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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*s sp. *bulgaricus*) were used in all treatments. *Bifidobacterium animlis* 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 for21 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_1Y-40-4.5 treatment (*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 therapeutical values. Although there is no world-wide agreement on viability of probiotics in food products, generally, the values of 10^6 and 10^7-10^8 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 10^6 cfu mL^{-1} and 10^5 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/stage-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 bacteriaon biochemical, microbiological, and sensory characteristics of Doogh.

Materials and Methods

Cultures

The *Bifidobacterium* strain used in the study was *Bifidobacterium animlis* spp. *lactis* PTCC (Persian Type Culture Collection-Iran) 1631 adapted to simulated 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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culture containing mixed culture of *Streptococcus thermophilus* and Lactobacillus *delbrueckii ssp. bulgaricus* (commercially known as Yf-3331) were supplied by Chr-Hansen (Horsholm, Denmark). The pouches were maintained according to manufacturer's instructions (at-18°C) until used.

Preparation of samples

Doogh milk with 6% of milk solid non-fat was prepared by reconstitution of skim milk powder and sterilized potable water. The mixture also contained 0.7% of sodium salt. The milk samples were heat treated at 95°C for 10 min. The samples were inoculated with B_1Y (*B. lactis* PTCC 1631 plus yogurt bacteria) or Y (yogurt bacteria only) cultures, and incubated at 40 or 45°C (± 1°C) until pHof 4.5 ± 0.02 was reached. Treatments inoculated with B_1Y were cooled down to 5°C, while the treatments fermented only with yogurt bacteria were cooled down to 15°C or were heat treated at 85°C for 10 min in order to inactivate the yogurt bacteria following cooling to 15°C. These treatments were then inoculated with *B. animalis* ssp. *lactis* PTCC 1631.

Chemical analysis

A pH value and redox potential of samples were measured at room temperature using a pH meter (MA235, Mettler, Toledo, Switzerland).

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 titrable 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 ($p<0.05$) usingMinitab 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 B_1Y-40-4.5 treatment (*B. animlis* 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.

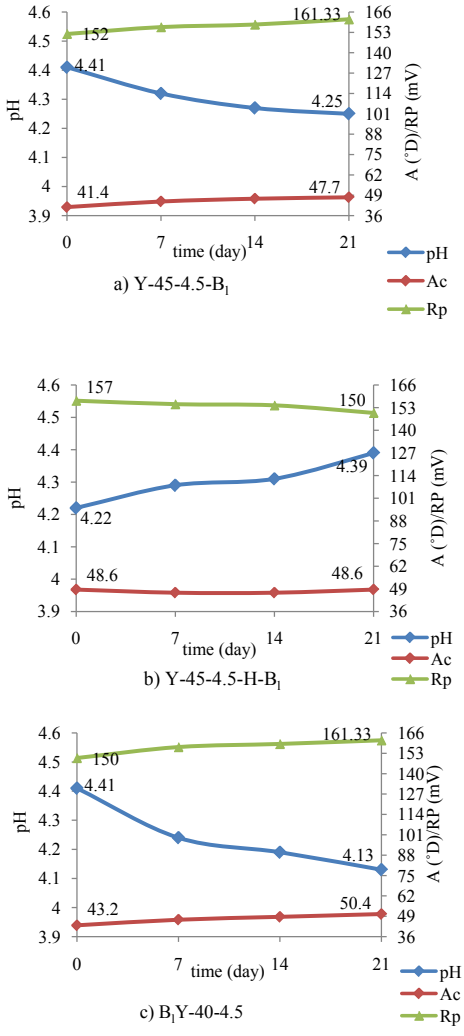

Figure 1: Changes in pH, titrable acidity, and redox potential in treatments during refrigerated storage.

Treatment**	Parameters					
	M-pH-DR***	M-A-IR	M-RP-IR	Peak time	Incubation time	Final acidity
	(pH/min)	(ºD/min)	(mV/min)	(min-min)	(min)	(ºD)
Y- 45 - 4.5 - B_L	0.0087^b	0.13^b	0.54^a	120-150	240^b	41.4^b
Y- 45 - 4.5 - H - B_L	0.0087^b	0.13^b	0.54^a	120-150	240^b	41.4^b
B_lY - 40 -4.5	0.0088^a	0.14^a	0.51^b	150-180	250^a	43.2^a

*Means in the same column with different letters are significantly different (p<0.05).
** Y= Yoghurt cultures (*S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*); B_l = *B. animalis spp. lactis*, B_b = *B. bifidum*; 40/45 = Incubation temperature; 4.5/= Final pH; H =Heat treatment after fermentation.
*** M-pH-DR = mean pH drop rate, M-A-IR = mean acidity increase rate, M-RP-IR= mean redox potential increase rate.

