CHAPTER 2

Traditional food and practices for health: Iranian dairy foods

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2.1 Introduction

Middle East has been identified as the origin of yogurt, and the evolution of fermented dairy products has been referred to this part of the world (Tamime and Robinson, 2007). Various types of milk and milk products, mainly fermented types, have been produced in Iran for centuries such as yogurt, which is called Mast, sour buttermilk, Doogh, kefir, different cheeses, Kashk, Qareh-Qurut, and many others. Similar to other countries, in Iran, the request for traditional homemade foods has increased recently, resulting in the growth of protected label of the origin (PDO). PDO is applied for foods, which are produced in a defined geographical area with distinctive natural characteristics. Nowadays, PDO products, such as Tarkhineh, Doogh, Siahmazgi cheese, Lighvan cheese, dried Kashk, and many others, have been produced in Iran.

Although, in the past, ewe and goat milk were utilized as the most important raw materials, today bovine milk is the main raw material for producing these products; meanwhile, some local products are made from sheep, goat, buffalo, and even camel milk. Despite the fact that the fermentation of raw milk has been used as a nonthermal preservation method, it is also used for creating unique organoleptic properties, flavor,

aroma, and texture in the final products. These features are applied for products produced only in specific areas of the country probably because of the particular properties of local lactic acid bacteria (LAB). Moreover, it has been demonstrated that food fermentation promotes the nutritional quality and safety of the product via improving the digestibility of food compounds as well as eliminating or decreasing the antinutritive components and toxins in the final products (Mathara et al., 2008).

2.2 The importance of lactic acid bacteria in Iranian traditional dairy foods

Several researchers have done the isolation and identification of LAB in fermented dairy foods of the country for decades to use the most suitable ones in the manufacturing of dairy products and maintaining the LAB diversity of particular areas. LAB are found in nature as well as fermented foods, for example, fermented milk and milk products. They are used as a starter culture in many types of cultured foods and present in many other fermented products as nonstarter LAB.

Fermentation process takes place using various components of milk, but LAB initially produce organic acids, mostly lactic acid by metabolizing the lactose, resulting in a reduced pH, increased titratable acidity (TA). Therefore they create unfavorable environments for both spoilage and pathogenic microorganisms (Aslim et al., 2005) also by producing a broad range of antimicrobial substances such as acetoin, bacteriocins that are antimicrobial peptides, or proteins synthesized by specific LAB strains, diacetyl, ethanol, formic acid, and hydrogen peroxide (Yang et al., 2014).

2.2.1 Lactic acid bacteria in raw milk

The presence of LAB in raw milk has been reported previously (Ortu et al., 2007; Sharma et al., 2013). Yousefi et al. (2011) investigated new strains of bacteriocinproducing LAB with antimicrobial activity in sheep and goat milk samples collected from various parts of Iran. They found that two strains of *Enterococcus* producing enterocin-like substances had a broad antibacterial activity against *Listeria monocytogenes* and *Staphylococcus aureus*. These substances with molecular weights between 24 and 29 KD were stable in a broad range of pH (3–10), maintaining their activity after 28 days of storage at refrigeration temperature, and some of them were also heat-stable (Yousefi et al., 2011).

2.3 Sour buttermilk

For centuries, sour buttermilk is produced using sheep milk in different parts of mountain ranges of Zagros and Alborz in the west and north of the country, respectively. It is the aqueous part of full-fat yogurt that remained after churning in



Figure 2.1 Mashk used for making butter and buttermilk from sour full-fat yogurt.

