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Effect of High Pressure Homogenization on Viability and Physicochemical of Probiotic Stirred Yogurt

Z. Roostazadeh[1], A. R. Shahab Lavasani[1]*, S. Mirdamadi[2]

[1]Department of Food Science and Technology, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran.
[2]Biotechnology Department, Iranian Research Organization for Science and Technology (IROST), No. 71, ShahidMousavi Street, Enghelab Avenue, Tehran, Iran.

Abstract

The effect of milk processing on the viability and physicochemical properties of probiotic low-fat yogurt was studied. Skim milk fortified with concentration 10^8 and 10^9 of *Lactobacillus acidophillus* subjected to one and two stages homogenization (220 and 660 MPa) respectively. The results of this study showed that the use of two stages homogenization to treat milk before fermentation protected the viability of the *lactobacillus acidophilus* better than one stage homogenization. High pressure homogenization affected physicochemical properties such as pH and acidity (%) of all samples except for dry matter (%) and fat (%). Dry matter (%) and fat (%) content of all samples did not change at specified period of storage, however, pH and acidity changed continuously during period of storage. According to above mentioned, treatment 6, was the best among all treatments with due attention to survival rate and physicochemical properties.
Key word: Yogurt, Probiotic, Homogenization, Viability, Physicochemical

Introduction

Yoghurt is a fermented milk product obtained from the milk or the milk products by the lactic acid fermentation through the action of *Streptococcus salivarius* subsp. thermophilus, *Lactobacillus delbrueckii subsp. bulgaricus* (FAO/WHO, 1977).
The probiotic yoghurt, having probiotic effect is a fermented milk product with adjuvant microorganisms. There are numerous advantages of consuming fermented dairy products containing probiotic bacteria. A high population of probiotic organisms in the colon contributes to good intestinal health. Consequently consumption of products such as yoghurt containing viable probiotic organisms adds benefit to human gut health. Some of emerging technologies are focused on modifying yogurt texture, which may include enzymatic cross-linking by transglutaminase, on extending shelf-life, which may involve the use of carbon dioxide, and on improving several characteristics of yogurt such as, fermentation time, water-holding capacity (WHC) and post-acidification, which may include the use of high hydrostatic pressure. However, recently, ultra-high-pressure

*Corresponding Author: shahabam20@yahoo.com

homogenization (UHPH), which is based on the same principle as conventional homogenization but which works at significantly higher pressures (up to 350 MPa), is being investigated for the manufacture of yogurt In this previous work, UHPH samples were compared to both: heat-treated milk with and without skim milk powder (SMP) added, since the increase in nonfat solids content (to E14%) of the milkbase using SMP is the most common practice in the current dairy industry(Tamime & Robinson, 2007). The authors concluded that set-type yogurts prepared from milk UHPH treated at 200or 300MPa presented higher gel firmness in texture analysis, less syneresis and lower titrable acidity compared with conventionally treated milk, even fortified with 3% skim milk powder. Yogurt is usually classified in two basic types according to its physical state in the retail container: set and stirred yogurt. To obtain stirred yogurt, the coagulum is mechanically broken before cooling and packaging, which induces considerable changes in the rheological properties, although the physical properties of stirred yogurts are also affected by the original gel characteristics. However, in order to maintain the stability of the product during the shelf-life, some of the following approaches are adopted by manufacturers. So the objective of present study were to determine viability of probiotic microorganism and physicochemical characteristics of probiotic yogurt which influenced by different pressure of homogenization.

Material and method

Pressure treatments

Pressure treatments were carried out using an isostatic pressure system (Engineered Pressure Systems, Inc., Haverhill, MA, USA) having a chamber size of 0.10 m diameter and 0.25 m height. The medium for hydrostatic pressurization was 5% Mobil Hydrasol 78 water solution. One sample was subjected to HHP at 660 MPa for 5 min at room temperature and another one was subjected to HHP at 220 MPa for 5 min at room temperature. Targeted pressure was achieved in 4–5 min and the depressurization took less than 1 min.

Yogurt preparation

The processed milk (HHP) was inoculated ($10^8$and 10^9 cfu/ml) with freeze-dried yogurt starter cultures supplied by Danisco USA Inc. (Milwaukee, WI, USA). This starter culture contained a mixture of *S. thermophilus, L. delbrueckii ssp. bulgaricus, and probiotic cultures including Lactobacillus acidophilus,* and *Bifidobacterium lactis were purchased from Caminox Spain Inc*. The fermentation was carried out at 43 $^{\circ}$C. Each fermentation process was monitored by then stirred with a mechanical mixer for 30 s according to a standardized protocol, and stored at 4 $^{\circ}$C for 15–16 h. The characteristics of milk used for yogurt making has been shown in table 1. And the protocol of yogurt making as shown in figure 1.

