Review Article



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Insights into a traditional Iranian food, "Kashk" as a promising candidate for functional food: A literature Review

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ABSTRACT

The traditional foods of many nations can be a platform for the development and introduction of functional foods. Iranian *kashk* is a fermented milk product obtained by adding water to yogurt, churning butter, adding salt, and then drying the product. This review was aimed to provide insight into all aspects of introducing chemical and nutritional components, production stages, further improvement, and enrichment. This review will also look at the types of microbial and chemical contamination found in kashk. Traditional Iranian kashk contains valuable protein, mineral content, and probiotics (such as types of *Lactobacilli, Enterococci, Bifidobacteria,* and *Pediococci)*, alongside functional peptides with healing functionality. Therefore, kashk can be a good candidate for further development as a functional food. However, due to the traditional preparation and manufacturing process, the production of kashk contains all the challenges and contaminations associated with dairy foods. The presence of microbial contaminants, chemical toxins, and heavy metals such as *E. coli, Staphylococcus aureus, Clostridium* spp., *Aspergillus* spp., aflatoxins, phthalates, PCBs, iron, copper, lead, and arsenic must be considered. Moreover, it is necessary to adopt strategies to reduce them to develop a functional and safe product. This study, the first of its kind, filled the gap in the comprehensive evaluation of Iranian kashk and presented a comprehensive picture of its platform for the supply of functional foods.

Keywords: Functional Foods; Kashk, Probiotics; Lactobacillus spp.; Nutrition



INTRODUCTION

As a conventional definition, functional foods are considered foods that have beneficial effects and some specific functions in the body in addition to their nutritional impacts. The consumption of functional foods can lead to an improvement in health and/or a reduced risk of disease [1-3]. This traditional definition has been criticized for being too broad and confusing for several reasons. For example, it is not clear what healthy and fortified foods should be included. Therefore, a new definition of functional foods has been proposed that emphasizes innovative formulations that include a substance or microorganism that potentially promotes health or prevents disease in safe and adequate amounts [4, 5]. Bioactive compounds are considered the basic structural units and the biochemical components of functional foods and they are responsible for the therapeutic effects of functional foods [6]. Martirosyan et al., in several studies, have introduced a new definition, classification, and regulation of functional foods. They also proposed an approach for producing functional foods and bringing them to the market. Their proposal includes 17 steps from targeting a functional product to conducting post-market studies [7, 8]. They also proposed a 5-step regulatory paradigm for the certification of a functional product by the Functional Food Center as a proposed certification center [9]. Today, the study and application of functional foods have gained new dimensions. For example, the use of Quantum and Tempus theories in functional food studies to develop personalized nutritional interventions, prevent diseases, and promote health and well-being are recent iterations of this subject [10]. Additionally, the use of functional foods as accessible and natural resources can strengthen the approach of non-pharmacological interventions, preventive healthcare, and personalized nutrition [11]. Traditional foods are generally considered to be foods with a long history of consumption for many years and often belong to a specific geography and population, deeply reflecting the culture and customs of their original communities as well as their climatic, economic, and cultural conditions. A range of raw or cooked foods that have specific and distinguishable characteristics at each stage of raw materials, composition, and production or processing can be considered as traditional food [12, 13]. Also, looking at the traditional methods of food production and processing can lead to the production of foods that, in addition to their basic benefits, possess other health benefits, and lead to the development of creative and sustainable functional foods [14]. Furthermore, the role of traditional foods in achieving nutritional security is undeniable [15]. Functional foods are excellent candidates for the GRAS certification process because they fulfill all the requirements for GRAS (Generally Recognized as Safe) certification [16]. Consumption of fermented foods can lead to antimicrobial, antioxidant, and peptide production benefits due to their unique functional properties resulting from the presence of probiotic microorganisms [17]. For example, consuming fresh fermented milk is associated with a lower risk of type 2 diabetes compared to unfermented milk [18]. In Iran, there are also reports of infrequent consumption of milk and dairy products among adolescents, which can cause harm, especially in <u>FFS</u>

