The Effect of Heat Inactivating Process on Biochemical, Microbiological and Sensory Characteristics of Iranian Drink Based on Fermented Milk (Doogh)

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Abstract
This study investigates the effects of heat inactivating processing of yogurt bacteria compared with other sequential inoculation on biochemical, microbiological, and sensory characteristics of typical Iranian drink based on fermented milk (Doogh). The yogurt bacteria (Streptococcus thermophilus and Lactobacillus delbrueckii sp. bulgaricus) were used in all treatments. Bifidobacterium animalis spp. lactis PTCC 1631 was used as probiotic bacteria. A pH, titrable acidity, redox potential, fermentation time, and viability of probiotic organisms were analyzed during fermentation and over the refrigeration storage for 21 days at 5°C. Also, the sensory attributes of treatments were determined at the end of fermentation. The greatest (p<0.05) mean pH drop rate was observed in B. animlis spp. lactis PTCC 1631 was co-cultured with yogurt starter bacteria and incubated at 40°C until final pH 4.5. In addition, the greatest viability of bifidobacteria was observed in this treatment. The viability of bifidobacteria strains was significantly higher in heat inactivating treatments than non-heat treated treatments. This process didn’t have positive effect on sensory properties of Doogh. The most acceptability in taste, texture, and mouth feel and appearance tolerability were observed in co-culture treatment in this study.

Keywords: Bifidobacterium animalis; Heat inactivated processing; Probiotic; Doogh

Introduction
Bifidobacteria, discovered in 1899 by Tessier, are a major component of the gastrointestinal tract microflora [1]. The typical habitat of bifidobacteria is human, warm-blooded animal and honeybee intestinal tract [2]. The reported health benefits of bifidobacteria include stabilizing the gut mucosal barrier, modulation of immune response, modulation of intestinal microbiota, prevention of traveler’s diarrhea in children, reduction of necrotizing endocarditis in neonates, alleviation of atopic dermatitis symptoms in children, improvement of constipation, and antibacterial and anti-carcinogenic activities [3]. The positive health effects documented for Bifidobacterium only occur when the bacteria are viable and active [4].

‘Viability’ of probiotic microorganisms in the final product until the time of consumption is the most important qualitative parameter, since it determines their therapeutic values. Although there is no world-wide agreement on viability of probiotics in food products, generally, the values of 10⁶ and 10⁷ cfu mL⁻¹ or cfu g⁻¹ have been accepted as the minimum and satisfactory levels, respectively [5]. In Iran, National standard requires minimums of 10⁶ cfu mL⁻¹ and 10⁵ cfu mL⁻¹ viable probiotic cells in yogurt and Doogh (typical Iranian drink based on fermented milk), respectively [6,7]. Reaching these standards is generally a difficult issue due to the poor viability of probiotic microorganisms during the fermentation and storage periods [8].

Various factors significantly affect the viability of probiotic microorganisms in fermented milks which include type of probiotic strains used, pH, titrable acidity, molecular oxygen, redox potential, hydrogen peroxide, and addition of salt, sugar and prebiotic compounds. Some stage of processing including dry matter content of milk, packaging conditions, step-wise/step-wise fermentation, heat treatment of milk, incubation temperature, cooling rate of the product and etc. have effect on survival of probiotic bacteria [5,9,10]. In fermented milks, the final pH at the time of consumption could be significantly lower (e.g., 3.8-4.2) and it is the most critical factor that decreases the viability of probiotic organisms in fermented milks [11]. Another critical factor in production of fermented milks is adding the probiotic culture prior to fermentation, simultaneously with the conventional yogurt cultures or after fermentation [12].

Doogh is a traditional Iranian fermented milk drink that is very popular and highly consumed product in Iran with a considerable increasing demand for its consumption. It is known as ‘Iran National Drink’. It prepares in two form: heat treated and un-heat treated Doogh. In heat treated Doogh, the product is subjected to post-fermentation heat treatment in order to increase the shelf life [7,13]. The aim of this study was to assess the effects of heat inactivated processing of yogurt traditional bacteria on biochemical, microbiological, and sensory characteristics of Doogh.

