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COMPARISON OF CHEMICAL AND MICROBIOLOGICAL CHARACTERISTICS BETWEEN TRADITIONAL AND INDUSTRIAL KASHKS IN THE FARS PROVINCE (IRAN)

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Abstract
*In this study, it was aimed comparison of chemical and microbiological characteristics of traditional and industrial Kashk samples that produced in the Fars province of Iran. Moreover, 30 kashk samples were taken in different periods and the intended analyses were carried out .In the dried and industrial kashk samples respectively, mean dry matter was 90.80 ± 4.2 , 20.53 ± 1.7% **;** protein 70.34±6.3 ,7.5±0.72% **;** fat 3.7±0.3 , 2.14±0.59% **;** salt 5.61±0.85, 1.37±0.19% **;** acidity 3.61±0.37, 1.8±0.1 (% of acid lactic)**;** pH 4.04±0.52, 3.61±0.16 and moisture 9.1±4.2, 79.4±1.7% . Comparison of analytical results obtained in present study, there was a statistically significant correlation ($p < 0.01$) between dried and industrial liquid kashk samples chemical condition. In general, data obtain according to ISIRI codex (Institute of Standards and Industrial Research of Iran) .Microbiological analysis showed that in dried kashk samples the mean number of total aerobic bacteria was 4.51±0.32 log cfu/gr, molds and yeasts TNTC, coliform 2.1±0.1 log cfu/gr, Staphylococcus aureus 2.83±0.06 cfu/gr and contained neither E. coli bacteria. isolated strains of PCA , MRS and M_{17} culture medium were include Staphylococcus , Yeast, Corynebacterium , Clostridium and Bacillus . A total of 20 Lactic Acid Bacteria isolates were determined and were identified as lactic acid cocci . Biochemical tests showed 95% of the strains (15 isolates) were S. thermophilus and 25% of the strains (5 isolates) were S. intestinalis. But in industrial kashk bacteria could not find in all samples because conducted industrial liquid kashk production under heat treatment with high temperature and hygienic conditions in industrial units.*

Keywords: dried kashk, industrial kashk, microbial contamination, nutritional value.

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1.INTRODUCTION

Fermentation and drying are of the oldest and most economical methods of producing and order to improve the shelf life and preserve foodstuffs for a long time (Mashak et al., 2014). Middle East is the origin of different and traditionally fermented dairy products (Soltani et al., 2013). Kashk is a fermented dairy product manufactured traditionally in dried form and produced industrially in liquid form in Iran , 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 (Hungary) and Atole (Scotland)(Mashak et al., 2014, Noori et al., 2013). Dried kashk is a concentrated yogurt-type product produced with dehydration of homemade yogurt by sun-drying in summer months by nomads and villagers in the different regions of Iran and spices are added in it. The shape of the 'Kashk' is cubic balls or conic, sold in sacks in Bazaars in Iran. Industrial liquid kashk is a dairy product that is produced from yogurt in industrial units (Noori et al., 2013, Sevgi Kirdar, 2012). Yogurt is a product obtained through milk fermentation by a specific yoghurt starter culture consisting of a mixture of two species of lactic acid bacteria (LAB), Lactobacillus delbrueckii subsp. bulgaricus (L. bulgaricus) and Streptococcus thermophilus (S. thermophilus) (Chaves et al., 2003, Meydani & Ha., 1999, Chaves et al., 2002, El-Shenawy et al., 2012). The main roles of this mixed starter in the production of yogurt are development of the typical yogurt flavor that relatively high