deprived areas of the country [19]. Research and development are underway in Iran in the field of probiotic and functional foods [20, 21]. Kashk is one of the traditional fermented dairy foods of the Middle East, especially Iran, which has attracted much attention as a promising functional food for several reasons, including the high presence of valuable nutrients such as calcium, protein, probiotics, and functional peptides. Kashk is the target of this study. Fermented milk containing probiotics as an appropriate food matrix has shown several health benefits [22]. Based on the definition of functional foods originating from the Functional Food Center, they are considered "natural or processed foods that contain biologically active compounds, which, in defined, effective, non-toxic amounts, provide a clinically proven and documented health benefit utilizing specific biomarkers, to promote optimal health and reduce the risk of chronic/viral diseases and manage their symptoms" [4]. Additionally, bioactive compounds are defined as the chemicals found in small amounts within plant/food products that have actions on the body that promote optimal health [23]. The Iranian kashk properly fits within the previously mentioned definition, as they contain some bioactive compounds that invoke positive responses within individuals. The present study aimed to investigate the comprehensive aspects of Iranian kashk as a functional food, including the introduction of the traditional and industrial production process, nutritional and probiotic compounds, ways to optimize the physicochemical, rheological, organoleptic, and nutritional properties of the product, as well as the challenges facing this product, including microbial contamination, fungal and chemical toxins, and antibiotics, based on studies conducted in Iran.

Literature search and databases: In order to conduct a comprehensive assessment, the keyword "kashk" (both in Persian and English) was searched in international

databases such as PubMed and Google Scholar, as well as regional and national Iranian databases such as ISC, Magiran, and Civilica, SID. All records were reviewed in both Persian and English and without time limits. The criteria for selecting articles for inclusion in the study were their focus on all aspects of introducing Iranian kashk, its nutritional composition, identification of functional components, enrichment, optimization, and the assessment of all microbial and chemical hazards of Iranian kashk. Due to the focus of this study on Iranian kashk, articles focusing on similar products in geographies other than Iran, as well as review studies, were excluded. The included original articles were divided into different groups based on their field of study (Figure 2).



Figure 2. Included articles based on thier filed of study

The process of producing traditional and industrial Kashk: In the simplest traditional way in Iran, kashk is made by boiling skimmed yogurt, concentrating it, adding salt and some local aromatic vegetables, and drying it in the sun in diverse forms (Figure 3) [24]. In some lesserknown versions, such as Zaboli yellow kashk or Tarhana, there are also other ingredients such as wheat bulgur, garlic, dill seeds, cumin seeds, coriander seeds, and turmeric powder. The stages of industrial production of kashk are as follows: first, samples are taken from the received milk, the necessary preliminary tests are carried out, and then the milk is weighed and strained in a relatively separate place from the production line. Then, the fat is separated from the milk by a fat separator. The milk is pasteurized and converted into yogurt at the appropriate temperature. This fat-free sour yogurt is heated to a boiling point, then cooled and drained. After adding salt, it is pasteurized in a double-walled steel boiler for half an hour at 65 °C. The pasteurized liquid Kashk is packaged in suitable and authorized containers and is ready for sale. To supply the product in dry form, the prepared liquid Kashk is dried in an incubator or cooking tunnels at the appropriate temperature, and, after adjusting the moisture, it is powered by a mill and packaged in suitable containers.



Figure 3: Stages of producing traditional dry Kashk and industrial liquid Kashk from it in Iran

Nutritional value, chemical composition, and standards: Kashk possesses a high protein content, minerals, phosphorus, and iron, and a low content of fat. Kashk is also rich in vitamin B, but poor in vitamin C and fatsoluble vitamins. The chemical composition of different kashks depends on the production method [25]. Kashks containing bulgur or wheat flour are high in carbohydrates and fiber. For instance, yellow kashk contains 61.9–71.43% carbohydrates [26]. Dried and

liquid kashk have an average protein content of 13.7%

and 8.6%, salt content of 0.8-1.5% depending on the geographical location of production, and calcium and phosphorus content of 0.19-0.25% and 0.18-0.31%, respectively) [27-29]. Iranian kashks are high in protein because they are made mainly from milk and without the addition of bulgur [30]. Also, the National Standards Organization of Iran has developed standards for the nutritional and microbial composition of liquid dry kashk, which can be observed in Table 1 [31-34].