Table 1. Milk characteristics used for yogurt making

Type of milk	**Characteristics of milk**						
Medium fat milk	**Dry matter%**	**Fat %**	**Acidity (Dornic basis)**	**Density**	**Antibiotic**	**Coagulation**	**pH**
	8.2	3.1	14.8	1.0295	Negative	Negative	6.76

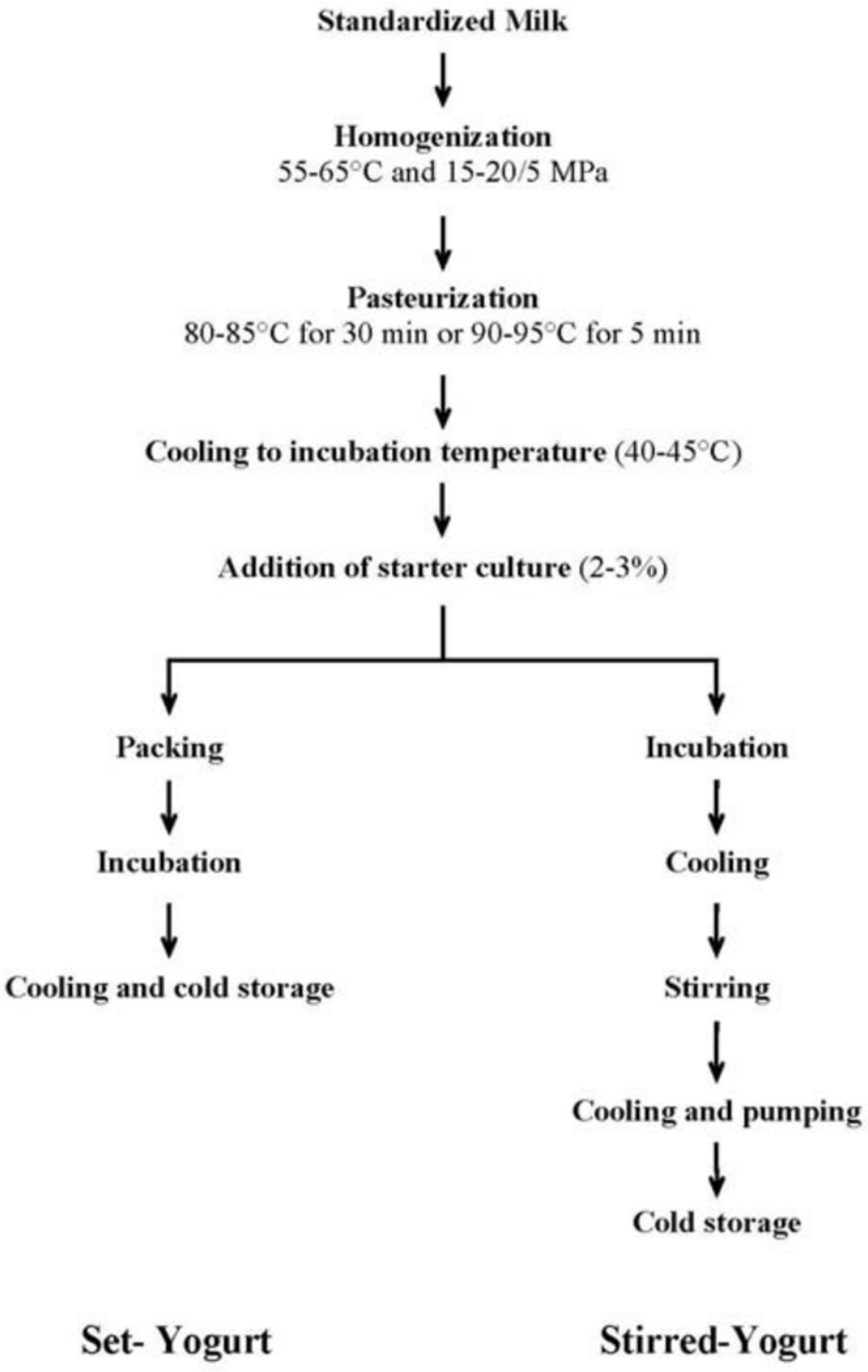

Figure 1. Main processing steps in the manufacture of set and stirred yogurt.

The preparation of probiotic organism

Ten gram of sample was poured in tube and phosphate buffer added to it in determined volume after that shaked the tube for 45 °C.

