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Chemical and microbial properties of two Iranian traditional fermented cereal-dairy based foods: Kashk-e Zard and Tarkhineh

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Abstract

This study was designed to investigate the chemical and microbial composition of two hitherto unstudied Iranian cereal-dairy based fermented foods. 40 samples of Kashk-e Zard and 40 samples of Tarkhineh were collected during 2012. Chemical (moisture and dry matter content, crude protein, crude fat, carbohydrate, salt, ash, pH and acidity, calcium, and phosphorus) and microbiological composition (*S. aureus*, Molds and yeast, *B. cereus*, *Clostridium perfringens*, Coliform and *E. coli*) of these foods were assessed using standard methods. Kashk-e zard and Tarkhineh are healthy and nutritious which make them proper for children, the elderly and medical patients.

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Introduction

Fermentation is one of the oldest and most economical methods of producing and preserving food (Chavan and Kadam, 1989; Blandino et al., 2003). Fermented foods are considered as one of the major dietary constituents in numerous developing countries because of their keeping quality under ambient conditions - thereby contributing to food security - and because they affect nutritional value through enhancing digestibility and soluble fractions, improving food safety and quality of the proteins (increase in bioavailability of lysine), increasing water-soluble vitamins and declining antinutritional factors (Holzapfel, 2002; Rolle and Satin, 2002; Paredes-lópez and Harry, 1988; Guermani et al., 1992; Sahlin, 1999). Regarding health effects of fermented foods, probiotic effect, flatulence reducing effect, anticholesterolemic effect, effect on transit time, bowel function and glycemic index, anticancerogenic effect, and Immunoactive effects can be mentioned (Sahlin, 1999; Strandhagen et al., 1994; Hosono and Hisamatsu, 1995; Marteau and Rambaud, 1993). Fermentation also leads to a general improvement in the shelf life, texture, taste and aroma of the final product (Blandino et al., 2003). Furthermore, antimicrobial effects of the lactic acid bacteria from cereal-based fermented foods have been reported (Marshall and Mejia, 2001).

Fermented foods are produced world-wide using various manufacturing techniques, raw materials and microorganisms (Blandino *et al.*, 2003). In Iran, Tarkhineh and Kashk-e Zard are of reputed cereal-dairy based fermented foods. These foods are still mainly prepared at the household level and marketed through informal routes. They thus remain beyond any official control for their compliance to national standards, and they may put consumer health at risk occasionally.

Tarkhineh, Tarkhowana or Doowina in Kurdish, is a unique product made traditionally in the west of Iran (Kurdistan, Kermanshah and Hamedan provinces). Wheat meal (*bulgur* or cracked and bran-free parboiled wheat) is soaked or boiled in sour doogh (a

beverage prepared by beating unflavored yogurt until smooth), and it is then fermented for 7-10 days. Subsequently, some flavoring dried vegetables, salt and spices are added to dough-like mixture and exposed to sunlight in small pieces to get dried.

Kashk-e Zard is a popular product in the southeastern part of Iran (Sistan and Balouchestan province) produced by mixing cereal flour (mainly wheat flour), voghurt, and a variety of vegetables, salt and spices followed by lactic and alcoholic fermentation for several days. Kashk-e zard is usually a mixture of 65% yoghurt and 35% wheat flour. A two-step fermentation is performed to make the final product. Firstly, wheat flour is transformed into a dough-like product by adding yoghurt and salt is then added. For one week, in a closed container and warm place, the product is let to be fermented. Secondly, yoghurt is added again and the dough is kneaded in order to homogenize the product. At this stage spices and garlic are also added. Another period of 7-10 days fermentation is needed in this step. Finally, the product is spread out outdoor to lose moisture. After getting dried, it milled to be granulated in size of 1-3 mm. Similar products in other countries are produced called Tarhana (Turkey and Greece), Kishk (Lebanon and Egypt), Kushuk (Iraq), Madeer-Oggt (Saudi Arabia), Kichk (India), Talkuna (Finland), Tahanya (Hungrey) and Atole (Scotland) (Taymuri, 2007; Jahandideh et al., 2006).