sheepskin, Mashk traditional device made from hide (Fig. 2.1) and Tulum (a traditional device made from the wood of the tree and depleted inside of tree) for butter making. As a refreshing and pleasant beverage, it is consumed in spring and summer months when ewe milk is available. Mohamadi et al. (2012) investigated the chemical composition and physical properties of samples of sour buttermilk or butter Doogh collected from the East-Azarbayejan province. The percentages of total solids (TS), crude protein (CP), lactose, ash, fat, and salt content were in the ranges of 3.51-5.47, 1.99-2.75, 0.31-0.97, 0.29-0.79, 0.16-0.45, and 0.16-0.27, respectively. The density, pH, and TA of the product were found to be in the ranges of 1.0191-1.0264 g/cm³, 3.64-3.81 and 0.91-1 (LA%). They also demonstrated that the lactation period has a significant effect on both the chemical and physical properties of buttermilk (Mohamadi et al., 2012). Iranmanesh et al. (2012) investigated the presence of LAB in ewe milk, sour yogurt, and buttermilk produced in Mashk. Pediococcus acidilactici was found in both milk and buttermilk, and hence it was assumed that this isolate was able to tolerate high processing temperatures and survived in the final product (buttermilk) or might be added to the product after boiling probably originated from Mashk (Iranmanesh et al., 2012). The same researchers isolated LAB-producing bacteriocin-like inhibitory substances from milk, yogurt, and buttermilk (Fig. 2.2). The inhibitory actions of selected strains were examined against S. aureus, Salmonella enteritidis, and L. monocytogenes. Lactobacillus brevis, Lactobacillus pentosus, P. acidilactici, and Lactobacillus paracasei showed inhibitory action against the tested pathogens. Although their inhibitory action was completely inhibited by proteolytic enzymes, this ability remained unaffected by the action of pH neutralization and hydrogen peroxide (Iranmanesh et al., 2014). The ability of isolated strains was also characterized by cholesterol assimilation. The highest level of cholesterol reduction was found in L. brevis, and all the other strains were also able to reduce cholesterol to a lesser extent.

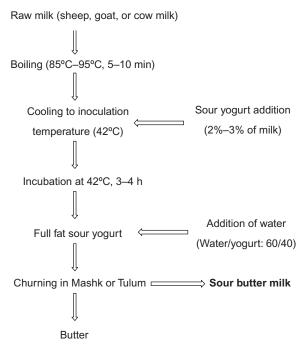


Figure 2.2 Schematic representation of sour yogurt and sour buttermilk production.

2.4 Kashk

Dried Kashk as a self-stable high protein traditional product has been produced from sour buttermilk (Fig. 2.3) in rural areas of Iran. It is a fermented/acidified, concentrated, salted, and heated product with the shelf life of 4 years at ambient temperature. The average TS, CP, NaCl, fat, and ash of dried Kashk were found to be 95.6%, 54.4%, 8.69%, 7.89%, and 3.82%, respectively, and acidity was found to be 9.05 (LA%) (Taleban and Renner, 1972). It is produced throughout the region between the eastern Mediterranean and the Indian subcontinent for centuries (Tamime et al., 2000). The name of the product differs based on used ingredients/additives, as well as the region. Tamime and Robinson (1999) reported that products containing Bulgar, parboiled cracked wheat, or flour are called as follows: Kishk in the Arab countries, Tarhana in Turkey and Greece, Chura in Tibet and Nepal, Zhum in Yemen, and Kadhi in India (Tamime and Robinson, 1999).

The main usage of dried Kashk is to prepare a kind of milk-based sauce after pulverizing and solubilizing in water (Fig. 2.4). Today, a new type of liquid Kashk is available in the market, prepared from industrially manufactured yogurt from bovine milk. However, the quality of liquid Kashk strongly depends on the type of incoming materials used, dried Kashk from ewe milk, or yogurt made from cow milk, and also

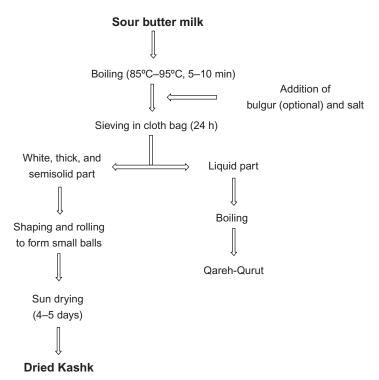


Figure 2.3 Schematic representation of dried Kashk production.

ingredients added such as Bulgur or flour. The percentages of nonfat solids, CP, NaCl, and fat in liquid Kashk are 20-25, >13, 3, and 1, respectively, and a pH less than 4.5 (Shiroodi et al., 2012).

The high-quality liquid Kashk is produced from traditional dried Kashk made from sour buttermilk and is very popular among Iranians because of its unique aroma and taste. Recently, Iranmanesh et al. (2018) characterized the volatile compounds in dried Kashk collected from various areas of Iran made from bovine or ovine milk and prepared using various production methods by producers (Fig. 2.3). Gas chromatography-mass spectrometry (GC-MS) analysis using the solid-phase microextraction detected 602 compounds in dried Kashk gathered from 11 parts of the country. They claimed that the volatile profiles of Kashk prepared using bovine or ovine raw milk were quite different. Alkanes, aldehydes, free fatty acids (FFA), esters, terpenes, sulfur compounds, ketones, alcohols, and terpenoids were the main compounds responsible for the flavor in dried Kashk. Alkenes and aldehydes were found more abundantly in samples from ovine milk than cow's milk. Higher content of aldehydes was found in samples containing bulgur or flour. A higher lipolysis rate of fat during the process of preparing sour buttermilk from sour full-fat yogurt in Mashk or Tulum is probably

