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The Effect of Homogenization Pressure and Stages on the Amounts of Lactic and Acetic Acids of Probiotic Yoghurt

Massoud, R.[1], Fadaei Noghani, V.[1]*, Khosravi-Darani, K.[2]

1- Department of Food Science & Technology, Faculty of Agriculture, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran

2- Department of Food Technology Research, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences, Food Science and Technology, Shahid Beheshti University of Medical Sciences, P. O. Box 19395-4741, Tehran, Iran.

Abstract

Nowadays the usage of probiotic products especially yogurt due to having wonderful and health properties has become popular in the world. In this study, the effect of homogenization pressures (100, 150 and 200 bar) and stages (single and two) on the amounts of lactic and acetic acids were investigated. Yoghurts were manufactured from low- fat milk treated using high pressure homogenization at 100,150 and 200 bar and at 60°C. The amounts of lactic and acetic acids were determined after days 1, 7, 14 and 21 of storage at 4ºC. Experiments were set up using a completely randomized design. With increasing pressure and stages of homogenization, the amounts of lactic and acetic acids increased ($p<0.01$). The results obtained in the present study indicated that the greatest amounts of lactic and acetic acids during the storage period were observed in the sample homogenized at a pressure of 200 bar and two stages.

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Correspondence to
Fadaei Noghani V. Department of Food Science & Technology, Faculty of Agriculture, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran.
Email: vn.fadaei@gmal.com

1. Introduction

The manufacture of fermented milks containing probiotic microorganisms especially yogurt, has become popular and commercially significant, resulting in the availability of many products of this kind in the world market [1,2,3]. Fermented dairy products are perceived by consumers as healthy products [4]. Viability of probiotic microorganisms in the final product until the time of consumption is their most important qualitative parameter; generally, the values of 10^6 and 10^7–10^8 CFU/ml or CFU/g have been accepted as the minimum and satisfactory levels, respectively. In Iran, national standard requires minimum of 10^6 CFU/ml viable probiotic cells in yogurt [2,5,6].

The use of probiotic (health-promoting) microorganisms in different fermented milks or yoghurt-like products has reinforced the acclaimed healthful properties and increased the consumption of these products [7]. To exert these healthy benefits in the host, the probiotic microorganisms need to be viable, active, and sufficiently abundant throughout the specified shelf life [8]. The species *Lactobacillus acidophilus* and *Bifidobacterium lactis* are frequently used in production of probiotic fermented milks [2,9]. Homogenization reduces the size of fat globules to prevent creaming [10]. When a large fat globule is disintegrated to a number of small droplets, a tremendous increase in surface area of the fat occurs. Onto the surfaces of these newly created droplets, casein adsorbs and stabilizes the droplet [11]. In single-stage homogenization, the whole pressure drop is used over one device. It is used for products with low fat content and in products requiring a high viscosity. Two-stage homogenizers are used in breaking down the fat globule in two stages. This is effective for high fat content, high solids content, or products in which low viscosity is desired [11]. In homogenization which is conducted by applying pressure in two stages, the first stage pressure reduces the average milk fat globule diameter size and the second stage is designed to break the clusters of fat globules apart to inhibit creaming in milk [12].

For the manufacture of yoghurt, milk is normally homogenized (15–20 MPa) and heat-treated to reduce microbial load, to increase the yoghurt stability, consistency and texture, and also to decrease whey separation during storage [13, 14]. In yoghurts from milk treated by HPH (High Pressure Homogenization) (300 MPa), the higher activities of probiotics indicated by organic acid results, especially lactic acids were detected [15]. HPH seems to be one of the most promising technological alternatives to pasteurization, which is useful for inactivation of dairy phages. The viability of bacteriophages of lactic acid- and probiotic bacteria was increased in higher pressures (>100 Mpa) [16].

Investigating the amount of non-volatile acids (lactic or pyruvic) and volatile acids (butyric or acetic) in yoghurts made from ultra-high pressure homogenized milk (200 or 300 MPa) showed that all four acids were increased and pyruvic acid was much higher in the yoghurts than others [17]. Shah and Dave reported that the accumulation of acids like lactic and acetic resulted in decreasing pH and increasing acidity value [5,18]. Acetic and lactic acids are known to be utilized during the fermentation process and accumulate over time during storage in yoghurt, reflecting the decrease in metabolic activity of *L. delbrueckii* subsp. *bulgaricus* [19,20]. Although there have been some research about organic acids available at yoghurt, there are still many aspects that remain unclear, mainly those related to the relationships between homogenization and organic acids in yoghurt. Further investigations are needed to establish the precise functions of homogenization on producing organic acids in low fat probiotic yoghurt. So, in this research, the simultaneously effect of increasing pressure and the number of homogenization stages of the low-fat milk used for producing probiotic yoghurt on the amounts of lactic and acetic acids were investigated.