Regarding the importance of traditional foods made by simple and convenient ingredients especially in third world countries, this study focuses on two of Iranian indigenous fermented foods which have not received the scientific attention nationally and internationally they deserve until now.

Material and methods

Sample collection and preparation

40 samples of Kashk-e zard from household producers and market retailers throughout Sistan, Zahedan and Zabol regions as well as 40 samples of Tarkhineh form Kurdistan, Kermanshah and Hamedan were collected over different seasons of 2012. All the samples were brought to the laboratory of food control and hygiene in Islamic Azad University (Karaj Branch) via plastic striled bags and they were ground in order to homogenize and to make them more impressible toward chemical reactions.

Chemical Analysis

Difference between sample weight before and after drying on the weight before drying was considered as moisture percentage (AOAC). Measuring the level of pH for all samples was done by a pH meter (Metrohm, 632). The amount of salt, fat, ash and protein were determined using Volhard, Soxhilet, ashing(muffli) at 525°C, and Kjeldahl techniques, respectively (AOAC). Finally, percentage carbohydrates calculated was as follows: Carbohydrate=total solid-(protein+ fat+ ash) (FAO, 2003). Spectrophotometry was applied to measure the phosphorous and calcium content of the samples (AOAC).

Microbial Analysis

In the current study presence of Coliform and *E. coli*, *Staphylococcus aureus*, *Clostridium perfringens*, Molds and yeast, and *B. cereus* were investigated. Violet Red Bile Agar (VRBA), specialized culture for identification of Coliform bacteria was used with incubation in 35-37°C for 48h. Tubes showing gas production and turbidity in most probable number (MPN) were transferred to Brilliant Green Broth (BGB) tubes which were incubated at 37°C for 24-48

h. A loopful from the BGB tubes testing positive was streaked on Eosin Methylene Blue (EMB) to investigate the presence of *E. coli*. (37°C for 24-48 hours). *S. aurous* was determined by surface plating on Baird Parker Agar (BPA) supplemented with egg yolk-tellurite emulsion (37°C for 24-48 hours). Sulfite polymyxin sulfadiazine (SPS) was utilized for examination of *Clostridium perfringens* followed with incubation in 37°C for 24-48 hours. Molds and yeast were enumerated in Sabouraud Dextrose Agar (SDA) after incubation at 20-25°C for three to five days. Selective agar (BCSA) culture followed with 37°C for 24-48 hours was applied for determining and counting *B. cereus*.

Statistical analysis

All data were imported to the SPSS software (version 17.00). Statistical descriptions and Pearson correlations among chemical compositions were estimated for all of the investigated factors.

Results and discussion

Chemical analysis

Chemical composition of Kashk-e Zard and Tarkhineh are shown in Table 1 and 2, respectively. Pearson correlations among these compositions are also presented in Table 3. Generally, geographical differences, ingredients type and their proportion, and processing techniques are of influential factors on chemical compositions and organoleptic properties of these foods (Marshall and Mejia, 2001).

Table 1. Chemical composition of Kashk-e Zard with minimum, maximum and three percentiles.

Component	Mean ±SE	max	min	25%	50%	75%
Dry matter	95.08±0.07	95.94	94.45	94.72	94.97	95.30
Moisture	4.92±0.07	5.55	4.06	4.70	5.02	5.26
Protein	12.68±0.16	16.00	11.16	11.95	12.45	13.27
Ash	7.74±0.29	11.90	4.50	6.04	7.59	8.98
Fat	2.23±0.12	3.64	0.99	1.52	2.43	2.77
Salt	7.43±0.05	8.00	6.80	7.20	7.40	7.60
Ca (mg/100gr)	109.0±5.05	220.00	60.00	90.00	100.00	130.00
P (mg/100gr)	233.35±16.26	450.00	24.00	150.00	235.00	305.00
pН	4.31±0.08	5.90	3.83	3.99	4.10	0.44
Acidity	3.60±0.19	5.80	1.20	2.52	3.90	4.60
Carbohydrates	72.41±0.41	77.70	66.22	71.14	72.21	73.96