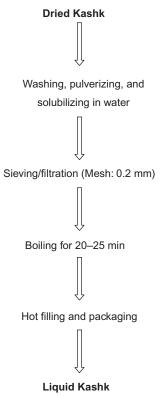


Figure 2.4 Schematic representation of liquid Kashk production.

the main reason for the high content of FFAs in Kashk samples. Higher alkenes were found in samples, which were made from ovine milk (Iranmanesh et al., 2018).

2.5 Kashk-e Zard and Tarkhineh

In the west of Iran, Hamedan, Kermanshah, and Kurdistan Provinces, Tarkhineh has been produced for centuries and it is called Tarkhowana or Doowina in Kurdish. Bulgar is soaked or boiled in sour buttermilk, followed by fermentation for 7-10days. Ingredients such as salt, spices, and condiments are added to the semisolid product, shaped to small pieces, and sun dried.

One of the most popular fermented products in Sistan and Baluchestan Provinces is Kashk-e Zard. It is made by mixing cereal flour (mainly wheat flour), sour yogurt, salt, condiments, and spices followed by lactic and alcoholic fermentation for several days. The ratio of yogurt to cereal flour is 65/35. It is produced by two-step fermentation; first, a dough-like product is made by cereal flour followed by the addition of the first portion of yogurt and salt and kept to be fermented in a closed container in a warm place for 7 days. Then, another portion of yogurt is added and is homogenized by kneading the dough-like product. Then, spices, condiments, and garlic are added and are kept to ferment for 1 week. The fermented dough as the product is sun dried followed by grinding to granule with 1-3 mm dimensions.

Mashak et al. (2014) studied the chemical composition and microbial quality of Kashk-e Zard and Tarkhineh, as traditional Iranian cereal-dairy-based fermented foods. They gathered samples of Kashk-e Zard from household producers and market retailers of Sistan and Baluchestan Province and Tarkhineh samples from Kurdistan, Kermanshah, and Hamedan Provinces. Both products contained high TS (95% <) and carbohydrate (72% <) contents. Higher protein content was found in Tarkhineh (14.66%) compared to Kashk-e Zard (12.68%), and low-fat contents were reported in Tarkhineh (1.59%) as well as Kashk-e Zard (2.23%). pH in Tarkhineh (4.91) was higher than that in Kashk-e Zard (4.31). Although they isolated *S. aureus* from 67.5% samples of Kashk-e Zard and 77.5% samples of Tarkhineh as well as *Bacillus cereus* from all samples of Kashk-e Zard and 62.5% samples of Tarkhineh, they claimed that the levels of microbial contamination are very low and are not considered as hazards. They also recommended changing unhygienic traditional methods of production to ameliorated conditions to eliminate or reduce pathogenic microorganisms in the products (Mashak et al., 2014).

2.6 Iranian traditional cheese types

2.6.1 Pot cheese

Pot cheese is an Iranian traditional cheese that is renowned for its unique aroma and flavor (Fig. 2.5). Nonstarter LAB are the main responsible microflora of Pot cheese as no starter culture is used for producing this type of cheese. Ghaderi et al. (2013) studied the type of LAB involved in the ripening process of Pot cheese produced in Sardasht city in West Azerbaijan Province, northwest of Iran. They isolated 51 strains; the dominant genera were *Lactobacilli* (37.3%), *Enterococci* (25.5%), *Lactococci* (19.6%), *Leuconostoc* (9.8%), and *Pediococci* (7.8%). In ripened Pot cheese, dominant isolated species was *Enrococcus faecium* (8 isolates), *Lactococcus lactis* spp. *Lactis* (7 isolates), *Lactobacillus casei* spp. *casei* (4 isolates), and *P. acidilactici* (4 isolates). They assumed that these species contribute to the ripening process and development of unique aroma and flavor of the Iranian Pot cheese (Ghaderi et al., 2013).