concentration of acetaldehyde found in yogurt (Elli et al., 2006, Erkus., 2007, Irkin. & Eren., 2008., 2008, Azadnia et al., 2009). These organisms are claimed to offer some health benefits for humans (Vaningelgem et al., 2003). This feature said that to be caused by the starter in yogurt and then given kashk . The importance of consumption dried kashk despite its small size, a diet material miraculous that includes all the properties of milk and contain calcium, fat, salt, protein, niacin vitamin and to have cause organic acids, disinfectants digestive tract (stomach, small and large intestine). Kashk is a flatulent, collector and firming intestine food. In addition, high protein, calcium and phosphorus available in kashk can be effective in the prevention of osteoporosis (Poursalimi, 2009).With respect to high production and consumption of kahsk in the traditional form by the villagers and nomads and in the industrial form in dairy units, few studies have been done on this dairy product. The aim of this study was to comparison of chemical and microbiological characteristics of traditional and industrial Kashks in the Fars province (Iran).

2. MATERIALS AND METHODS

2.1. Sample collection

A total of 15 samples of traditional kashk were randomly collected from 5 local areas of Fars in Iran including Sarvestan, Firozabad, Byza, Sheikh Aboud, Sarhad and put into in sterile plastic bags and 15 samples of industrial liquid kashk were taken from dairy units (Fars) . Then all these samples were stored at 4°C and processed in less than 24 h.

2.2. Chemical Analysis

The pH values of yogurt samples were measured using a pH meter (Knick- Germany). For total titratable acidity (TTA), samples were diluted with equal volume of water and titrated with a 0.1 N NaOH. The results were expressed in Dornic degrees (˚D). To determination of dry matters, samples were dried to constant weight in an air oven (Electronic Fater Feb 50 liters) regulated to 102±2°C (ISIRI number 637). Specific gravity was determined using Thermo-lactodensimeter (ISIRI number 638). To determination of moisture, samples were dried to constant weight in an air oven (Electronic Fater Feb 50 liters) regulated to 102±2°C (ISIRI number 9874). For fat percent of kashk samples determined according to the Gerber method (ISIRI number 366).The salt contents in kashk samples were determined by Volhard method (ISIRI number 1809). Total protein content in kashk samples was measured by Kjeldahl's method (ISIRI number 639).

2.3. Microbial Analysis

For this purpose, in quite sterile conditions, 10 grams of each sample dried kashk and liquid kashk were weighed aseptically and homogenised in 90 ml of sterile Ringer's solution before used (Azadnia et al., 2014). Then, one mililiter (ml) of samples cultured in petri dishes by the pour plate method. Violet Red Bile Agar (VRBA), specialized culture for identification of Coliform bacteria was used with incubation in 37^0C for 24- 48 hours (Al-Otaibi., 2012, ISIRI number 5486). Escherichia coli cultured in Lauriyel Sulfate Tryptose Broth incubation in 37^0C for 24h (Al-Otaibi, 2012).Yeast Extract Dextrose Chloramphenicol Agar was used for molds and yeasts cultured and incubating at 25ºC for 5 days (Al-Otaibi., 2012, ISIRI number 10154). Staphylococcus aureus was determined by surface plating on Baird Parker Agar (BPA) and incubating at 37^0C for 24-48h (IRISI number 6806-3).

2.4. Isolation of Lactic Acid Bacteria

Sample preparation is done like the previous step and then, sequential decimal dilutions of the homogenate were obtained .One ml aliquot of the appropriate dilutions were used for the isolation of LAB .Following culture media and conditions (pH and incubation temperatures) were used. Plate Count Agar (Merck, Germany) incubated at 30˚C for 72h for enumeration of total aerobic mesophilic