Kashk-e Zard and Tarkhineh have low moisture content (4.92 and 3.03 g/100g respectively) with high

dry mater (95.08 and 96.96 g/100g). Previous studies showed more moisture content for Tarhana [9.8

(Georgala, 2013), 6–9 (Haard et al., 1999; Blandino et al., 2003), 6–10 (Ozdemir et al., 2007)] and Kishk [8.37 (Tamime et al., 1999-1), 8.3 to 12.1(Tamime et al., 2000)], the most similar products to ours. Scientific investigations have reported that low moisture content in food samples increased the storage periods of the food products (Alozie, 2009), while high moisture content in foods encourage microbial growth followed with food spoilage (Assohoun et al., 2013; Temple et al., 1996).

Crude protein content was 12.68 g/100 g and 14.66 m

g/100g for Kashk-e Zard and Tarkhineh, respectively. A range with a little more quantities to that we obtained can be found in literature on Tarhana [15.3 (Georgala, 2013), 16.79 (Erbas et al., 2005), 12-20 (Ozdemir et al., 2007)] and Kishk [17.75 (Tamime et al., 1999-1), 18.7-21.3 (Tamime et al., 2000)]. The main reason for variation in protein content may be due to the type and amount of yoghurt used in preparation of these foods or the different cereal and legume flour used (Temiz et al., 1991; Yucecan et al., 1988; Kose et al., 2002; Oner, et al., 1993).

Table 2. Chemical composition of Tarkhineh with minimum, maximum and three percentiles.

Component	Mean ±SE	max	min	25%	50%	75%
Dry matter	96.96±0.16	99.20	95.26	96.12	96.75	97.72
Moisture	3.03±0.16	4.74	0.80	2.27	3.24	3.88
Protein	14.66±0.50	22.83	10.73	12.07	13.86	16.63
Ash	7.30±0.40	13.51	3.80	4.99	7.46	8.39
Fat	1.59±0.14	4.60	0.20	0.94	1.55	1.80
Salt	4.35±0.28	7.37	1.69	2.53	4.71	5.98
Ca (mg/100gr)	203.18±7.51	287.00	113.00	156.5	203.0	236.7
P (mg/100gr)	282.72±10.26	399.00	132.00	241.2	293.5	327.0
pН	4.91±0.06	5.82	4.33	4.69	4.83	5.07
Acidity	5.23±0.38	12.42	1.28	3.77	4.73	6.52
Carbohydrates	73.41±0.90	82.02	57.15	70.22	72.56	79.01

There was a notable difference between salt content of Kashk-e Zard (7.43 g/100g) and Tarkhineh (4.35 g/100g), which is in consistent with prior reports on Tarhana [1.9 (Georgala, 2013), 6.48 (Erbas *et al.*, 2005)] and Kishk [2.84 (Tamime *et al.*, 1999-1), 3.2 (Tamime *et al.*, 2000)]. Such diversities can refer mainly to variation of salt addition during manufacturing of the foods. However, such level of salt is diluted when cooking them.

Ash content of both studied foods were almost the same [7.74 g/100g for Kashk-e Zard and 7.30 g/100g for Tarkhineh]. In literature various percentages have been reported for ash content of Tarhana [3.2 (Georgala, 2013), 8.94 (Erbas *et al.*, 2005), 1.5-4 (Ozdemir *et al.*, 2007)] and Kishk [7.03 (Tamime *et al.*, 1999-1), 3.3-6.4 (Tamime *et al.*, 2000)].

Fat percentage of Kashk-e zard (2.23 g/100g) was higher than that of Tarkhineh (1.59 g/100g). Both foods in our study exhibited lower levels of fat content than their peers, Tarhana [3.9 (Georgala, 2013), 3.92 (Erbas *et al.*, 2005), 1-9 (Ozdemir *et al.*, 2007)] and Kishk [6.39 (Tamime *et al.*, 1999-1), 3.7-10.7 (Tamime *et al.*, 2000)]. Fat content varies due to the difference in type of dairy fraction of the foods. High level of yoghurt means high fat percentage which can be easily reduced by exposing to light or oxygen and makes the product bitter over a long time.