2. Materials and Methods

2.1 Starter culture

The commercial lyophilized ABY (Acidophilus Bifidobacter Yoghurt) culture (containing *Lactobacillus acidophilus* LA-5, *Bifidobacterium lactis* BB-12, *Lactobacillus delbrueckii* ssp. *bulgaricus*, and *Streptococcus thermophilus*) that are known as 'FD-DVS ABY-1' were supplied by Chr-Hansen (Horsholm, Denmark). The cultures were maintained according to the manufacturer's instructions at -18ºC until used.

2.2 Milk treatment and production of yoghurt samples

According to the method of industrial manufacturing ice cream, the low fat milk (1.5% fat) used for producing yoghurt samples was preheated at 60°C and homogenized; then, heated at 85°C for 30 minutes. After cooling milk up to fermentation temperature (42ºC), it was inoculated with mixture starter culture ABY1 and incubated. During fermentation, pH dropped until it reached 4.5. After fermentation, yoghurt samples prepared were held in refrigerator (4ºC). To evaluate the amount of acetic and lactic acids content in yoghurt, analysis were performed on days 1, 7, 14 and 21 of storage.

2.3 Separation and quantification of organic acids by HPLC

Quantification of lactic and acetic acids was carried out by high-performance liquid chromatography (CE 4200- Instrument, Cecil, Milton Technical Center, Cambridge CB46AZ, UK) [18]. Briefly, for extraction of acids, 6.0 g of sample was diluted to 5 mL with 0.1 N H_2SO_4 homogenized and centrifuged at 5000 ×g for 10 min. The supernatant was filtered through Whatman no. 1 filter paper and through a 0.20ml membrane filter and was immediately analyzed. A Jasco UV-980 detector and an ODS-5 column (Macherey–Nagel, Duren, Germany) were used. The mobile phase was 0.009 H_2SO_4 at a flow rate of 1.0 ml/min. The wavelength of detection was optimized at 210 nm. The standard solutions of lactic and acetic acids (Merck, Darmstadt, Germany) were prepared in distilled water [19].

2.4 Statistical analysis

Statistical analysis on a Full factorial Design was performed for ANOVA using SAS software (version 9.1; Statistical Analysis System Institute Inc., Cary, NC, USA). Duncan's multiple range tests were used to compare means at the significant level of 0.05 (P<0.05). All experiments were replicated two times.

3. Results and Discussion

3.1 Evaluation of acetic acid content of probiotic yoghurt samples affected by different pressures and stages of homogenization

There was significant difference between the amount of acetic acid in yoghurt samples during storage and at different stages and pressures of homogenization (p<0.01). As shown in Table 1, the highest and lowest acetic acid values were observed in T6 and T1, respectively (p<0.05). The results showed that an increase in homogenization pressure and stages induced a significant increment in acetic acid content.

There was significant difference between the amount of lactic acid in yoghurt samples during storage and at different stages and pressures of homogenization (p<0.01). As shown in Table 2, the highest and lowest lactic acid values were observed in T6 and T1, respectively (p<0.05). The results showed that an increase in homogenization pressure and stages induced a significant increment in lactic acid content.

The catabolism of lactose by *Lactobacilus delbrueckii* results mainly in the production of lactic acid [14]. In this study, the highest amount of lactic acid was observed in T6 (from 40.60 to 61.96 mg/kg), the yoghurt sample homogenized at 200 bar and in two stages; and the lowest amount was related to the T1 (from 30.73 to 36.10 mg/kg), the yoghurt sample homogenized at 100 bar and in single stage.