Materials and Methods
Cultures
The Bifidobacterium strain used in the study was Bifidobacterium animalis spp. lactis PTCC (Persian Type Culture Collection-Iran) 1631 adapted to simulate gastrointestinal fluid [14] and obtained from microbial culture stock of ‘Department of Drug and Food Control’ (Tehran University Culture Collection Center, Tehran, Iran). The Direct Vat-Set (DVS) pouches of commercial lyophilized Y-type

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namely, 6 for flavor, 3.5 for oral texture and mouth feel, and 2 for each sensory parameter were multiplied to the relevant coefficients, acceptable, 3 = satisfactory, and 4 = excellent. The given numbers for homogeneity). Each of these parameters was scored in a five-point standard for plain Doogh (Anon-b). Sensory parameters were flavor, oral texture and mouth feel, appearance (color, syneresis and texture, acidity), and bile by Sigma-Aldrich, Inc., Reyde, USA) was used for the selective enumeration of bifidobacteria strains according to Mortazavian et al. [10]. The plates were incubated anaerobically at 37°C for at least 72 hours. Anaerobic conditions were produced using the GasPac system. The titrable acidity was determined after mixing 10 mL of sample with 10 mL of distilled water and titrating with 0.1 N NaOH using 0.5% phenolphthalein according to Dave and Shah [15].

Parameters of pH mean drop rate, mean acidity increase rate, and mean redox potential increase rate were calculated according to Mortazavian et al. [11]:

- pH drop rate = (final pH value - initial pH value) / incubation time [pH value/min]Acidity increase rate = (final acidity value – initial acidity value) / incubation time [Dornic degree/min]
- Redox potential increase rate = (final value - initial value) / incubation time [mV/min]

The ‘peak time’ was defined as the highest increase in titratable acidity during fermentation period for every 30 min intervals.

Microbiological analysis

MRS-bile agar medium (MRS agar by Merck, Darmstadt, Germany and bile by Sigma-Aldrich, Inc., Reyde, USA) was used for the selective enumeration of bifidobacteria strains according to Mortazavian et al. [10]. The plates were incubated anaerobically at 37°C for at least 72 hours. Anaerobic conditions were produced using the GasPac system (Merck, Darmstadt, Germany).

Sensory analysis

Nine trained consumer panel analyzed and compared the treatments using “scoring methodology” according to Iran national standard for plain Doogh (Anon-b). Sensory parameters were flavor, oral texture and mouth feel, appearance (color, syneresis and texture homogeneity). Each of these parameters was scored in a five-point Hedonic scale including 0 = un-consumable, 1 = un-acceptable, 2 = acceptable, 3 = satisfactory, and 4 = excellent. The given numbers for each sensory parameter were multiplied to the relevant coefficients, namely, 6 for flavor, 3.5 for oral texture and mouth feel, and 2 for appearance.

Statistical analysis

Experiments were performed as completely randomized design in triplicate and the comparison of the means was done using one Way ANOVA Test significance level of 0.05 (p<0.05) using Minitab software (Co. Name, City, State).

Results and Discussions

Biochemical characteristics

In this research, we surveyed the effects of heat inactivated processing of yogurt traditional bacteria on biochemical, microbiological, and sensory characteristics of Doogh. Table 1 shows mean pH drop rate, mean acidity increase rate, mean redox potential increase rate, incubation time, peak time, and final titrable acidity in different treatments during fermentation and at the end of fermentation. Changes in pH drop, acidity increase and redox potential increase during 21 days of refrigerated storage are shown in Figure 1. According to Table 1, the greatest (p<0.05) mean pH drop rate was determined for BY-40-4.5 treatment (B. animilis spp. lactis PTCC 1631 was cultured with yogurt starter bacteria and incubated at 40°C until final pH 4.5 was reached). The lowest mean acidity increase rate was found in two treatments which Doogh was prepared by yogurt starter bacteria only.
During the refrigerated storage, in heat treated treatment (Y-45.5-B-L), the viability of bifidobacteria strains was significantly higher than non-heat treated treatment (Y-45.5-B-L). This phenomenon occurred as a result of inactivating yogurt starter bacteria. The highest survival of \textit{Bifidobacterium} strains was observed in B.\textit{animalis} \textit{spp. lactis} PTCC 1631 survived 8.78 log cfu.mL$^{-1}$, 8.76 log cfu.mL$^{-1}$ and 8.67 log cfu.mL$^{-1}$ on days 7, 14 and 21, respectively.