Tarkhineh was more richful than Kashk-e zard regarding calcium and phosphorus amount, 203 and 282 mg/100g for Tarkhineh versus 109 and 233 mg/100g for Kashk-e zard, respectively. Tarhana with

109 calcium and 105 phosphorus (Ozdemir *et al.*, 2007), and Kishk with 45-63 calcium and 43-48 phosphorus (Tamime *et al.*, 2000) were contained less Ca / P than Tarkhineh and Kashk-e zard. The calcium content is affected by cereal flour and yoghurt rate in the recipe as well as by the type of

yoghurt used (Georgala, 2013). Reduction in phytate due to fermentation increases the amount of soluble calcium and phosphorus several folds (Nout and Ngoddy, 1997; Kohajdová and Karovičová, 2007; Blandino *et al.*, 2003).

Table 3. Pearson correlations among chemical composition for Kashk-e Zard (above diagonal) and Tarkhineh (below diagonal).

	Moisture	Dry matter	Protein	Ash	Fat	Salt	Ca	P	Hd	Acidity	Carbohydrates
Moisture	-	-1**	-0.01	0.08	-0.13	0.05	-0.30	-0.04	0.22	-0.12	-0.19
Dry matter	-1**	-	0.01	-0.08	0.13	-0.05	0.30	0.04	-0.22	0.12	0.19
Protein	-0.09	0.09	-	0.16	0.30	-0.08	0.24	0.37	-0.05	0.23	-0.56**
Ash	0.23	-0.23	0.62**	-	0.13	0.39*	0.16	0.14	0.10	-0.03	-0.84**
Fat	0.17	-0.17	0.25	0.36	-	-0.20	-0.13	0.05	-0.16	0.37	-0.49**
Salt	0.19	-0.19	0.55	0.78**	0.34	-	0.25	0.08	0.01	-0.32	-0.20
Ca	-0.41	0.40	0.51**	0.31	0.36	0.16	-	0.29	-0.22	0.07	-0.11
P	-0.30	0.30	0.06	0.02	0.29	0.01	0.24	-	-0.19	0.04	-0.25
pН	-0.00	0.00	-0.19	-0.49	-0.41	-0.43	-0.46	-0.11	-	-0.28	-0.04
Acidity	-0.16	0.17	0.29	0.42	0.46	0.30	0.51	0.29	-0.81**	-	-0.16
Carbohydrates	-0.26	0.21	-0.86**	-0.89**	-0.49**	-0.75**	-0.40	-0.03	0.39	-0.39	-

Kashk-e Zard showed a pH of 4.31 and acidity of 3.60, while they were 4.91 and 5.23 respectively for Tarkhineh. Figures of 3.5-5 (Ozdemir *et al.*, 2007) and 3.77 (Tamime *et al.*, 1999-1) were previously obtained for Tarhana and Kishk, respectively. The total titratable acidity was calculated as percentage of lactic acid. The concentrations of these organic acids increased continuously during the fermentation. The undissociated forms of the acetic and lactic acids at low pH exhibit inhibitory activities against a wide range of pathogens. The low pH (3.8–4.2) makes fermented foods a poor medium for pathogens and spoilage organisms (Haard *et al.*, 1999; Blandino *et al.*, 2003; Assohoun *et al.*, 2013).

Concerning carbohydrate content, Kashk-e zard (72.41 g/100g) and Tarkhineh (73.41 g/100g) were almost equal and were in accordance with amounts reported already for Tarhana [67.6 (Georgala, 2013), 40-75 (Ozdemir *et al.*, 2007)] and Kishk [68.75

(Tamime et al., 1999-1), 63.9-72.1 (Tamime et al., 2000)].

