition and quality of yoghurt. *Journal of Food and Dairy Sciences, 9*(1):31-35. https://doi.org/10.21608/jfds.2018.35164

Torres, I. C., Amigo, J. M., Knudsen, J. C., Tolkach, A., Mikkelsen, B. Ø., & Ipsen, R. (2018). Rheology and microstructure of low-fat yoghurt produced with whey protein microparticles as fat replacer. *International Dairy Journal*, *81*, 62-71. https://doi.org/10.1016/j.idairyj.2018.01.004

Trujillo, A. J., Capellas, M., Saldo, J., Gervilla, R., & Guamis, B. (2002). Applications of high-hydrostatic pressure on milk and dairy products: a review. *Innovative Food Science & Emerging Technologies*, *3*(4), 295-307.