Table 1: Mean pH drop rate, acidity increase rate, redox potential increase rate, incubation time, final acidity, and peak time in treatments during fermentation or at the end of fermentation*.

During storage time, pH value increased in heat treated treatments, whereas redox potential decreased, and acidity remained constant. These changes would be probably due to heat shock and inactivating of yogurt starter culture. The reason we should be concerned with the low ability of *Bifidobacterium* strains, which were added after fermentation, produces acid. Ina similar investigation, Marshall and Tamime [16] reported that the probiotic bacteria could acidify in a slowly manner.

During the storage period, almost in majority of treatments pH drop, acidity increase, and redox potential increase except heat treated samples. The rise of acidity (post-acidification) in these treatments is mainly due to the growth of yogurt starter cultures during refrigerated storage, also their proteolytic activity continued in refrigerated temperature. Donkor et al. [9] reported that the increase in acidification of probiotic yogurt was mainly due to the growth of *L. delbrueckii* spp. *bulgaricus* Lb1466, *S. thermophilus* St1342 during refrigerated storage. Similarity Bonczar et al. [17] reported that the pH of yogurt decreased and the titrable acidity increased during storage.

Microbiological characteristics

Table 2 shows changes in the counts of *B. animlis* spp. *lactis* PTCC 1631 at the end of fermentation and during refrigerated storage. Results showed that in two samples which Doogh carried the *Bifidobacterium* strain, number of bifidobacteria was not significantly different (p>0.05), as they had not passed fermentation process. In contrast, the viability of *B. animlis* spp. *lactis* PTCC 1631 increased approximately 1 log cycle in co-cultured treatment in which bifidobacteria were inoculated with traditional yogurt bacteria. Several factors affected the growth of bifidobacteria in co-cultured treatments during the fermentation process. First, the presence of bifidobacteria throughout fermentation process increased the consistency of these bacteria to environmental condition such as pH, titrable acidity, and redox potential changes. Also, proteolytic and β-galactosidase activity of yogurt bacteria *(L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*) could stimulate the growth of bifidobacteria. Moreover, the incubation temperature (40°C) which is appropriated for these strains could be considered as another reason. The results in this study were consistent with Hansen [18] findings where bifidobacteria strains had low proteolytic activity that they would be able to grow better if they were co-cultured with proteolytic bacteria or addition of casein hydrolysates. Also, Samona and Robinson [19] observed that the presence of yogurt cultures declined growth of bifidobacteria. The highest viability of bifidobacteria was observed in B_lY-40-4.5 treatment, which the viable cells of *B. animlis* spp. *lactis* PTCC 1631 was 8.69 log cfumL^{-1} of Doogh, at the end of fermentation.

During the refrigerated storage, in heat treated treatment (Y-45-4.5- H- B_l), the viability of bifidobacteria strains was significantly higher than non-heat treated treatment (Y-45-4.5-B_l). This phenomenon occurred as a result of inactivating yogurt starter bacteria. The greatest survival of *Bifidobacterium* strains was observed in B_lY-40-4.5 treatment in which *B. animalis* spp. *lactis* PTCC 1631 survived 8.78 log cfumL^{-1}, 8.76 log cfumL^{-1},and 8.67 log cfumL^{-1} on days 7, 14 and 21, respectively.

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 flavor, appearance, oral texture and mouth feel was observed in B_lY-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-45-4.5-H-$B_l$treatment had the least acceptability in taste, texture, mouthfeel, and appearance tolerability. In thistreatment, *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.

Conclusions

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

Treatment*	Parameters			
	Flavor	Oral texture and Mouth feel	Appearance	Total score
Y- 45 - 4.5- B_L	14.6^b	8.5^b	5.1^{ab}	28.2^b
Y – 45-4.5-H - B_L	9.3^c	5.4^c	3.5^b	18.2^c
B_LY – 40 -4.5	18.6^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.
** Y= yoghurt cultures (*S. thermophilus*, *L. delbrueckii* ssp. *bulgaricus*), 45 / 40 = Incubation temperature, 4.5 = Final pH, H = Heat treatment, B_L = *B. animalis spp. lactis*.
*** Initial population (Log cfu/mL) = 7.63

