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Original article

Effect of temperature and salt concentration on microbial changes during Tarkhineh fermentation

F. Tabatabaei-Yazdi*, B. Alizadeh-Behbahani, A. Mortazavi
Department of Food science and technology, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran.

*Corresponding author; Department of Food science and technology, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran.

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ABSTRACT

Tarkhineh is a traditional Iranian fermented cereal food produced both commercially and in homes. It is mainly used in the form of a thick and creamy soup consumed at lunch or dinner and is easily digested. In this study, Tarkhineh were fermented at three different temperatures (10, 20, 37) °C and two different salt content of (0.5, 1) %. The purpose of this study was to determine the amount of micro flora of Tarkhineh. Another purpose is studying changes in microbiological composition of Tarkhineh during fermentation. The results show that the naturally occurring lactic acid bacteria (LAB) load was found to vary between 1.97×10^5 cfu/gr to 4×10^5 cfu/gr at 10 °C. The yeast and mold counts decrease from 1.04×10^5 cfu/gr to 0 cfu/gr at 10 °C. Lactic acid bacteria load was found to vary between 1.97×10^5 cfu/gr to 4.3×10^5 cfu/gr at 20 °C. The yeast and mold counts decrease from 1.04×10^5 cfu/gr to 3×10^4 cfu/gr at 20 °C and salt content 0.5%. Lactic acid bacteria load was found to vary between 1.97×10^5 cfu/gr to 1.1×10^6 cfu/gr at 37 °C. The yeast and mold counts decrease from 1.04×10^5 cfu/gr to 3×10^4 cfu/gr at 37 °C and salt content 0.5%. The largest increase in the numbers of LAB was noted during the first 24 h of fermentation and further incubation led to decrease. Maximum total acids produced in Tarkhineh at 37°C. At 37°C, the optimum ripening period was 1 day. The results show that Tarkhineh fermentation is complex and is due mainly to certain LAB and yeasts naturally present in raw materials. Among the many factors affecting Tarkhineh fermentation, salt content and temperature are the most important. Also the temperature and salt

content is effective on microbial changes during Tarkhineh fermentation.

1. Introduction

Tarkhineh is a traditional Iranian fermented product produced from a mixture of doogh and wheat grout. It is produced with flour, doogh, salt, Turnip; Leaven (mint, red pepper). In winter time, iranian people prefer traditionally prepared foods more in their diets. Furthermore, the dried Tarkhineh slices are consumed in different ways such as nugget or snack in local regions of Kermanshah. Baker's yeast is generally added to the Tarkhineh dough, leading to the lactic acid and alcohol fermentation (Bilgic and Elgun., 2005; Bozkurt and Gurbuz., 2008). (Daglıoglu., 2000) Cereal flours, doogh and a variety of vegetables are the primary ingredients and therefore a good source of B vitamins, minerals, organic acids and free amino acids which make it healthy for children, the elderly and medical patients.

Tarkhineh fermentation occurs mainly by the microorganisms naturally present in the raw materials that contain numerous micro flora including lactic acid bacteria (LAB). Various LABs may initiate fermentation, but hetero fermentative type LAB increased rapidly with organic acids accumulation and homo fermentative type LAB increased thereafter. LAB from doogh also aid in absorption of nutrients, which would otherwise, be indigestible or poorly digestible. LABs are a group of gram-positive bacteria linked to a constellation of morphological, metabolic and physiological characteristics. They are included in the group of non-spore forming, non-respiring cocci or rods, catalase-negative, devoid of cytochromes; non-aerobic but aero-tolerant fastidious, acid tolerant and strictly fermentative with lactic acid as the major end products during the fermentation of carbohydrates. LABs are widely distributed in nature. They have been isolated from grains, green plants, dairy and meat products, fermenting vegetables (Salminen etal,. 2011) and traditional Iranian foods such as Tarkhineh. (Tabatabaei Yazdi etal., 2012) report that After series of purification on MRS agar, 400 isolates were found to be Gram-positive and catalase negative from Tarkhineh. The isolates were identified as *Lactobacillus nagelii* (67%), *Lactobacillus bifermentans* (21.3%), *Leuconostoc cermoris* (6%), *Lactobacillus fructosus* (1.45%), *Lactobacillus fermentum* (1%), *Lactobacillus intestinalis* (0.9%), *Lactobacillus agilis* (0.9%), *Lactobacillus acidipiscis* (0.9%), and approximately %1 of isolated samples remained unknown.