2.6.2 Siahmazgi cheese

Siahmazgi cheese is made from ovine milk or a mixture of ovine and caprine milks in the Siahmazgi village, suburbs of Rasht, Gilan Province, and north of Iran. The curd is kept in special conditions in sheepskin during a 6 months ripening period. It has its



Figure 2.5 Ripened Pot cheese.

own distinct physicochemical and textural characteristics such as an extremely firm texture with some pea-sized holes, yellowish appearance and fermented taste (Farahani et al., 2014). Farahani et al. (2014) studied the effect of ripening time (6 months) on the chemical, physicochemical, rheological, and textural characteristics of Siahmazgi cheese. Rheological and textural properties were determined using a rheometer (frequency sweep) and texture analyzer (uniaxial compression). The measured values including pH, TA, TS, fat, protein, ash, salt content, water-soluble nitrogen (WSN) in total nitrogen (TN), and nonprotein-nitrogen in TN significantly increased during ripening (P < .05). They characterized this kind of cheese by its respectively high values of TS (59.95 \pm 0.08 g/100 g), salt (5.65 \pm 0.05 g/100 g), and ash (7.24 \pm 0.02 g/100 g) contents and categorized it as a kind of full-fat semihard cheese. Regarding rheological and textural properties, storage modulus (G'), loss modulus (G''), fracture stress (sf), and firmness increased while loss tangent and fracture strain decreased during ripening.

Zamani (2016) isolated LAB from this type of cheese and studied the probiotic potential of isolated LAB. He reported that one strain, labeled as Lb3, showed good probiotic properties, tolerance to low pH, bile, and simulated gastrointestinal tract conditions, resistance to Streptomycin, Vancomycin, and Polymixin B, and effective antibacterial activity against two Gram-negative pathogens, lacking hemolytic activity as well as high β -galactosidase activity. The strain Lb3 was identified as *Lactobacillus plantarum* CJLP55 using biochemical characterization and *16S rRNA* sequencing assay (Zamani, 2016).

2.6.3 Lighvan cheese

Lighvan cheese, the most famous Iranian ethnic cheese, is very similar to Greek feta cheese. It has been produced for hundreds of years mainly from raw sheep milk or a mixture of sheep and goat milk in northwest of Iran, East Azerbaijan province, and Lighvan village. Every year more than 4000 tons of Lighvan cheese is produced where one fourth is exported to Japan, Canada, United States, and Malaysia. More than 200 dairies produce the artisanal Lighvan cheese in the Lighvan village with a population of about 5000 (Donnelly and Kehler, 2016). This kind of white brined cheese has a sour and salty flavor, and probably umami taste. The brittle semisoft Lighvan cheese has round shape cheese holes, which look like tears.

The TS and fat contents in sheep milk are much higher than those in bovine milk (Fox et al., 2017). Different attributes of sheep milk products, for example, Lighvan cheese are influenced by the composition of sheep milk. Table 2.1 shows the composition of sheep milk, Lighvan, and other brined cheeses.

According to Abrahamsen and Rysstad (1991), the most abundant amino acids in cow, goat, and sheep milk are glutamic acid (Glu), followed by proline (Table 2.2).

Lighvan cheese, as a sort of brined cheese, needs ripening to develop the required properties. Similar to other brined cheese, this kind of unpasteurized cheese is produced in warm climates and the usage of salt as a preservative is essential. The procedure of Lighvan cheese production starts from raw milk, mostly sheep milk and 20%-30% goat milk followed by renneting the cheese milk without the addition of starter culture. In other words, the LAB in the production of cheese originate particularly from raw milk. After curdling, fresh curd is poured in fabric baggage in triangular molds and maintained until complete drainage takes place. The triangular curds with a thickness of 20 cm are removed from the bags and cut into 3D shapes, salted with granular salt, and on the next day are transferred to tins. Although the bottom of the tins is filled with salt, this is distributed between curd cubes. Salted curds are kept at $14^{\circ}\text{C}-16^{\circ}\text{C}$ for 4-5 days followed by whey removal and filling with brine (6%-8% NaCl). Then, tins are closed and stored at $14^{\circ}C-16^{\circ}C$. After 2 weeks, they are transferred to cold stores to start ripening. The ripening period for Lighvan cheese is usually 3-12 months. The pH value in ripened Lighvan cheese is about 4.87, whereas in other brined cheeses is 4.2-4.8. Aminifar et al. (2010) investigated the physiochemical

	Moisture	Total solids	Protein	Fat	Fat in dry matter	Salt	Salt in moisture
Sheep milk	81.5	18.5	5.15	6.82	_	_	_
Lighvan cheese Brined cheese	53.75 50-58	46.25 42-50	_ 17 ≤	17.27 —	37.34 45-50	3.68	6.85 5.5—9

Table 2.1 Gross composition of sheep milk, Lighvan cheese, and brined cheese (percentage).