bacteria (Al-Otaibi, 2012). De Man Rogosa Sharpe (MRS) agar with pH 5.7 (Merck, Germany) for isolation of lactobacilli and M_{17} agar with pH 7.15 (Himedia, Hindi) for isolation of streptecocci, lactococci and enterococci were used (Nikolic et al., 2008). To prevent the growing of mold and yeasts were supplemented with 50 mgL^{-1} of natamaycin (Botes et al., 2007). MRS plates were incubated under aerobically and anaerobilcally conditions using the gas pack system (Merck Anaerocult type A) at 37˚C for 48 -72 h (Bossyouni et al., 2012). M_{17} Agar plates were incubated under aerobically and anaerobilcally conditions at 30˚ C and 42˚C for 24 - 48h (Azadnia et al., 2009). Morphologically distinct colonies from PCA , M_{17} and MRS agar plates were randomly-picked and were sub-cultured and purified by streak plating using the same medium and incubate at 37˚C for 72h. Then the bacterial strains were subsequently kept in at 4°C. Pure strains were further tested for gram reaction, catalase production, oxidase activity, spore formation, and cell morphology. Isolates that gram positive and catalase negative, oxidase negative, non-spore, cocci or rod were selected as presumptive LAB, and were preserved in MRS or M_{17} broth medium or Tryptic soy broth (TSB) medium which contained 20% (v/v) glycerol as frozen stocks at -80°C (Azadnia et al., 2009).

2.5. Biochemical Identification

Biochemical identification of the isolated bacteria was carried out using Bergey's manual of determinative bacteriology. For biochemical identification, each isolate was activated in 5 ml M_{17} or MRS broth for 24 h at 37 ºC before use. Therefore, overnight cultures were used during all the identification procedures (Nikita & Hemangi., 2012). Growth at different temperatures and different NaCl concentrations- growth at pH 9.6- arginine hydrolysis and gas production from citrate- reduction methylene blue. Isolates were also characterized on the basis of their sugar fermentation profiles. Six different sugars were used (mannitol, raffinose, ribose, galactose, maltose, lactose). For each test, fifty µl of strains were inoculated in 5 ml Phenol red base broth media , then 150µl of filter sterilized (0.22µm, Millipore) 10% sugar solutions were pipetted into each tubes . They incubated for 48h at 37˚C .The change of the color from red to yellow taken as the evidence for cell growth.

2.6. Statistical Analysis

All the analyses of chemical composition were performed in triplicate. The data were recorded as means ± standard deviation and analyzed using SPSS v.21 for windows (SPSS Inc, Chicago). One-way analysis of variance (ANOVA) was performed. Significant differences between means were determined by t- test. ($p<0.01$ was considered as a level of significance.

3. RESULTS AND DISCUSSION

3.1. Chemical analysis

The importance of consumption of fermented dairy products for human health is confirmed by different researches. Kashk is a fermented dairy product which is manufactured to the two forms including dried and industrial liquid, which geographical differences, ingredients type and their proportion, and processing techniques are of influential factors on chemical compositions and organoleptic properties of this food (Mashak et al., 2014). Chemical composition of dried and industrial kashks is shown in Table 1. Comparison of analytical results obtained in present study from pH, acidity, moisture, protein, fat and dry matter value. There was a statistically significant correlation ($p < 0.01$) between dried and industrial liquid kashk samples chemical condition that indicate a significant differences ($p < 0.01$) .The mean pH and acidity (% lactic acid) value of kashk samples was affected pH and acidity yoghurt the basic raw material used for production and heat treatment applied during the production. In the during yogurt fermentation by lactic acid bacteria, the lactic acid is produced from lactose contributes to the