Numerous chemical, physical, and biological factors may contribute directly to the growth of microorganisms and the extent of fermentation. Tarkhineh-like food consumed in other countries such as Iraq, Turkey and Egypt.

Salt is one of the key factors for controlling the Tarkhineh fermentation and preservation of good quality of Tarkhineh at various temperatures. However, salt concentrations of those Tarkhineh are all different depending on the makers. The most important factor affecting Tarkhineh fermentation is temperature, since the Tarkhineh fermentation occurs mainly by the microorganisms naturally present in raw materials.

Ibanoglu et al., (1995) and Bilgiçli (2009) report that Tarkhineh is a good source of minerals, organic acids and free amino acids which make it healthy for children, the elderly and medical patients. In addition, it is a good source of vitamins such as thiamine, riboflavin and vitamin B12 Ascorbic acid, niacin, pantothenic and folic acid are also present (Ekinci, 2005, Ekinci and Kadakal 2005).

The purpose of this study was to determine the amount of micro flora of Tarkhineh. Another purpose is studying changes in microbiological composition of Tarkhineh during fermentation.

2. Materials and methods

In this study, the ingredients (wheat, doogh, yeast, turnip, salt and leaven) used in Tarkhineh preparation were purchased from local markets in Mashhad, Iran. The protein content of wheat, based on total weight, was 12.2%. The doogh used was (pH 3.7) made from cow's milk and had a fat content of 3.0% (wet basis). Yeast was baker's yeast in active dry form.

2.1. Production of Tarkhineh

Production method of Tarkhineh with ingredients ratio is presented in Figure (1) and table (1). Turnip was grated and blended with doogh, salt and leaven. Then wheat grout added to this mixture. They were kneaded in a bowl for formed Tarkhineh dough. At the end of kneading, it was separated in to tree parts. first part (a) of dough was fermented at 10^0C, the second part (b) was fermented at 20^0C and the third part was fermented at 37^0C for 3 days in an incubator. It was kneaded daily to facilitate fermentation. Samples were taken at 0th, 1st, 2nd, and 3rd days of fermentation to investigate the chemical and microbiological changes. (Erbaş etal., 2005) Before fermentation, all samples were filled in small jars and closed. Samples were analyzed at 0th, 1st, 2nd, 3rd days of the storage period.

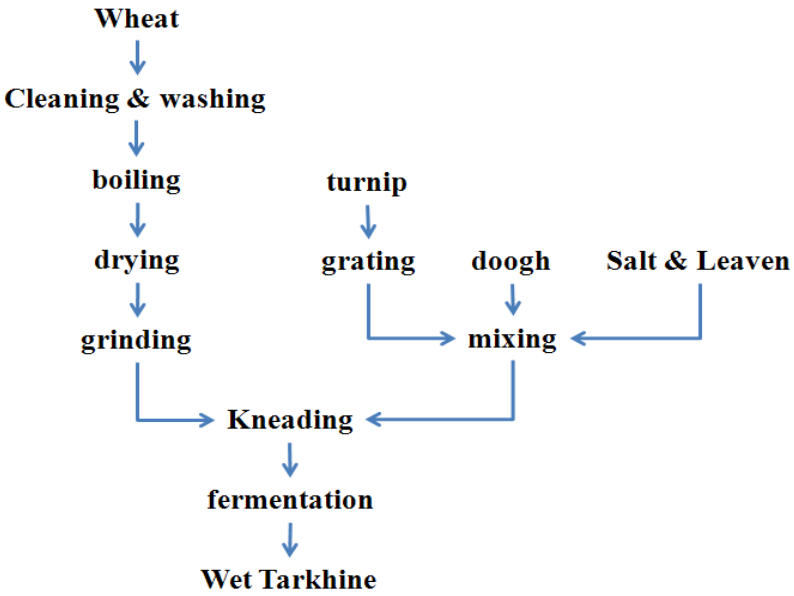

Fig. 1. Flow diagram for traditional Tarkhineh production.