Table 1. Acetic acid content (mg/kg) of probiotic yoghurt samples affected by different pressures and stages of homogenization during storage at 4°C*

Treatment \ Day	0	7	14	21
T1	5.73±0.05^{g}	9.66±0.20^{c}	11.83±0.15^{b}	12.10±0.10^{a}
T2	11.93±0.11^{t}	12.90±0.10^{o}	16.53±0.05^{i}	18.10±0.10^{f}
T3	7.46±0.23^{l}	10.16±0.05^{h}	14.90±0.10^{e}	14.50±0.12^{d}
T4	14.06±0.15^{u}	18.43±0.11^{r}	21.03±0.05^{m}	22.90±0.17^{k}
T5	8.53±0.15^{p}	11.16±0.25^{n}	17.83±0.20^{j}	30.96±0.15^{g}
T6	15.90±0.11^{v}	20.70±0.10^{s}	23.26±0.15^{q}	25.70±0.10^{op}

*Mean ± Standard Deviation (SD) values with different superscript letters differ significantly in treatments during storage (P<0.05).
T1: Homogenization at 100 bar and as single stage, T2: Homogenization at 100 bar and as two stage, T3: Homogenization at 150 bar and as single stage, T4: Homogenization at 150 bar and as two stage, T5: Homogenization at 200 bar and as single stage, T6: Homogenization at 200 bar and as two stage.

Table 2. Lactic acid content (mg.kg) of probiotic yoghurt samples affected by different pressures and stages of homogenization during storage at 4ºC*

Treatment \ Day	0	7	14	21
T1	30.73±0.11^{j}	32.06±0.34^{h}	34.40±0.17^{e}	36.10±0.15^{c}
T2	41.03±0.05^{m}	43.06±0.05^{i}	45.30±0.17^{g}	47.46±0.55^{f}
T3	40.76±0.11^{m}	43.00±0.30^{d}	46.10±0.17^{b}	47.33±0.20^{a}
T4	46.83±0.05^{p}	50.60±0.10^{o}	53.00±0.10^{n}	58.60±0.17^{l}
T5	47.70±0.10^{o}	53.86±0.05^{n}	56.70±0.10^{l}	58.00±0.17^{k}
T6	40.60±0.10^{t}	55.90±0.10^{s}	59.50±0.26^{r}	61.96±0.15^{q}

*Mean ± Standard Deviation (SD) values with different superscript letters differ significantly in treatments during storage (P<0.05).
T1: Homogenization at 100 bar and as single stage, T2: Homogenization at 100 bar and as two stage, T3: Homogenization at 150 bar and as single stage, T4: Homogenization at 150 bar and as two stage, T5: Homogenization at 200 bar and as single stage, T6: Homogenization at 200 bar and as two stage.

This observation is in line with that of Serra [15], who reported producing higher lactic acid in probiotic yoghurt at pressure 300 Mpa in comparison with 200 Mpa; and it was related to the increased activity of probiotics. The amount of lactic acid increased during cold storage. Lactic acid content increased during storage in all samples, as a result of the continuing activity of the starter cultures [15]. This finding is agreement with Mortazavian and Sohrabvandi [9], who reported the same result in this field. The increment in lactic acid during storage is correlated with the lactose consumption by Lactobacilli [23].

There appears to be a relationship between the concentration of acetic acid and viability of bifidobacteria. Because these bacteria produce appreciable amounts of acetic acid during fermentation, the amount of this acid represents bifidobacteria growth and activity [21,24]. In this study, the highest amount of acetic acid was observed in T6 (from 15.90 to 25.70 mg/kg), the yoghurt sample homogenized at 200 bar and in two stages; and the lowest amount was related to the T1 (from 5.73 to 12.10 mg/kg), the yoghurt sample homogenized at 100 bar and in single stage. This is in agreement with results obtained by Serra [15], who reported producing higher acetic acid in probiotic yoghurt at pressure 300 Mpa in comparison with 200 Mpa; and it was related to the increased activity of probiotics. The amount of acetic acid increased during cold storage. This is in accordance with the result of Mortazavian and Sohrabvandi [9], which has shown that acetic acid increased in probiotic yoghurt during cold storage.

In general, the homogenization pressure could induce some conformational changes in whey proteins and caseins, which could increase their susceptibility to proteolysis, and therefore the availability of free amino acids, as reported by Patrignani [25] for yoghurts from milk treated at HPH (>100MPa). So, this could increase the activity of probiotics and producing lactic and acetic acids.

4. Conclusion

In this research, the simultaneously effect of increasing pressure and the number of homogenization stages of the low-fat milk used for producing probiotic yoghurt on the amounts of lactic and acetic acids were investigated. The highest amount of acetic acid was observed in the yoghurt sample homogenized at 200 bar and in mtwo stages; and the lowest amount was related to the yoghurt sample homogenized at 100 bar and in single stage. The amounts of acetic and lactic acids increased during the cold storage.

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