sour taste of yogurt by decreasing pH. Low pH and high acidity could enhance the shelf life and microbial safety, improve texture, and contribute to the pleasant sensory profile of the end product. In the study conducted on chemical and microbial properties of Iranian traditional kashk-e-zard the mean pH and acidity (% of lactic acid) value of samples were 4.31 ± 0.08, 0.35%-3.60% respectively. The pH of all samples studied was lower than 4.6 and acidity 1.54±0.10% (Noori et al., 2013). The pH level in industrial liquid kashk samples is 3.78±0.05 (Soltani et al., 2013). According with national standards pH value industrial kashk is Maximum 4.2, acidity 1.3–2% (ISIRI number 6127). The moisture content of dried kashk is low, because the dehydration of doogh (drinking yogurt) and drying in the sun are done. Drying involves removing sufficient moisture from the food to prevent the growth of pathogens and spoilage organisms. Moisture value in industrial liquid kashk is dependence the amount of water which is added to yogurt during kashk production. High moisture value has several effects on food stability, palatability, and overall quality and it can cause the growth of various microorganisms. Previous research reported that the moisture content of traditional kashks in Iran has a range of 4.92 ± 0.07% (Mashak et al., 2014) and in industrial kashk is 81.41 ± 0.15 % (Soltani & Guzeler, 2013).. According with national standards, moisture content in dried and liquid industrial kashks are Maximum10% and Maximum 82% respectively. Some researchers reported that the fat percentage in dried kashk samples was 2.23 ±0.12%, 2.46% to 3.35% and protein was range 13.83 ± 0.48 % (Mashak et al., 2014; Noori et al., 2013). In the present study, fat and protein content varies due to the difference in type of yoghurt used for kashk samples production. Milk fat imparts important physical and organoleptic properties to dairy products, is a source of energy and essential fatty acids, and helps deliver fat-soluble vitamins A, D, E, and K. In a study on liquid kashk samples in Iran, the average percentage of fat was 1.65 ± 0.06% that is lower than values determined for strained yogurt and protein content was 8.59±0.22%(Soltani & Guzeler., 2013). Milk proteins play a crucial role in achieving and maintaining the desired textural and sensorial properties of almost all dairy products. Moreover, milk proteins contribute to the nutritional and health effects of dairy products. Kashk is a diet rich in protein and vitamin D which contributes to bone health. Due to their high protein, vitamin D, and calcium content, dairy foods are a good choice for maintaining strong bones, and required for growth and repair. Proteins component are important and it is valuable in terms of biological value especially when they derived from animal sources (Soltani et al., 2013). According with national standards, fat percentage of dried and industrial kashks are Maximum10 and least1 respectively and Protein percentage of dried and industrial kashks are least50 and least8 respectively. Another component is salt. Salt content of dried and industrial liquid kashk samples depending on the amount added to the product. The salt is added during the manufacture of industrial liquid kashk formed the source of salt rate in the final product (Soltani & Guzeler, 2013). Historically, the main reason for the addition of salt to food was for preservation, and plays a role in reducing the growth of pathogens and organisms that spoil products and reduce their shelf life because it reduces the water activity of foods. During the survey by (Noori et al. 2013) of traditional kashk samples in Iran reported that salt level was range of 0.89 ± 0.05 % to 1.52 ± 0.06 % , 4.35 ± 0.28 % (Mashak et al., 2014) . Industrial liquid kashk the mean salt value was 1.69 ± 0.07 % (Soltani & Guzeler, 2013). According with national standard, the salt percent of dried and industrial kashks are (Maximum 9 and Maximum 2 respectively). The mean dry matter value of samples was found (90.80 and 20.53%). The dry matter is a measurement of the mass of something when completely dried. The dry matter of food would include carbohydrates, fats, proteins, vitamins, minerals, and antioxidants. In addition , data obtain according to ISIRI codex (Institute of Standards and Industrial Research of Iran) except fat percent in dried kashk samples

because during production by nomads and villagers separated fat globules of milk and doogh (drink yogurt) then used as butter.

Table 1 .The chemical composition of dried kashk and industrial liquid kashk samples

Chemical parameters	Dried kashks (Mean ± SD)	Industrial kashks (Mean ± SD)
pH	4.04 ± 0.52	3.61 ± 0.16
Acidity (lactic acid %)	3.61 ± 0.37	1.8 ± 0.1
Moisture (%)	9.1 ± 4.2	79.4 ± 1.7
Fat (%)	3.7 ± 0.37	2.13 ± 0.59
Protein (%)	70.34 ± 6.3	7.5 ± 0.72
Salt (%)	5.61 ± 0.85	1.37 ± 0.19
Dry matter (%)	90.80 ± 4.2	20.53 ± 1.7