Table 1. Standard limits of dried and liquid Kashk by Iran National Standard Organization ([31-34])

Parameters / Kashk type	Dried	Liquid
Chemical parameters		
Moisture (weight %)	≤ 10	-
Ash (weight %)	≤13	≤ 4
Protein (weight %)	≥ 50	≥13
Fat (weight %)	≤11	-
Salt (weight %)	≤9	≤3
Total dry matter (weight %)	-	≥ 25
рН	-	≤ 4.5
Phytosterols (%)	-	≤ 3
Mycotoxins - (ppt)	0	0
Aflatoxin M1 (100 ppt)	100	100
Microbial parameters		

Parameters / Kashk type	Dried	Liquid
Coliforms (MPN/g)	0	10
Escherichia coli /g	-	0
Coagulase-positive Staphylococcus spp. CFU /g	0	0
Yeasts and molds CFU/g	-	≤ 10 ²
Sulfite-reducing Clostridiums CFU/g	≤ 10 ²	≤ 10 ²

Probiotic and antimicrobial properties: Probiotic means "for life" in Greek. This word was first suggested by Lilly and Stillwell as a way to describe a microbe promoting the growth of another microbe. Probiotics are defined as live, specific microorganisms that, when administered in sufficient numbers through inoculation or colonization, alter the microflora in a part of the host's body and have beneficial effects on the host's health [35, 36]. Probiotics can be considered a main part of functional foods since they possess health benefits in addition to their traditional nutrition function [37]. The most common probiotic bacteria are Bifidobacteria and Lactobacilli [38]. To be considered a probiotic, a microorganism must possess certain characteristics. These characteristics can include several items, such as resistance to gastric acidity and bile acid, ability to adhere to mucus and/or human epithelial cells and cell lines, antimicrobial potential against pathogens, inhibitory effect on pathogen adhesion to surfaces, bile salt hydrolase activity, and improving the viability of probiotics [39-44]. Therefore, Tajabadi et al. by adding bile acid and salt to a selective enrichment medium for lactic acid-producing bacteria (MRS broth), isolated a number of strains with antibiotic potential from traditional Iranian dairy products, including traditional Kashk [45]. Among the Kashk samples, three Lactobacillus isolates that were resistant to acid and bile salts were identified. Two of the isolates had populations above 10⁷ CFU after acid treatment. Saboori et al., (2022) also isolated eight lactic acid bacteria including Enterococcus faecium KKP 3772, Enterococcus faecium C1, Pediococcus pentosaceus H11, Pediococcus pentosaceus VNK-1, Lactococcus lactis RSg, Enterococcus faecalis P190052, Enterococcus mundtii CECT972T, and Lactiplantibacillus plantarum PM411,

from kashk samples of two provinces of Iran (Fars and Khorasan Razavi). Among these isolates, six of them were resistant to acidity and had appropriate antimicrobial activity [46]. Cell surface hydrophobicity is a key feature that is a major factor in the ability of LAB to adhere to intestinal cells and further proliferation. In this study, the highest and the lowest hydrophobicity belonged to the Pediococcus pentosaceus VNK-1 with 66.7% and Enterococcus mundtii CECT972T with 22.4%, respectively [47]. In Golestan Province, Iran, 35 Lactobacillus spp. isolates were isolated from traditional local kashks, including L. casei, L. acidophilus, L. bifermentans, L. animalis, L. buchneri, L. delbrueckii, L. sakei, L. brevis, L. fructivorans, and L. helveticus in order from highest to lowest abundance. Among these, L. casei, L. acidophilus, and L. bifermentans showed the broadest antimicrobial effect against four gastrointestinal pathogens especially *Shigella dysenteriae* by forming inhibitory growth zones [48]. In addition to rod-shaped bacteria, cocci such as Enterococci can also have probiotic potential. In East Azerbaijan province, about seventeen Enterococci samples that were resistant and tolerant to acid and bile salts, including E. avium, E. faecium, E. durans, and E. faecalis, were isolated from traditional dairy products including kashk. E. avium and E. durans showed the broadest antimicrobial effect of some pathogens [49]. In addition, the presence of bacteriocin-encoding genes in isolates originating from traditional dairy products can also confirm their probiotic potential. In this regard, *Enterococcus faecalis* was isolated from about 40% (8/20) of traditional kashk samples from the Chaharmahal and Bakhtiari provinces in Iran, and some of them contained Enterocin-encoding genes [50]. The casein-derived peptides of kashks of Fars province can inhibit the

antibiotic-resistant *S. aureus* isolates which were isolated from patients with atopic dermatitis by 45% [28]. Additionally, *Lactobacillus plantarum* TW29-1 isolated from Zaboli yellow kashk had all the appropriate probiotic properties, including tolerance to bile and acid, broad antimicrobial properties against a range of grampositive and gram-negative pathogens, hydrophobicity, auto-aggregation, and inhibition of pathogen attachment to intestinal cell lines [47]. In another study, *Lactobacillus* spp., *Pediococcus* spp., and *Streptococcus* spp. were dominantly present in the yellow kashks of the Sistan and Baluchistan provinces [26].