The preparation of Antibiotics

2mg of clindamycin was soluted by 10 mg of distilled water after that solution filtered by biological filter (pore size= 0.22 micron), then solution collected in vial and stored in freeze temperature.

20 mg ciprofloxacin was soluted by 10 mg of distilled water and then filtered by biological filter (pore size= 0.22 micron) after that the whole solution collected in vial and stored in freeze temperature

Media

200 ml of MRS agar was placed in retort at 121 °C for 15 min then 0.1 ml clindamycin and 1 ml ciprofloxacin were added to it at 40 °C.

Counts of yoghurt cultures and probiotic bacteria

The enumeration of bacteria was carried out at the beginning and at the end of the fermentation when the sample reached a pH of 4.5 and at days 2, 7 and 14 of the storage to assess bacterial growth and viability. A 10 g yoghurt sample was diluted in 90 mL of sterile peptone 0.1 % (w/w) solution (Oxoid, Basingstoke, UK), then serially diluted using 9 mL of sterile peptone solution and added to the media using a pour-plate technique. MRS agar (Oxoid, Basingstoke, UK) supplemented with clindamycine (0.1 mg L^{-1}) was used for the selective enumeration of L. acidophilus La-5. MRS agar supplemented with cysteine hydrochloride (0.5 g L^{-1}), lithium chloride (1 g L^{-1}) and dicloxacillin (0.5 mg L^{-1}) was used for the enumeration Bifidobacterium animalis BB-12. Clindamycin and dicloxacilin were supplied by Sigma Aldrich (NSW, Australia) while cysteine hydrochloride and lithium chloride were obtained from Merck (VIC, Australia). The inoculated plates were incubated under anaerobic conditions using an anaerobic gas jar (Oxoid, SA, Australia) at 37 °C for 72 h. Plates with 25–250 colonies were selected for manual counting.

Physicochemical properties

Samples of cheese were analyzed for pH (Metrohm Model 632 pH-meter; Herisau, Switzerland) and percentages of titratable acidity, dry matter and total fat (AOAC 2000).

Treatments including:

Treatment 1: sample without *Lactobacillus acidophilus* and one stage homogenization as a control sample 1

Treatment 2: sample with concentration of *Lactobacillus acidophilus* 10^8cfu/ml and one stage homogenization

Treatment 3: sample with concentration of *Lactobacillus acidophilus* 10^9cfu/ml and one stage homogenization

Treatment 4: sample without *Lactobacillus acidophilus* and two stages homogenization as a control sample 2

Treatment 5: sample with concentration of *Lactobacillus acidophilus* 10^8cfu/ml and two stages homogenization

Treatment 6: sample with concentration of *Lactobacillus acidophilus* 10^9cfu/ml and two stages homogenization

Results and discussion

After comparison of several selective media for isolation and enumeration of lactobacillus acidophillus, under the conditions in this study, mMRS agar modified with L-cysteine HCl (0.05%) clindamycin and ciprofloxacin was the best for cell count of *lactobacillus acidophillus*. *Lactobacillus acidophilus* bacteria increased slightly until 7 days of storage period and then decreased up to 14 days of storage periods however, two stages homogenization process protected *lactobacillus acidophilus* better than one stage homogenization because of more availability of nutrients (Table 2). The Fermented Milks and Lactic Acid Bacteria Beverages Association in Japan introduced a standard that stipulates that the minimum concentration of viable lactobacillus acidophilus per gram or milliliter of product defined as a probiotic food should be at least 7.0 log10 cells. This concentration should ensure the therapeutic minimum dose of 5.0 log10 viable cells/g or ml of product. Other international food associations and results from several studies have proposed that the concentration should range between 6.0 and 7.0 log10 cfu/g or ml (Gardiner *et al*., 1999). Intrinsic characteristics of the yogurt (low pH, high acidity) could have caused severe cellular stress that reduced cell recovery.