3.2. 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 (Mashak et al., 2014). In our study, microbiological analysis of dried kashk samples showed that Escherichia coli could not find in all samples. But, Coliform bacteria could not find in Sarvestan samples but in other samples coliform colonies were observed (2.1±0.1cfu/gr). Coliform bacteria include a large group of many types of bacteria that occur throughout the environment. Most types of coliform bacteria are harmless to humans, but some can cause mild illnesses and a few can lead to serious waterborne diseases. Fecal coliform can be present in foods because of the post-sanitization or post-process contamination, often caused by a lack of hand hygiene on the part of food handlers (Soltani & Guzeler, 2013). In study on microbial properties of Iranian traditional kashk-e-zard reported that number of coliform (1.67±1.49 MPN/gr), that 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 feed and environment (Mashak et al., 2014). According ISIRI number 1188, coliform must not exist in dried kashk, in our study was contamination because used of contamine water and lack hygiene during production. Molds and yeasts, several dilutions were cultured and all plates were uncountable, or TNTC (Too Numerous To Count) that it can be often due to the production and drying it in the free air. In a study, 17.5% of traditional Kashk-e-zard (3.11±3.11 log cfu/gr) samples were suggested a contamination with molds and yeast (Mashak et al., 2014). Yeasts and molds are highly efficient at causing foods to spoil and they are a problem for most food manufacturers. Contamination may occur during processing, packaging or storage of raw materials or finished products. In our study all samples of dried kashk were positive for staphylococcus aureus (2.83±0.06 log cfu/gr), while according

national standard staphylococcus aureus count much in any gram of dried kashk is negative. These bacteria multiply quickly at room temperature to produce a toxin that causes illness. Also, these bacteria have a high salt tolerance, heat resistant and cannot be destroyed through cooking. In previous studies, it was reported that 31 (11.5%) samples of dried kashk (Soltani & Guzeler, 2013) and 67.5% (3.11± 3.34 log cfu/gr) (Mashak et al., 2014) indicated S. aureus contamination. It can be expressed that dairy products are a known source of staphylococcal poisoning. The average of total aerobic mesophilic bacteria counts was 4.51±0.32 log cfu/gr. 50 isolates were picked of PCA medium and sub culturing , pure isolates after gram reaction, catalase production, oxidase activity, isolated strains were include Staphylococcus , Yeast and Corynebacterium. Some Corynebacterium species are known for their pathogenic effects in humans and other animals but nonpathogenic species of Corynebacterium are used for very important industrial applications. 20 isolates were determined of MRS medium also after gram reaction, catalase production, oxidase activity, isolated strains were Fungi, Clostridium, Bacillus and Lactobacillus could not find in all samples. Clostridium contains around 100 species that include common free-living bacteria, as well as important pathogens. In a review, Mashak et al. (2014) reported that 12.5% of traditional kashk samples have Clostridium perfringens contamination. Bacillus spp. is aerobic spore forming rod bacteria that stain gram positive or gram variable. Despite the widespread distribution of Bacillus organisms they are rarely implicated with actual infections and are more frequently isolated as a culture contaminant. In study all samples of Kashk-e-zard were positive for B. cereus (1.79 ± 1.89 log cfu/gr) (Mashak et al., 2014). 20 isolates were obtained of M_{17} agar medium that were gram positive, catalase and oxidase negative, non-spore were identified as lactic acid cocci. In order to identify of cocci genus isolates physiological and biochemical differentiation tests were performed that characteristics are shown in Table 2. Regarding their ability to grow at 2% NaCl and ferment different carbohydrates was used to distinguish. Upon evaluating the results, 95% of the strains (15 isolates) were S. thermophilus and 25% of the strains (5 isolates) were S. intestinalis. Streptococcus is a genus of coccus (spherical) gram-positive bacteria belonging to the phylum Firmicutes and they grow in chains or pairs. Streptococcus thermophilus is an essential lactic acid bacterium used for commercial purposes and a powerful probiotic strain that has well researched health benefits .Some potential benefits may result from growth and action of the bacteria during the manufacture of cultured foods. Streptococcus intestinalis small (1mm in diameter), alpha hemolytic (sometimes non-hemolytic), non-pigmented colonies, grow at 37˚C (30- 45℃), aerobic or facultative anaerobic, growth on complex media. In a study about identification of Lactobacilli genus in traditional Iranian dairy products (yogurt, cheese, butter, doogh, kashk and fermented milk), Lacto bacillus species were isolated from kashk samples. Lactobacillus species were L. plantarum and L. agilis (Tajabadi Ebrahimi et al., 2011). In present study and previous studies have been shown that traditional kashk samples are high contamination but identification pathogenic strains of Corynebacterium and Bacillus necessary to carry out biochemical tests. Despite the dose used of natamaycin some of fungi and yeast strains were grown on PCA, MRS and M_{17} media. Therefore data obtained of microbial analysis present study disagreement with national standard Iran. As mentioned, the main reason can be the lack hygiene at raw material, during production and storage. Microbiological analysis of industrial liquid kashk samples showed that coliform bacteria, Escherichia coli, molds and yeasts were less than 10 cfu/gr. Staphylococcus aureus did not find in all samples. On PCA agar, MRS agar and M17 agar medium wasn't grown bacterial, because conducted industrial liquid kashk production under heat treatment with high temperature. In addition, there are hygienic conditions in industrial units and this conditions controlled by HACCP principles.