Other health benefits of Kashk: Iranian kashk also has been shown to possess other health benefits, such as skin-repairing activity. Folliero et al. (2022) illustrated that, based on the scratch and vitality tests, the treated keratinocytes with kashk peptide extract would leave a major wound-healing impact on the damaged epithelia and positively lead to tissue recovery [51]. Also, in a randomized clinical trial study on 70 overweight or obese women in Yazd province, it was shown that consuming a low-energy diet plus 50 grams of kashk daily can improve various anthropometric and biochemical indicators. For instance, the reduction of triglycerides and low-density lipoprotein cholesterol levels, body mass index, fat percent, and waist circumference was observed in the intervention group compared to the control group from the outcomes of the consumption of this diet [52]. Moreover, in a cross-sectional study on a large population of Iranian adults, an inverse relationship was observed between kashk consumption and anxiety symptoms. [53].

Optimization and fortification of Kashk: Efforts have also been made around the world to fortify dairy products to enhance their health benefits, such as improving gut microbiota [22, 54]. In Iran, several studies have added different substances and additives to fortify kashk and assessed their impact (Figure 4). The use of plant

products by inhibiting bacteria and fungi. On the other hand, it can reduce the survival of probiotics in kashk [55, 56]. Two studies in Iran using essential oils from the mint family (Lamiaceae), reported a decrease in the survival of probiotics in kashk with an increase in its concentration compared to the control group [56, 57]. Kashk has a low omega-3 fatty acid content, so Rashidi et al. (2021) reported that adding different percentages of flaxseed extract to kashk increased pH, moisture, fat, and viscosity, and decreased acidity, protein, and solubility of the product. However, the presence of this extract in the product reduced the color and flavor scores at high levels [58]. On the contrary, adding inulin and guar gum to the kashk can improve its flavor, mouth feel, and texture. Also, it can increase adhesiveness and acidity and reduce the syneresis and dry matter of the liquid kashk [59]. Amaranth seed powder can also increase the fiber and dry matter content of kashk as well as its mineral elements, including calcium, phosphorus, potassium, iron, and magnesium. However, adding it to liquid kashk can lead to lower overall acceptance and texture scores of the product [60]. One of the problems of industrial liquid kashk is the phase separation of the product, which hurts its rheological, textural, and microbial quality properties during storage. For this purpose, Mohammad Nejad et al. (2024) used pectin hydrocolloid as a stabilizer in liquid kashk formulation. They reported increased acidity, water holding capacity, firmness, and improvement in sensory evaluation indices, including apparent syneresis, texture, consistency, color, appearance, and overall product acceptance [61]. Also, adding modified tapioca starch as another stabilizer to liquid kashk can increase the product's acidity, hardness, adhesiveness, viscosity, and overall acceptability [62]. Tragacanth gum, when added in 0.5% (w/w) to the liquid kashk, reduces the syneresis due to a significant increase in viscosity of the continuous phase simultaneously with

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essential oils can, on the one hand, lead to diversification