Table 1
Ingredients ratio of Tarkhineh.

Ingredient	%	
	Type 1	Type 2
wheat grout	23.7	23.66
Doogh	66	65.7
Turnip	8	7.89
Leaven	0.5	0.47
Salt	1.8	2.28

2.2. Samples

In the present study, 6 samples of laboratory fermented Tarkhineh were prepared. 25g from samples were homogenized with 225mL sterile sodium citrate solution 2% (w/v), in a Stomacher 400 (Seward Medical, London, UK). Serial decimal dilutions (10^{-2} to 10^{-5}) were made in 0.1% (w/v) peptone solution.(Abdi etal.,2006) Decimal dilution of these samples were mixed with MRS medium (AEB, France) and incubated at 37ºC for 48-72 h under anaerobic conditions.(Lengkey etal., 2009) The numbers of LAB were measured by the plate count on MRS agar (Difco Laboratories, Detroit, USA) mold and yeast were counted on Potato Dextrose agar (Nissui) incubated for 72 h at 30°C. Each LAB colony was purified twice by streaking on MRS agar. Colonies were counted as viable numbers of Microorganisms (colony forming unit (CFU) g^{-1} of Tarkhineh) (Duan etal., 2008)0.1 mL of the diluents were streaked on Nutrient agar for total bacteria counts.

2.3. Determination of pH Values and acidity of the Test Samples

The pH values of each sampels were determined at 25C using a pH meter (WTW-Inolab Level 3 Terminal, Weilheim, Germany) (Yilmaz et. al., 2010). Acid content was determined by titrating with 0.1N NaOH with the indicator of phenoluphetalin until it's Color changes to pink.

2.4. Statistical analysis

All the assays were carried out in triplicates. The experimental results were expressed as mean ± standard deviation. The data were analysed using one way analysis of variance (ANOVA) using SPSS version 17.

3. Results and discussion

The number and species of major microorganisms in Tarkhineh fermentation vary widely, and are influenced by raw materials and other ingredients. However, the growth, activity, and role of the microorganisms participating in the fermentation are influenced more by environmental conditions, especially by temperature and salt concentration. Generally, total LAB was highly distributed throughout the whole fermentation period. However, they were slightly declined as the acidity increased. The microbial changes in during fermentation at 10 °C and salt content 0.5 % of Tarkhineh are shown in (Figure2 and Table 2). The results shows that the naturally occurring lactic acid bacteria load was found to vary between 1.97×10^5 cfu/gr to 4×10^5 cfu/gr at 10 °C and salt content 0.5%. The yeast and mold counts decrease from 1.04×10^5 cfu/gr to 0 cfu/gr at 10 °C and salt content 0.5%. The microbial changes in during fermentation at 10 °C and salt content 1 % of Tarkhineh are shown in (Figure3 and Table 2). Lactic acid bacteria load was found to vary between 1.97×10^5 cfu/gr to 1.05×10^6 cfu/gr at 10 °C and salt content 1%. The yeast and mold counts increased from 1.04×10^5 cfu/gr to 6.6×10^5 cfu/gr at 10 °C and salt content 1%. The microbial changes in during fermentation at 20 °C and salt content 0.5 % of Tarkhineh are shown in (Figure4 and Table 3). Lactic acid bacteria load was found to vary between 1.97×10^5 cfu/gr to 4.3×10^5 cfu/gr at 20 °C and salt content 0.5%. The yeast and mold counts decrease from 1.04×10^5 cfu/gr to 3×10^4 cfu/gr at 20 °C and salt content 0.5%. The microbial changes in during fermentation at 20 °C and salt content 1 % of Tarkhineh are shown in (Figure5 and Table 3). Lactic acid bacteria load was found to vary between 1.97×10^5 cfu/gr to 1.05×10^5 cfu/gr at 20 °C and salt content 1%. The yeast and mold counts increased from 1.04×10^5 cfu/gr to 5.1×10^5 cfu/gr at 20 °C and salt content 1%. The microbial changes in during fermentation at 37 °C and salt content 0.5 % of Tarkhineh are shown in (Figure 6 and Table 4). Lactic acid bacteria load was found to vary between 1.97×10^5 cfu/gr to 1.1×10^6 cfu/gr at 37 °C and salt content 0.5%. The yeast and mold counts decrease from 1.04×10^5 cfu/gr to 3×10^4 cfu/gr at 37 °C and salt content 0.5%. The microbial changes in during fermentation at 37 °C and salt content 1 % of Tarkhineh are shown in (Figure 7 and Table 4). Lactic acid bacteria load was found to vary between 1.97×10^5 cfu/gr to 0 cfu/gr at 37 °C and salt content 1%. The yeast and mold counts decrease from 1.04×10^5 cfu/gr to 0 cfu/gr at 37 °C and salt content 1%. The pH changes of Tarkhineh are shown in (Figure 8, 9 and 10) and (Table 5, 6 and 7). In the present investigation, the pH of tharkhineh ranged between 3.93 and 4.48. The total acid changes of Tarkhineh are shown in (Table 5, 6 and 7).