Table 2 . Differential characteristic of cocci shaped of Lactic Acid Bacteria

Characteristics	S. thermophilus	S. intestinalis
gram reaction	+	+
catalase production	-	-
oxidase activity	-	-
spore formation	Non - spore	Non - spore
Growth at pH 9.6	-	-
Reduction Methylene Blue	-	-
Growth at 10 °C	-	-
Growth at 40 °C	+	+
Growth at 45 °C	+	+
Growth in 2% NaCl	±	-
Growth in 4% NaCl	-	-
Growth in 6.5% NaCl	-	-
Hydolysis of arginine	-	-
CO2 from citrate	-	-
Acid formed from:		
Mannitol	-	-
Raffinose	-	-
Ribose	-	-
Galactose	-	+
Maltose	-	-
Lactose	+	-

4. CONCLUSIONS

Yogurt was used as a raw material for production of dried and industrial liquid kashks but different production methods leads to differences in the microbiological and chemical properties of these two types of kashk. The chemical properties of industrial liquid kashk were reported normal but dried kashk is rich of protein, calcium and fat that are most important necessary micronutrients for the human body. Fat, protein, calcium play an essential role in bone formation and metabolism. Microbial properties of dried kashk samples have been high microbial contamination but consists LAB (lactic acid bacteria) that of this point, it is a valuable animal origin food. Unlike samples of industrial liquid kashk lack each bacteria species (LAB) and microbial contamination. In general, dried kashk has more micronutrient and LAB bacteria superior than industrial kashk, but the contamination is a problem. Dried kashk due to fragrant taste and flavor more popular in Iran, as well as doctors are advised to eat it by the osteoporosis patients, children, pregnant women and elderly people for having high calcium and protein. Unfortunately, some people are not aware of the contamination of this traditional product. On the other hand, industrial kashks are poor in viewpoint nutrition and useful bacteria, therefore aren't special benefits for the body. Industrial kashk manufacturers in dairy units can improve their product according to the interests of consumers. They can apply some strategies for converting the kashk with commercial probiotic food products that help to improve the product such as using of lower temperatures during production for surviving lactic acid bacteria or adding the starter culture after heating to eliminate the pathogenic bacteria.

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