Chemical characterization, sensory evaluation, microbial properties and effect on foodborne pathogens of synbiotic yogurt containing Persian shallot and probiotic bacteria

Abstract

Keywords: *Bifidobacterium bifidum, Lactobacillus acidophilus*, Natural Additives, Probiotic

۱. Introduction

Dairy products are one of the most important parts of human nutrition (Mahmoudi et al., 2015). Due to the amount of energy, proteins, fats and most importantly vitamins and minerals, dairy products have a very essential role in nourishing human communities (Granato et al., 2018). Among the many types of dairy products, yogurt consumption has a special place today. Yogurt is a fermented dairy product that has a starter as an initiator bacteria and also has probiotic properties (Mahmoudi et al., 2014).

In addition to the many benefits of dairy products, the role of probiotic bacteria in increasing the nutritional benefits of these products is very important today (Mehdizadeh et al., 2019). Probiotics are living microorganisms that, when used in appropriate amounts through ingestion or topical application, cause one or more health effects on the host body (Mahmoudi et al., 2015). Bifidobacteria and Lactobacillus are the most common group of bacteria due to their strong anti-pathogenic effect, which are added to food for their probiotic properties (Mehdizadeh et al., 2019).

One of the most important factors in the consumption of dairy products, including probiotic yogurts, is the survival rate of beneficial bacteria in the final product (Ertem & Cakmakci, 2018). There should be at least 10^6 cfu/gr of alive probiotic bacteria in final product (Fazilah, et al., 2018). Today, much research is being done on increasing the properties and survival of probiotic bacteria in dairy products. The use of essential oils and plant extracts as natural substances as well as enhancers of bacterial viability and effect on foodborne pathogens in the food product has a special place (Aliza Sigdel et al., 2018; Turgut & Cakmakci, 2018; Mahmoudi et al., 2014).

Persian Shallot (*Allium hirtifolium* Boiss) is one of the most widely used medicinal plants in Iran, whose main habitat is the Zagros Mountains in western and central Iran. This plant belongs to the genus *Allium* and the family *Alliacea*. Use of *Allium* species such as garlic and onions for treatment of various diseases has been reported. Antibacterial, antifungal, anticancer and antihelminthics effects have been proven to many members of this genus. Persian shallot has widely been used as a traditional herb and spice. It is added to a variety of foods such as salads, pickles, yogurt and different sauces (Ghahreman, 1984; Harris et al., 2001; Hirsch et al., 2000).

According to the studies and also the role of probiotic and synbiotic yogurts in communities, in this study the synbiotic properties of persian shallot in probiotic yogurts and its effect on sensory and microbial characteristics were investigated. Also in this study, the effect of persian shallot on foodborne pathogenic bacteria in probiotic yogurts was investigated to determine the extent of their inhibitory effects in the food model.

۲. Materials and Methods

۲،۱. Preparation of bacteria

Lactobacillus acidophilus (LA_5) (PTCC1608) and *Bifidobacterium bifidum* (BB_12) (PTCC1644) were prepared as lyophilized vials from the Bacterial and Fungal Collection Center of the Iranian Scientific and Industrial Research Organization. MRS broth (Merck, Germany) inoculated with probiotic bacteria suspension was incubated separately at 37 °C and 5% carbon dioxide anaerobically for 24 hr. Then, 200 µl of the original culture was transferred to test tubes containing 10 ml of sterile MRS broth and incubated anaerobically at 37 °C for 24 h. The cultured tubes were then centrifuged at 1500 rpm for 15 minutes. The bottom of the tubes was washed twice with 0.1% sterile peptone water and centrifuged (at 1500 rpm for 2 minutes). Finally, 5 ml of 0.1% sterile peptone water was added to the tubes and vortexed. Finally, the prepared bacterial suspension contained about 10^8-10^9 cfu/ml of probiotic bacteria. Then 1 ml of the prepared bacterial suspension was added to 1 L of pasteurized milk at 35 °C and finally used to prepare yogurt (Mehdizadeh et al., 2019).