in flavor and aroma and increase the shelf life of food

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the aggregated casein particles [24]. Karimi et al. (2024) used chitosan-coated nano-liposomes as carriers for hydrolyzed and un-hydrolyzed maize zein in order to enhance the functionality and shelf life of probiotic kashk and reported the improvement of rheological properties and probiotic viability and consumer acceptance [63]. To increase the protein content of kashk, a part of the original initial milk can be replaced with soy milk. Although soy milk can significantly increase the moisture, viscosity, consistency, and protein content of kashk, its addition at particularly high levels can reduce the product's flavor, color, and overall acceptability [64]. In Iran, efforts have also been made to optimize and enrich yellow kashk. For example, replacing part of the wheat flour with barley flour increased the protein, phytic acid, phenolic content, and antioxidant properties. Also, replacing the type of starter including Saccharomyces cerevisiae PTCC5052, Lactobacillus delbrueckii PTTC1737, Lactobacillus plantarum PTCC1058 alone and in combination can increase protein digestibility, zinc antioxidant properties, absorption, and overall acceptability of yellow kashk and reduce the amount of phytic acid [65]. Also, adding unprocessed and hydrothermal wheat bran increased total fiber levels and improved zinc absorption. Adding unprocessed bran increased protein levels and adding hydrothermal bran reduced it. In addition, adding hydrothermal bran improved protein digestibility [66]. To preserve the nutritional value, sensory properties, and functional characteristics of milk and consequently kashk, nonthermal techniques such as high-pressure processing, ultrasound treatment, pulsed electric fields, ultraviolet light, and oscillating magnetic fields were used to ensure maximum nutritional and functional value and the health benefits derived from them, as well as microbial safety in kashk [67].



Figure 4: Different additives and supplements added to kashk in Iranian studies

Microbial contamination of Iranian kashk: Milk, as the main and primary ingredient for the production of kashk, can become microbially contaminated in various ways.

The bulk milk can become contaminated by three main sources: bacteria from the external surface of the udder and teats, the surface of the milking equipment, and

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mastitis organisms from within the udder. Milk after secretion can be contaminated by bacteria from different sources, including animal feces, soil, air, feed, water, bedding material, animal hide, udder and teats, milk containers, and milking staff [68-71]. Studies in Iran have also investigated the microbial contamination of kashk. For example, Dehkordi et al. (2014) reported that 8.33% of traditional Iranian kashks were contaminated with E. coli. Strain O157 was the dominant strain (26%) among them. Also, virulence genes including stx1, stx2, eae, ehly, cnf1, cnf2, iutA, cdtB, pap A, tra T, sfaS were present among these isolates [72]. Additionally, traditional liquid kashks from Hamedan also had unacceptable microbial contamination in terms of the number of coliforms, Staphylococcus spp., mold, and yeast [73]. Traditional kashk samples from the tribes of Chaharmahal and Bakhtiari province were not contaminated with E. coli and coagulase-positive Staphylococcus aureus, and only 25% of the kashk samples had a total count of microorganisms, mold, and yeast above the standard level [74]. In accordance, the traditional kashks of Isfahan province had no E. coli contamination [75]. The traditional kashks of Gilan province also had no contamination by Clostridium botulinum and botulinum toxin [76]. Another study from Gilan province also revealed that only 8 and 12% of traditional liquid kashks exceeded the standard levels for coliform and coagulasepositive S. aureus, respectively and the rest were in acceptable ranges for all microbial indices [77]. Fallahi et al. (2014) also reported that only 15% of kashks in Isfahan City, Isfahan province had mold counts above the permissible limit, with the dominant species of Aspergillus niger among them [78].

Aflatoxin contamination: Mycotoxins are natural contaminants of dairy products and the secondary metabolites of molds. They are mainly produced by *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp.,

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under inappropriate temperature and humidity conditions of storage. They are transmitted to the lactating animals and consequently to humans from contaminated feed through poor control conditions [79-81]. Aflatoxin is one of the most important mycotoxins which is present in Iranian dairy and kashk. According to a report by Amirpour et al. in 2015, about 90.6% of the industrial pasteurized liquid kashk sold in Tehran was contaminated with aflatoxin M1 (AFM1) in the range of 6.2-336.6 ppt with an average of 60.2 ± 75.5 ppt [82]. Bahrami et al. (2016) and Fallah et al. (2011) also reported the levels of AFM1 in traditional kashk as 62.1 ± 2.2 and 53.1 ± 1.1 ng/kg, respectively [83, 84]. Based on the pooled data of a systematic review and meta-analysis study in Iran, 12.63% of kashks were contaminated by AFM1 in concentrations in amounts exceeding the permitted standard levels. Also, according to this study, the average prevalence of aflatoxin in kashk was 50.052% (95% CI: 31.339-68.764) [85]. However, it has been shown that the process of industrial production of kashk in the same concentration between initial milk and commercial kashk decreased the AFM1 by 91% [86].