\textbf{Sensory evaluation}

Table 3 shows the results of sensory characteristics in the first day of storage. In the first day of storage, the highest acceptability for \textit{flavor}, \textit{appearance}, \textit{oral texture} and \textit{mouth feel} was observed in B.\textit{lytor} - 40 - 4.5 treatment. The primary reason for this highest acceptability was that the yogurt starter cultures made desirable flavor and texture by producing acetaldehyde, diacetyl, etc. Co-cultured treatments had better appearance since they had lower phase separation. Syneresis occurred in co-cultured treatments since they had not undergo heat treatment. Dave and Shah [15] reported that producing the fermented milks only by probiotic bacteria, as starter culture was impossible, because increases of the fermentation time led to the unfavorable taste products.

As represented in Table 3, Y-45.5-H-L treatment had the least acceptability in taste, texture, mouthfeel, and appearance tolerability. In this treatment, \textit{Bifidobacterium} strain was added after fermentation and heat shock process (85°C for 10 min). Heat deactivating process resulted in denaturation and accumulation of milk proteins which led to decrease in the consistency and viscosity of Doogh. Our findings were in contrast with Marshall [20] found that the heat shock process led to stabilization of product flavor in his study.

\textbf{Conclusions}

The results in this study revealed that heat inactivating process and step-wise of probiotic inoculation significantly ($p<0.05$) affected the

### Table 2: Viable counts (log cfu.mL$^{-1}$) of \textit{B. animalis} \textit{spp. lactis} in treatments at the end of fermentation and during refrigerated storage.

| Treatment* | Flavor | Oral texture and Mouth feel | Appearance | Total score |
|------------|--------|-----------------------------|------------|-------------|
| Y-45 - 4.5-B-L | 14.6$^a$ | 8.5$^a$ | 5.1$^{bc}$ | 28.2$^{bc}$ |
| Y-45 - 4.5-H-B-L | 9.3$^b$ | 5.4$^b$ | 3.5$^{b}$ | 18.2$^b$ |
| B.Y - 40 - 4.5 | 16.8$^a$ | 12.4$^a$ | 7.3$^a$ | 36.8$^a$ |

* Means shown with different small and capital letters represent significant differences ($p<0.05$) in the same columns (between the days of storage) and rows (among the treatments), respectively.

### Table 3: Sensory evaluation of probiotic Doogh in treatments at the end of fermentation (Day 0).

| Treatment** | Storage time (day) |
|------------|-------------------|
| Y-45 - 4.5-B-L | 7.63$^{ab}$ | 7.32$^{abc}$ | 7.11$^{bc}$ | 7.39$^{abc}$ |
| Y-45 - 4.5-H-B-L | 7.63$^{ab}$ | 7.5$^{ab}$ | 7.39$^{bc}$ | 7.30$^{ab}$ |
| B.Y - 40 - 4.5 | 8.69$^{a}$ | 8.78$^{a}$ | 8.76$^{a}$ | 8.67$^{a}$ |

* Yoghurt cultures (S. thermophilus, L. delbrueckii spp. bulgaricus), 45 / 40 = Incubation temperature, 4.5 = Final pH, H = Heat treatment, B.L = B.\textit{animalis} \textit{spp. lactis}.

** Initial population (Log cfu.mL$^{-1}$) = 7.63
viability bifidobacteria. The greatest (p<0.05) mean pH drop rate was determined for BY-40-4.5 treatment (B. animalis spp. lactis PTCC 1633 cultured with yogurt starter bacteria and incubated at 40°C until final pH 4.5 was reached), whereas in Y- 45 - 4.5 - B and Y- 45 - 4.3-H-B, treatments (B. animalis PTCC 1644 added after fermentation) showed the lowest mean pH drop rates. The highest viability of bifidobacteria in all treatments was observed in BY-40-4.5 treatment at the end of fermentation and during refrigerated storage; while Y- 45 - 4.5 - B, treatment which B. animalis PTCC 1644 added after fermentation and without heat shock processing demonstrated the lowest survival. The results showed that heat deactivation of traditional yogurt bacteria significantly increased the viability of bifidobacteria than the non-heat treated ones. The most acceptability for appearance, flavor, texture, and mouth feel was observed in BY-40-4.5, treatment and the least acceptability was observed in treatments having B. animalis spp. lactis PTCC 1631 species which were inoculated after fermentation and heated. As a result, the sequence of probiotic inoculation had significant effects on sensory characteristics of probiotic Doogh at the first day of refrigerated storage in this study.

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