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

Treatment**	Storage time (day)			
	0***	7	14	21
Y- 45 - 4.5 - B_L	7.63^{bA}	7.32^{cBC}	7.11^{cD}	7.39^{bB}
Y- 45 - 4.5 -H - B_L	7.63^{bA}	7.51^{bcB}	7.39^{bcC}	7.30^{bcD}
B_LY - 40 - 4.5	8.69^{aB}	8.78^{aA}	8.76^{aA}	8.67^{aC}

*Y= Yoghurt cultures (*S. thermophilus*, *L. delbrueckii* ssp. *bulgaricus*), 45 / 40 = Incubation temperature,
4.5= Final pH, H =Heat treatment, B_L = *B. lactis*

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

viability bifidobacteria. The greatest ($p<0.05$) mean pH drop rate was determined for B_lY-40-4.5 treatment (*B. animlis* 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_l and Y- 45 - 4.5-H-B_l 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 B_lY-40-4.5 treatment at the end of fermentation and during refrigerated storage; while Y- 45 - 4.5 - B_l 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 B_lY-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 after. 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.

References

1. Mitsuoka T (1990) Bifidobacteria and their role in human health. J Ind Microbiol 6: 263-267.
2. Vlkova E, Rada V, Trojanova I (2004) Enumeration, Isolation, and Identification of Bifidobacteria from Dairy Products. Acta agriculturae slovenica 84: 31-36.
3. Martinez-Villaluenga C, Gomez R (2007) Characterization of bifidobacteria as starter in fermented milk containing raffinose family of oligosaccharides from lupin as prebiotic. Int Dairy J 17: 116- 122.
4. Davidson RH, Duncan SE, Hackney CR, Eigel WN, Boling JW (2000) Probiotic culture survival and implications in fermented frozen yogurt characteristics. J Dairy Sci 83: 666-673.
5. Tamime AY, Saarela M, Sondergaard AK, Mistry VV, Shah NP (2005) Production and Maintenance of Viability Probiotic Micro-Organism in Dairy Products. In: Probiotic Dairy Products, A. Y. Tamime (Ed), pp. 39-97. Blackwell Publishing, UK.
6. Anon-a. Iran National Standard for plain yogurt: No. 695. Available on www.isiri.org [In Persian].
7. Anon-c. Iran national standard for probiotic Doogh; No. 11324. Available on www.isiri.org [In Persian].
8. Shah NP (2001) Functional foods from probiotics and prebiotics. Food Technology 55: 46-53.
9. Donkor ON, Henriksson A, Vasiljevic T, Shah NP (2006) Effect of acidification on the activity of probiotics in yogurt during cold storage. Int Dairy J 16: 1181-1189.
10. Mortazavian AM, Ehsani MR, Reinheimer J, Sohrabvandi S (2007) MRS-bile agar: its suitability for the enumeration of mixed probiotic cultures in cultured dairy products. Milchwissenschaft 62: 270-272.
11. Mortazavian AM, Khosrokhavar R, Rastgar H, Mortazaei GR (2009) Effects of Dry Matter Standardization Order on Biochemical and Microbiological Characteristics of Freshly Made Probiotic Doogh (Iranian Fermented Milk Drink). Ital J Food Sci 22: 98-104.
12. Shah NP (1997) Bifidobacteria: Characteristics and potential for application in fermented milk products. Milchwissenschaft 52: 16-20.
13. Anon-b. Iran national standard for plain Doogh; No. 2453. Available on www.isiri.org [In Persian].
14. Jamalifar H, Bigdeli B, Nowroozi J, Zolfaghari HS, Fazeli MR (2010) Selection for autochthonous bifidobacteial isolates adapted to simulated gastrointestinal fluid. Daru 18: 57-66.
15. Dave RI, Shah NP (1997) Viability of yogurt and probiotic bacteria in yogurts made from commercial starter cultures. Int Dairy J 7: 31-41.
16. Marshall VM, Tamime AY (1997) Starter cultures employed in the manufacture of biofermented milks. Int J Dairy Technol 50: 35-41.
17. Bonczar G, Wszolek M, Siuta A (2002) The effects of certain factors on the properties of yogurt made from ewe's milk. Food Chem 79: 85-91.
18. Hansen R (1985) Bifidobacteria have come to stay. North European Dairy Journal 3: 1-6.
19. Samona A, Robinson RK (1994) Effect of yogurt cultures on the survival of bifidobacteria in fermented milks. Int J Dairy Technol 47: 58-60.
20. Marshall VM (1992) Inoculated ecosystems in a milk environment. J Appl Bacteriol 73: 127S-135S.

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