 Table 2.2
 The amino acid contents in bovine, caprine, and ovine milk (mg amino acid/g total amino acid).

Species	n	Asp	Glu	Ser	Gly	His	Arg	Thr	Ala	Pro	Tyr	Val	Met	Cys	lle	Leu	Phe	Lys
Cow	4	70.5	208.2	56.1	18.1	24.1	34.1	42.1	32.1	100.4	47.1	52.2	26.1	9.1	47.1	99.1	50.1	86.2
Goat	2	75.1	209.1	49.5	18.2	26.1	29.1	49.1	34.5	106.8	38.1	61.1	25.2	9.1	48.1	96.3	47.1	80.1
Sheep	6	75.2	203.4	52.1	18.1	26.1	34.1	41.1	40.1	102.2	47.2	57.2	29.1	8.1	49.1	90.4	48.1	83.3

characteristics, microstructure, and texture of Lighvan cheese over a 90-day ripening period. They found that the moisture content of the cheese decreased during storage and the salt-in-moisture ratio increased during this period. The most important biochemical change of the Lighvan cheese during aging was the extent of proteolysis. The WSN to TN ratio increased significantly during ripening (Aminifar et al., 2010). The volatile compounds (acids, esters, alcohols, cyclic aromatic compounds, ketones, and aldehydes) and protein profiles of Lighvan cheese over a 90-day ripening period were investigated by Aminifar et al. (2014). They used sodium dodecyl sulfatepolyacrylamide gel electrophoresis (SDS-PAGE) for the assessment of protein degradation during the ripening period. They revealed that the degradation of β - and α S-casein was higher during the initial stage of ripening (first month) of ripening than the last stages. Lavasani et al. (2012) studied the physicochemical and biochemical changes of Lighvan cheese over 90 days of ripening in brine. Acidity, pH, dry matter, fat values, lipolysis level, WSN, TN, ripening index (RI), trichloroacetic acid-soluble nitrogen (TCA-SN), and organoleptic assessments were analyzed. Dry matter and fat values decreased during ripening. Lipolysis level, RI, TCA-SN values, and salt content increased continuously until the end of the ripening period, but TN decreased throughout a 90-day storage period.

2.7 Conclusion and future perspectives

Nowadays genetics, environment, and particularly diet are the main players of human health, and increasingly health promoting and prevention of diseases have been connecting to food and food ingredients. Although, for centuries, food and food components have been considered as a remedy for treating many diseases in Persia, recently, Iranian ethnic dairy products have attracted the attention of scientists, researchers, processors, and consumers owing to their nutritional value, health-promoting, and unique organoleptic properties. Nevertheless, bovine milk products are produced these days at an industrial scale, nonbovine milk (mainly sheep milk products) have been the main traditional dairy foods such as sour buttermilk, Kashk, Kashk-e zard, Tarkhineh, Pot cheese, Siamazgi cheese, Lighvan cheese, and many other Persian ethnic dairy foods. Iranians, like other communities, are seeking safe foods containing bioactive components and/or probiotics originating from tradition or novelty, to prevent or tackle illnesses. Therefore traditional dairy foods have considered over and above short-term commercial aspects and have been the point of focus for long-term scientific investigations. Self-carbonated sour buttermilk with hypocholestrolemic properties has been consumed by consumers who regularly intake high amounts of milk fat. Within this context, self-stable Kashk has provided high calcium and protein contents for Iranians specifically children and the elderly. Adapted LAB have acted as the most important factor for therapeutic properties and self-stability of sour Doogh and various types of cheeses such as Pot, Siamazgi, and Lighvan. Additionally, they have contributed to provide distinct aroma, flavor, taste, and structure to sour buttermilk, Kashk, Kashk-e zard, Tarkhineh, Pot cheese, Siamazgi cheese, and Lighvan cheese.

Safe, functional, and traditional Iranian dairy foods, with accepted organoleptic properties for a global consumer base, are yet to be investigated. In addition, the type and the amount of bioactive components/probiotic in a particular ethnic product should be optimized based on personalized nutrition requirements. The potential of those products needs to be proved by scientifically established clinical trials. The mechanism of controlling the pathogens by LAB or other factors remains to be better understood through the systematic study of Iranian traditional dairy foods.

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