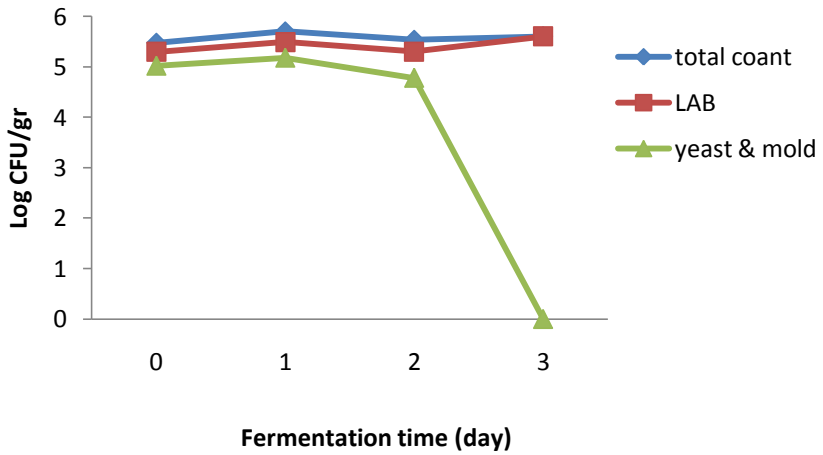

Fig. 2. Changes in microorganism during fermentation (10 °C and low salt).

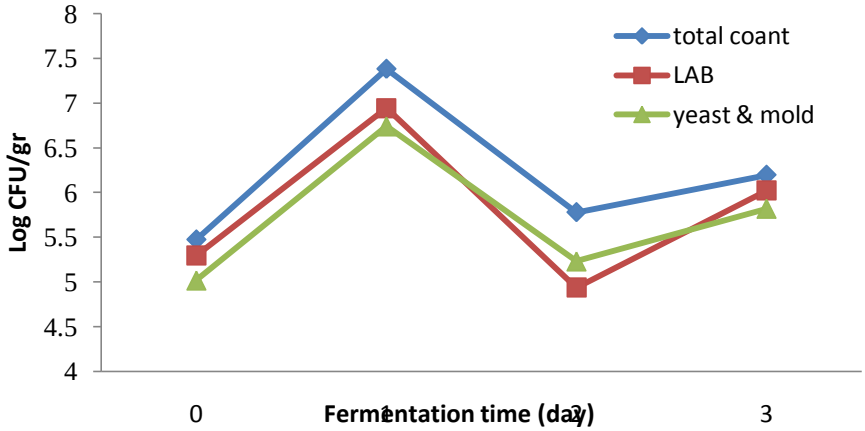

Fig. 3. Changes in microorganism during fermentation (10 °C and high salt).