Table 2. Viability† of *Lactobacillus acidophilus*‡ in each samples during 14 days of storage period

The days of storage	Treatments	Viability of *Lactobacillus acidophilus* cfu/ml
2	2	$6\times10^7\pm0.14\times10^7$ cd
	3	$1.5\times10^7\pm0.28\times10^7$c
	5	$45\times10^7\pm1.7\times10^7$b
	6	$69\times10^7\pm7.5\times10^7$a
7	2	$3.1\times10^7\pm0.66\times10^7$gh
	3	$20\times10^7\pm6\times10^7$g
	5	$80\times10^7\pm6.9\times10^7$f
	6	$89\times10^7\pm4\times10^7$e
14	2	$1.5\times10^7\pm0.28\times10^7$ghm
	3	$8\times10^7\pm0.57\times10^7$gl
	5	$26\times10^7\pm2\times10^7$fk
	6	$60\times10^7\pm20\times10^7$ei

†Data with different letter superscript had significant differences (P<0.05)
‡Mean value ±Standard Error

The level of pressure of homogenization had no significant impact on levels of pH of the yogurt treatments at (P>0.05) whereas this factor had significant effect on total acidity (P < 0.05).The pH values ranged from 4.2 to 4.5, and titratable acidity ranged from 69.2 to 82 ^{o}d (Table. 3). The titratable acidity and pH were significantly affected by the storage period (P < 0.05). The increase in titratable acidity during 14 days of storage in refrigerator was due mainly to the near completion of lactose fermentation and the liberation of amino and free fatty acids following proteolysis and lipolysis (Shahab lavasani *et al*., 2012).This parameter slightly increased during the cold storage in all treatments as a result of the persistent metabolic activity of starters, which has been

called post-acidification (Serra *et al*., 2009). Parallel to the variation in titratable acidity values values, pH decreased until the 14th day of storage. The dry matter (%) of the yogurt samples are shown in Table 2. The results obtained from dry matter (%) of all samples showed that there were no significance differences (P>0.05) between all treatments. The average range is from 10.29-10.79 % (Table. 3). According to USDA specification (2001) and FDA (2009), yogurt should contain not less than 8.25% SNF (Solids Non Fat) before the addition of bulky flavor. The present findings conform to this specification.The fat (%) content of all treatments did not differ significantly (P>0.05) and fat (%) content of all samples was constant just 1.2 %(Table. 3).

Table 3. Physicochemical properties of probiotic† yogurt‡

The days of storage	Treatments	Physicochemical properties of probiotic yogurt			
		pH	Acidity (Dornic)	Dry matter%	Fat %
2	1	4.5±0.005abc	70.1±0.1abc	10.55±0.00	1.2±0.00
	2	4.5±0.003a	69.26±0.14e	10.55±0.00	1.2±0.00
	3	4.51±0.003abcde	70.16±0.16a	10.55±0.00	1.2±0.00
	4	4.5±0.003ab	69.2±0.1ef	10.55±0.00	1.2±0.00
	5	4.5±0.00abcdef	70.23±0.14abcd	10.55±0.00	1.2±0.00
	6	4.5±0.00abcd	70.06±0.06ab	10.55±0.00	1.2±0.00
7	1	4.4±0.00ghi	75.7±0.13ghi	10.79±0.00	1.2±0.00
	2	4.4±0.003g	75.6±0.00l	10.79±0.00	1.2±0.00
	3	4.4±0.003ghikl	76.33±0.16g	10.79±0.00	1.2±0.00
	4	4.4±0.003gh	75.6±0.00lm	10.79±0.00	1.2±0.00
	5	4.4±0.003ghiklm	76.33±0.16ghik	10.79±0.00	1.2±0.00
	6	4.4±0.003ghik	76.5±0.00gh	10.79±0.00	1.2±0.00
14	1	4.3±0.00noq	82±0.00noq	10.29±0.00	1.2±0.00
	2	4.3±0.08n	81.13±0.13s	10.29±0.00	1.2±0.00
	3	4.3±0.003nqrs	81.8±0.2h	10.29±0.00	1.2±0.00
	4	4.3±0.00no	81±0.00st	10.29±0.00	1.2±0.00
	5	4.2±0.003noqrst	81.13±0.13noqr	10.29±0.00	1.2±0.00
	6	4.3±0.003noqr	81.66±0.33no	10.29±0.00	1.2±0.00

†Data with different letter superscript had significant differences (P<0.05)
‡Mean value ±Standard Error

Conclusion

The results of this study showed that the use of two stages homogenization to treat milk before fermentation protected the viability of the *lactobacillus acidophilus* better than one stage homogenization. High pressure homogenization affected physicochemical properties such as pH and acidity (%) of all samples except for dry matter (%) and fat(%). Taking into account, that all samples are homogenized, the only difference in this case is due to survival rate of *lactobacillus acidophilus*. It can be seen that two stages homogenization caused more viability of *lactobacillus acidophilus*. Dry matter (%) and fat (%) content of all samples did not change at specified period of storage, however, pH

and acidity changed continuously during period of storage. According to above mentioned, treatment 6, was the best among all treatments with due attention to survival rate and physicochemical properties.

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