As expected, moisture and dry mater content are completely correlated in a negative way (-1) (Table3). There were moderately to highly negative correlations between protein and carbohydrate (-0.56 for Kashk-e zard and -0.86 for Tarkhineh), fat and carbohydrate (-0.49 for both Kashk-e zard and Tarkhineh) as well as between ash and carbohydrate (-o.84 for Kashk-e zard and -0.89 for Tarkhineh) suggesting that most of the protein and fat are originated from dairy ingredients of these foods, whereas carbohydrate is mainly from cereal. The higher proportion of yoghurt is, the higher moisture will be, which leads to make a better environment for various microorganisms resulting in shorter shelf life. Previous statistical analysis on Kishk suggested a strong positive correlation (0.865) between protein and fat and strong negative correlations (-0.950 and -0.917)

between protein and carbohydrate, and between fat and carbohydrate, respectively. Thus, intending to produce Kashk-e zard and Tarkhineh, it seems necessary to consider a balance between the main parts, cereal and yoghurt/doogh. Since the amount of salt is a component of the ash content (Tamime *et al.*, 1999-1; Tamime *et al.*, 1999-2), it was observed that

salt was correlated to ash in both foods (0.78 and 0.39) and was not independent of protein or carbohydrate (Tamime *et al.*, 1999-1). Obviously, the higher acidity, the lower pH, which is confirmed by significant correlation coefficient of -0.81 in Tarkhineh.

Table 4. Microbiological analyses for Kashk-e Zard & Tarkhineh.

Microorganism	Kashk-e Zard		Tarkhineh			
	Mean ±SE (Log10)	Max (Log10)	Min (Log10)	Mean ±SE Max		Min (Log10)
				(Log10)	(Log10)	
Staphylococcus	3.11± 3.34	4.03	0	3.90±2.03	7.54	0
aureus (Cfu/gr)						
Molds and yeast	3.11 ± 3.11	3.54	0	5.03±1.41	7.96	2.00
(Cfu/gr)						
B. cereus (Cfu/gr)	1.79 ± 1.89	2.60	1.00	3.41±2.01	6.75	0
Clostridium	35(-), 5(+)	-	-	0	-	-
perfringens (+/-)						
Coliform (MPN/gr)	1.67±1.49	3.04	0	1.58±2.34	6.70	0
E. coli (+/-)	40 (-)	-	-	0	-	-

Microbiological analysis

Main sources of microbiological contamination in traditional foods can be mentioned as follows: staple, traditional method of manufacturing, flora of skin, mouth and nose of producer, fermentation time, and outdoor drying (Ghasemian, 2000). Dalgic and Belibagh (2008) carried out a study on safety of Tarhana based on the principle of hazard analysis critical control point (HACCP) revealing that critical control points during the manufacturing of this food were cooking, fermentation, drying and sieving (Dalgic and Belibagli, 2008).

The low pH (3.3–5.0) generated due to fermentation, release of organic acids, and low moisture content (6–10%) lead to a harsh environment (bacteriostatic effect) for pathogenic microorganisms, in which food spoilage may not occur and shelf life increases (Daglioglu, 2000; Daglioglu *et al.*, 2002; Bilgiçli and Ibanoglu, 2007).

Microbiological composition of Kashk-e zard and Tarkhineh are shown in Table 4. Salt as a dual function additive can improve palatability and hygienic condition of foods through plasmolysis phenomenon in microorganisms. However, some halophiles such as *S. aureus* can resist again high level of salt (Ghasemian, 2000). *S. aureus* was isolated from 67.5% of Kashk-e zard (3.11± 3.34 cfu/gr), and 77.5% of Tarkhineh (3.90±2.03 cfu/gr) samples, while according to ISIRI codex (Institute of Standards and Industrial Research of Iran) it must not exist in food products (ISIRI, NO: 2452 and 1759).