Table 2

Change in microorganism during fermentation at 10 °C.

Yeast & mold		LAB		Total count		day
High salt	Low salt	High salt	Low salt	High salt	Low salt	
1.04×10^5	1.04×10^5	1.97×10^5	1.97×10^5	2.97×10^5	2.97×10^5	0
5.5×10^6	1.5×10^5	2.88×10^7	3.1×10^5	7.9×10^7	5×10^5	1
1.7×10^5	6×10^4	8.6×10^4	2×10^5	6×10^5	3.4×10^5	2
6.6×10^5	0	1.05×10^6	4×10^5	1.57×10^6	4×10^5	3

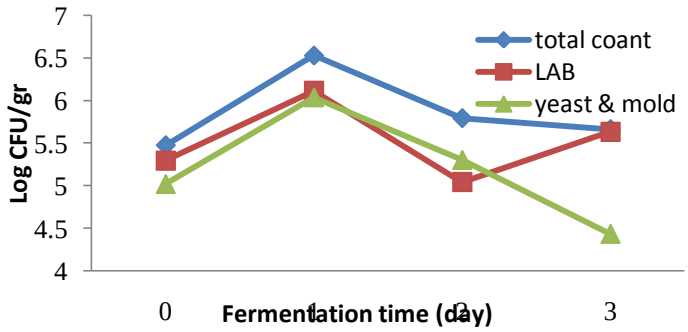

Fig. 4. Changes in microorganism during fermentation (20 °C and low salt).

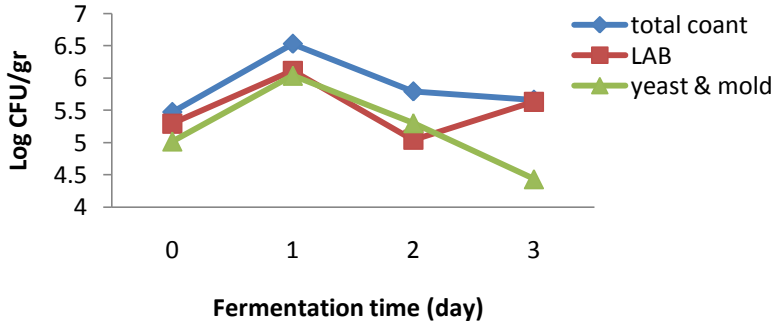

Fig. 5. Changes in microorganism during fermentation (20 °C and high salt).

Table 3
Change in microorganism during fermentation at 20°C.

Yeast & mold		LAB		Total count		day
High salt	Low salt	High salt	Low salt	High salt	Low salt	
1.04×10^5	1.04×10^5	1.97×10^5	1.97×10^5	2.97×10^5	2.97×10^5	0
$4^{\times}10^5$	1.09×10^6	$8^{\times}10^4$	3.4×10^6	2.5×10^6	1.3×10^6	1
3×10^5	2×10^5	4×10^4	1.1×10^5	3.5×10^5	6.2×10^5	2
5.1×10^5	3×10^4	1.05×10^5	4.3×10^5	1.7×10^5	4.6×10^5	3

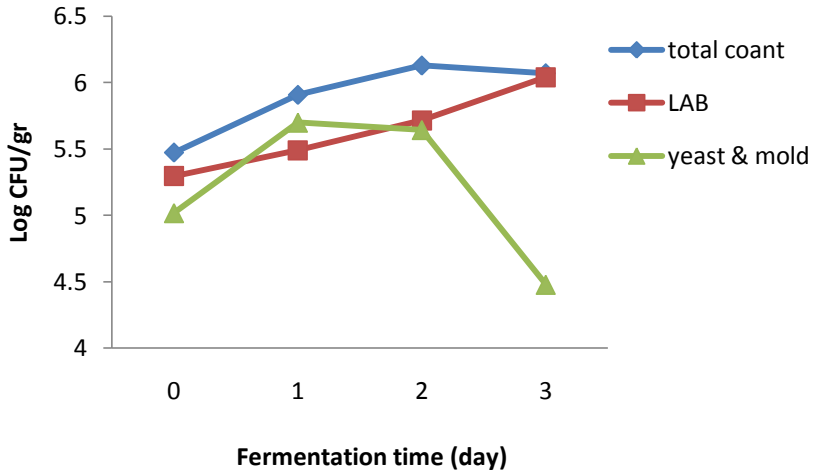

Fig. 6. Changes in microorganism during fermentation (37°C and low salt).