Chemical and antibiotic contamination: The excessive presence of heavy metals in dairy products and kashk is another challenge in its production and consumption in Iran. In this regard, Mohammadi Sani et al. (2010) compared the amount of heavy metals in liquid kashk prepared from traditional kashk and pasteurized yogurt and showed that the traditional process of producing kashk can lead to a significant increase in the presence of heavy metals in kashk. In this study, the amount of copper and iron in all samples was higher than the permissible limit. The amount of lead in kashk prepared from traditional dry kashk was higher than the permissible limit, but in kashk prepared from pasteurized yogurt was within the permissible limit. The amount of arsenic in both types of kashk was higher than the

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permissible limit. The contents of arsenic, copper, zinc, and iron were significantly greater in liquid kashk prepared from traditional dry kashk than in the type prepared from pasteurized yogurt. The entry of heavy metals into kashk can be caused by the conditions of traditional dried kashk production, especially drying and storage conditions, including contact with unsuitable containers and equipment, production and storage in outdoor conditions, and its supply under unsanitary conditions [87]. It seems that there is no scientific paper on the prevalence of antibiotic residue in kashk in Iran which notices its importance in drawing a comprehensive insight into the safety of Kashk.

Other contamination and challenges: Since milk and dairy products are the most important sources of human exposure to polychlorinated biphenyls (PCBs), Kiani et al. (2023), investigated the contamination of Iranian kashk with them and reported contamination of 18.66 ± 2.42 ng/g fat of Iranian kashk with 6-NDL-PCBs. However, after calculating the health risk assessment from kashk consumption, they stated that this amount of contamination does not pose a health risk to Iranian consumers [88, 89]. Also, the mean of Bis (2-ethylhexyl) phthalate (DEHP) and total phthalates (PAEs) in kashk samples were 2.43 ± 1.02 and $10.07 \pm 1.06 \ \mu g/kg$, respectively which were lower than the international permittable limits and their intake via Iranian kashk consumption pose no health risk [90]. The salt content of Iranian kashk is another feature that should be taken into consideration. Massomian et al. (2025) and Shiroodi et al. (2012), reported the salt content of 2.78 ± 0.25 (%) and 3% of kashk, respectively [24, 91]. However, Noori et al. (2013), stated that the salt content of traditional kashk was 0.89-1.52% [92]. In general, according to numerous studies conducted on its various aspects, Iranian kashk is a product with functional properties and, high nutritional <u>FFS</u>

composition, and also originates from the historical Iranian culinary tradition. To our knowledge, no study has so far comprehensively studied all aspects of Iranian kashk as a functional food and has not discussed the microbial and chemical hazards of its traditional production. Therefore, this could be an innovative aspect of the present study.

CONCLUSION

The cuisines of different nations reflect their culture and economy and are a potential source for the production and development of functional foods. Traditional Iranian kashk, which is derived from fermented milk and is produced in dried form with various ingredients and in liquid and industrial form, can be a suitable platform for introduction as a functional food. Iranian kashk is rich in nutritional components such as high protein content, minerals, and vitamin B, and also has suitable functional components such as potential probiotic microorganisms and functional peptides, as well as positive effects on women's physical indicators and wound healing potential. Several studies have also been conducted in Iran to optimize the formulation, and nutritional value and develop further functional behavior by adding functional components such as some stabilizers and functional materials. However, there are also challenges related to contaminants that may be added, especially during traditional production of kashk, such as heavy metals, bacterial and fungal contaminants, mycotoxins, PCBs, and PAEs, as well as strategies to reduce them.

List of abbreviations: LAB: Lactic Acid Bacteria, CFU: Colony Forming Unit, MPN: Most Probable Number, spp.: species, PTCC: Persian Type Culture Collection, INSO: Iranian National Standards Organization, pH: potential of hydrogen, w/w: weight by weight, AFM1: Aflatoxin M1, ppt: parts per trillion, ng/kg: nanograms per kilogram, µg/kg: micrograms per kilogram, CI: Confidence Interval, DEHP: Di(2-ethylhexyl) phthalate, PAEs: Phthalate Acid Esters, PCBs: polychlorinated biphenyls, 6- NDL-PCBs: 6non-dioxin-like polychlorinated biphenyls, ISC: Islamic World Science Citation Center, SID: Scientific Information Database.

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