Listeria monocytogenes (ATCC19115) and *Escherichia coli* O157:H7 (ATCC1533) were collected from the collection of the Food Hygiene and Quality Control Department of the Faculty of Veterinary Medicine, Urmia Universitym, Urmia, Iran. Then dilution of 10^3 cfu/ml of pathogenic bacteria was added to the milk samples and yogurt was prepared (Al-Nabulsi et a., 2016).

۲,۲. Preparation of persian shallots

In this study, persian shallot bulbs were first purchased from the bazaar of Urmia and approved by the Department of Agriculture, Faculty of Agriculture, Urmia University.

۲,۳. Yogurt production

The milk used was first analyzed. To prepare yogurt, fresh and whole cow's milk was pasteurized and before starting the various stages of yogurting, the milk temperature was raised to 35 35 °C and 500 ml of milk was added to each sterile container for making yogurt and probiotic bacteria were added. Then the milk temperature was raised to 41 °C and yogurt starter including Lactobacillus delbrueckii ssp. Bulgaricus and Streptococcus thermophilus (Ch.Hansen, YC-X11, Horsholm, Denmark) was added in a ratio of 1/1. Finally, persian shallots were added with a concentration of 14 g/L. The samples were then incubated for 24 hr and then refrigerated and stored for 21 days for the desired tests. The experiments were performed one week apart (days 1, 7, 14 and 21) (Mehdizadeh et al., 2019).

A total of 12 yogurt treatments were used for this study, including: probiotic-free and shallot-free yogurt (C), Bifidobacterium probiotic yogurt (PB), Lactobacillus probiotic yogurt (PL), Bifidobacterium and Lactobacillus probiotic yogurt (PB-L), Bifidobacterium and shallot probiotic yogurt (PB-M), Lactobacillus and shallot probiotic yogurt (PL-M), Bifidobacterium and Lactobacillus with shallot probiotic yogurt (PBL-M), Yogurt with shallots (CM), Bifidobacterium and Lactobacillus probiotic yogurt containing shallot and *E. coli* (PBL-M: *E. coli*), Bifidobacterium and Lactobacillus probiotic yogurt containing shallot and *Listeria monocytogenes*

(PBL-M: *L. monocytogens*), Bifidobacterium and Lactobacillus probiotic yogurt containing *L. monocytogens* and *E. coli* without shallot (PBL: *L. monocytogens* and *E. coli*).

۲،٤. Pathogenic bacteria addition

100 µl of both bacteria (*E. coli* and *Listeria monocytogenes*) cultured in the BHI media were removed from the previous day and transferred to the new BHI media and incubated at 37 °C for 24 hr. Using a spectrophotometer with a wavelength of 600 nm, the number of bacteria was adjusted to 10^8 CFU/ml. After this step, dilution was performed in five tubes and 10^3 CFU/ml of pathogenic bacteria was added to milk and then yogurt was prepared and the pathogenic bacteria were cultured on specific culture media for 21 days and counted (AL-Nabulsi et al., 2016).

۲،٥. Physicochemical characteristics

The amount of fat was evaluated by the Mojonnier ether extraction procedure (Association of Official Analytical Chemist (AOAC, 1995). The obtained fat is dried to a steady weight and determined as a percent of fat per weight. Solids not fat (SNF) was evaluated by determining total solids and fat measurement (AOAC, 1995). The percentage of fat was deducted from the total solids and used to calculate the SNF. The protein level was evaluated by the micro Kjeldahl procedure based on AOAC (2000).

۲،٦. Measurement of acidity and pH

To determine the acidity, exactly 9 g of yogurt was poured into a bekker, the equivalent of distilled water without carbon dioxide was added, and 0.5 ml of 5% alcohol phenolphthalein reagent was added to it, and the desired profit solution was added drop by drop to the first drop when a pale pink color appears. Then, the amount (ml) of profit was multiplied by 10 to obtain acidity degrees (AOAC, 1997).

To determine the pH of the yogurt, after calibrating the pH meter, the pH meter was placed directly inside the yogurt samples and the pH was read.

۲،۷. Measurement of syneresis

Centrifuges are used to measure syneresis as one of the most important physical factors in yogurt production. 15 g of the each samples was carefully weighed in each of the centrifuge falcons, and after closing the lid, they were centrifuged at 3500 rpm for 30 minutes at 4 °C. The drains are then drained out of the supernatants and the falcons weighed again.