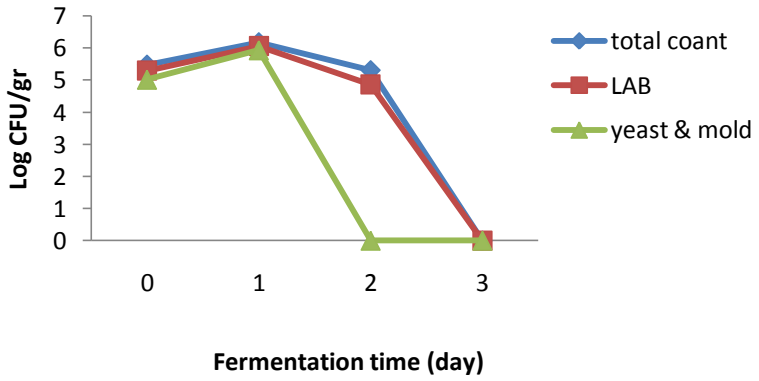

Fig. 7. Changes in microorganism during fermentation (37°C and high salt).

Table 4
Change in microorganism during fermentation at 37°C.

Yeast & mold		LAB		Total count		day
High salt	Low salt	High salt	Low salt	High salt	Low salt	
1.04×10^5	1.04×10^5	1.97×10^5	1.97×10^5	2.97×10^5	2.97×10^5	0
8.6×10^5	5×10^5	$1.13^{\times}10^6$	3.1×10^5	1.48×10^6	8.1×10^5	1
0	4.4×10^5	7.2×10^4	5.2×10^5	2×10^5	1.35×10^6	2
0	3×10^4	0	1.1×10^6	0	1.18×10^6	3

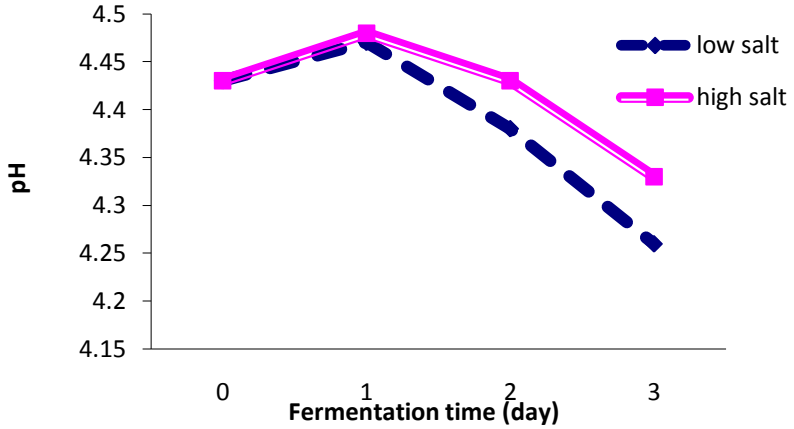

Fig. 7. Changes pH during Tarkhineh fermentation at temperature 10 °C.

Table 5
Change of total acid and PH during Tarkhineh fermentation at 10 °C.