17.5% of Kashk-e zard (3.11 \pm 3.11 cfu/gr) and 10% of Tarkhineh (5.03 \pm 1.41 cfu/gr) samples were suggested a contamination with molds and yeast. However, average numbers of Mold and Yeast in these samples were at safe ranges. Erbas *et al.* (2005) reported that yeast and molds count of stored in 4°C was significantly higher than others because yeast and molds could have survived in low temperature.

In our study, all samples of Kashk-e zard were positive for *B. cereus*, whereas only 62.5% of Tarkhineh samples were contaminated with these bacteria. Nevertheless, according to dangerous range reported in literature (10^5 - 10^8 cfu/gr), the bacteria range in our study (1.79 ± 1.89 cfu/gr for Kashk-e

zard and 3.41±2.01 cfu/gr for Tarkhineh) is not hazardous for human health (Rezoiler, 2000; Ghasemian, 2000).

Clostridium perfringens is gram-positive, anaerobic and spore form bacteria contaminating food through diverse ways, and this group is really pivotal in food poisoning. Sulphite-reducing bacteria were detected only in 12.5% of Kashk-e zard and none of Tarkhineh samples.

Microbiological contamination of different Kishks made from different cereals by Tamime et al. (2000) was: total viable: 4.01-6.37, Mesophilic spores: 2.15-Thermophilic spores: 1-3.54. Change in microbiological counts of the Tarhana dough during fermentation was studied by Erbas et al. (2005), showing a significant decrease in the count of total mesophilic aerobe bacteria, lactic acid bacteria, acid content, and formation of components such as carbon dioxide, hydrogen peroxide, diacetyl, ethanol and bacteriocins acid production to reach the lowest quantities of 5.95, 5.44 and 5.78 cfu/gr, respectively (Erbas et al., 2005). Furthermore, storage type and different retention periods had significant effects on total mesophilic aerobe bacteria, lactic acid bacteria, and yeast and molds. The counts of all these categories decreased continuously during the storage period due to the restricting factors such as temperature, acid content, osmotic pressure and water activity (Erbas et al., 2005).

Number of coliform in Kashk-e zard (1.67±1.49 MPN/gr) and Tarkhineh (1.58±2.34 MPN/gr) in our study was more than what has been determined by ISIRI. Since these bacteria can't survive in pasteurization and pH below 4.6, this contamination primarily comes from cereal grains and environment (ISIRI, NO: 2406). Coliforms and fecal coliforms were reported by Roy *et al.* (2007) in some legume-based traditional fermented foods in India. No coliforms were recovered from any Tarhana samples during fermentation and storage periods, indicating no contamination occurred from environment and raw materials (Erbas *et al.*, 2005). *E. coli* is the most

important species of coliform contaminating foods via feces and it wasn't found in any sample of Kashk-e Zard and Tarkhineh in our study. Viable cells count indicates MPN indices of 0.3 per gram for total coliforms and fecal *E. coli* in groundnut seed cake (Elgasim *et al.*, 2012). The gradual decrease in loads of coliforms and fecal coliforms during fermentation has been reported, which may be a result of the activity of the lactic acid bacteria. Addition of combo salt to Tunjanee one day before the end of fermentation decreased significantly the load to 0.3 MPN/gr for total and fecal coliforms (Elgasim *et al.*, 2012).

Conclusion

Fermentation is a low-input enterprise and provides individuals with limited purchasing power, access to safe, inexpensive and nutritious foods (Marshall and Mejia, 2011). Kashk-e Zard and Tarkhineh are good sources of minerals, organic acids and free amino acids which make them healthy for children, the elderly and medical patients. Nowadays, many countries are interested in manufacturing fermented foods in the Ready to Eat and conserve or powder-like forms which can be valuable for consuming in hospitals, schools and homes. However, under traditional circumstances, outdoor drying, grinding the lumps in order to make a granule-like product, and packaging should be ameliorated so that a product with lower pathogenic agents is obtained.

The flora responsible for the fermentation is in many cases indigenous and includes different strains of lactic acid bacteria, yeast and molds (Blandino *et al.*, 2003), which can be a proper subject for further studies on Kashk-e Zard and Tarkhineh.

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