۲،۸. Microbial analysis

MRS culture media containing ciprofloxacin and clindamycin antibiotics as well as bile was used to count *Lactobacillus acidophilus*. Then, the culture was done linearly and pour-plate, and then the plates were incubated anaerobically at 37 °C for 72 hr. For bacterial count of *Bifidobacterium bifidum*, MRS agar modified with lithium chloride (0.3% w/v), neomycin sulphate (0.1% w/v), nalidixic acid (0.0015% w/v) and L-cysteine (0.05% w/v) was used. After pour-plate culturing, the plates are incubated under anaerobic conditions at 37 °C for 72 hr using a type A anaerocult (Type A) gas pack system.

VRBA (Violet Red Bile Agar) culture media was used to count coliform bacteria (according to the instructions of the Standard Organization of Iran). Yeast Extract Dextrose Chloramphenicol Agar medium at 25 °C was used to evaluate and count mold and yeast (according to the instructions of the Iranian Standard Organization).

PALCAM agar media was used to evaluate and count of *Listeria monocytogenes* (akhondzadeh Basti et al., 2007). The counts of *E. coli* were enumerated on MacConkey agar-sorbitol and incubated at 358C for 18–24 hr (Mehdizadeh et al., 2018).

۲,۹. Sensory evaluation

Sensory analysis was done based on a 5-hedonic (0-4) scale test according to method described by Mehdizadeh et al (2018) with some modifications (Mehdizadeh et al., 2018). In this method, 0 is the lowest evaluation score and 4 is the highest. In this sensory analysis; taste, texture, appearance and overall evaluation on the last day of storage were examined.

۲,۱۰. Statistic analysis

All experiments were done triplicates and two samples from each treatment were tested. Analysis of variance (ANOVA) was performed using statistical software (SPSS Statistical Software Inc., Chicago, III). Duncan test to compare means and significant differences between different treatments and combinations with confidence limits ($P < 0.05$) was used.

Results

The amounts of fat, SNF and protein measured in the samples of probiotic yogurt are shown in Table 1. Based on the results, the amount of SNF and protein in the sample of yogurt with shallots was significantly higher than samples without shallots ($p<0.05$).

Table 1. Amounts of Fat, SNF and Protein

	Treatments							
	C	CM	PB	PL	PB-L	PB-M	PL-M	PBL-M
Fat (%)	2.40±0.18[a]	2.45±0.06[a]	2.38±0.11[a]	2.42±0.08[a]	2.40±0.19[a]	2.47±0.21[a]	2.46±0.19[a]	2.51±0.16[a]
Protein (%)	3.01±0.03a	3.21±0.07b	3.03±0.06a	3.04±0.12a	3.06±0.08a	3.29±0.05b	3.32±0.06b	3.31±0.14b
SNF (%)	8.13±0.09a	9.20±0.15b	8.19±0.11a	8.20±0.13a	8.28±0.09a	9.16±0.06b	9.18±0.13b	3.01±0.03a

Non-identical letters in each column for each parameter indicate a significant difference ($P<0.05$).

The pH, titratable acidity and syneresis of probiotic yogurt samples were measured and the results are shown in Table 2. According to the results, the addition of shallots to the samples of prepared yogurts has significantly increased the pH. Also, the pH level decreased during storage (21 days). The rate of watering in samples containing shallots was significantly higher ($p<0.05$).

Table 2. pH, titratable acidity and syneresis

Parameter	Treatment	Days			
		1	7	14	21
pH	C	٤.٤٠±٠.٠٩^{ab}	٣.٤١±٠.٠٦^{ab}	٣.٤٠±٠.٠٤^{ab}	٣.٤٦±٠.٠٣^{ab}
	CM	٤.٦٧±٠.١٤^{ab}			
	PB				
	PL				
	PB-L				
	PB-M				
	PL-M				
	PBL-M				
Acidity	C				
	CM				
	PB				
	PL				
	PB-L				
	PB-M				
	PL-M				
	PBL-M				
Syneresis	C				
	CM				
	PB				
	PL				
	PB-L				
	PB-M				
	PL-M				
	PBL-M				

Discussion

Conclusion

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