Total acid		pH		day
High salt	Low salt	High salt	Low salt	
0.1035	0.1035	4.43	4.43	0
0.081	0.0765	4.48	4.47	1
0.0855	0.0855	4.43	4.38	2
0.072	0.066	4.33	4.26	3

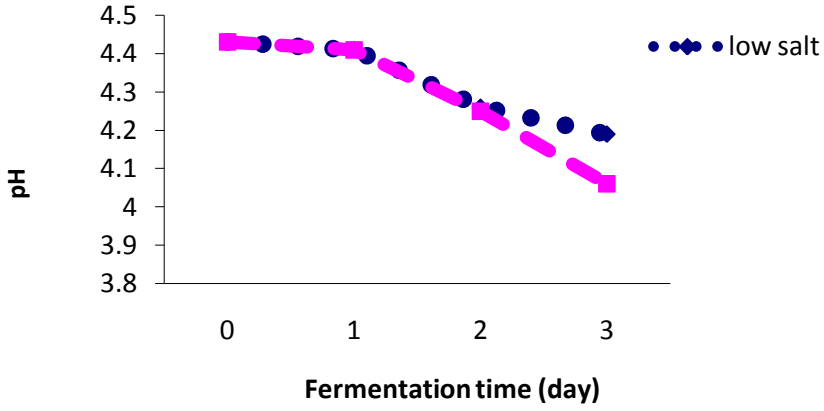

Fig. 8. Changes pH during Tarkhineh fermentation at temperature 20 °C.

Table 6
Change of total acid and PH during Tarkhineh fermentation at 20 °C.

Total acid		pH		day
High salt	Low salt	High salt	Low salt	
0.1035	0.1035	4.43	4.43	0
0.09	0.0945	4.41	4.41	1
0.072	0.099	4.25	4.26	2
0.09	0.099	4.06	4.19	3

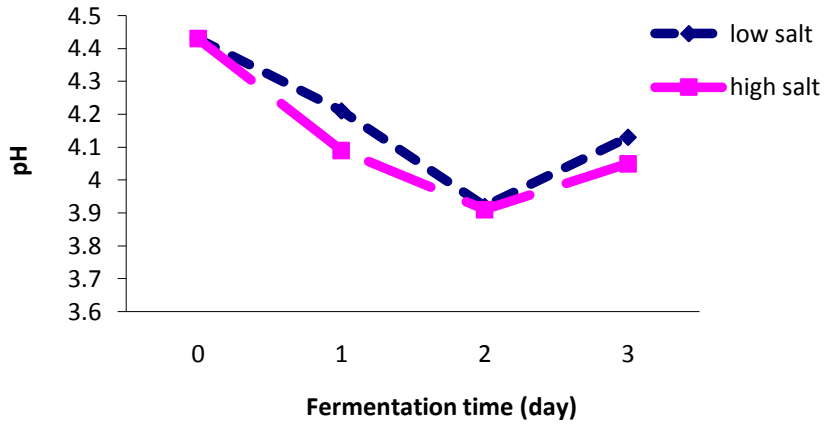

Fig. 9. Changes pH during Tarkhineh fermentation at temperature at 37°C

Table 7
Change of total acid and PH during Tarkhineh fermentation at 37°C.

Total acid		pH		day
High salt	Low salt	High salt	Low salt	
0.1035	0.1035	4.43	4.43	0
0.0945	0.0855	4.09	4.21	1
0.117	0.135	3091	3092	2
0.117	0.0765	4.05	4.13	3

Salt is one of the key factors for controlling the Tarkhineh fermentation and preservation of good quality of Tarkhineh at various temperatures. However, salt concentrations of those Tarkhineh are all different depending on the makers. The most important factor affecting Tarkhineh fermentation is temperature, since the Tarkhineh fermentation occurs mainly by the microorganisms naturally present in raw materials. During the preparation and fermentation of Tarkhineh using of lactic acid bacteria decrease in pH to 3.93 and 4.48 within 24 to 48 h. 37°C and salt content 1% was mostly acceptable fermentation temperature and salt content industrially due to its lowest production cycle industrially to achieve the desirable pH of the Tarkhineh. Maximum total acids produced in Tarkhineh at 37°**C**. At 37°**C**, the optimum-ripening period was 1 day and the edible period was also 1-2 days. The reason of increasing in pH levels on the third day related to death of mold and yeast. So the yeast and molds have been removed from media and the medium being unfavorable for the growth of lactic acid bacteria, it causes their loss and increasing pH.

4. Conclusion

The results this study shows that the temperature and salt content is effective on microbial changes during Tarkhineh fermentation.

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