



Fungal contamination and chemical assessment of Iranian Doogh marketed in Hamadan, Iran

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Introduction

Doogh as a native and traditional Iranian fermented drink has high value of nutrients and therapeutic properties. This product is also consumed in other countries such as Azerbaijan, Turkey, Armenia, Iraq, Syria, the Balkans, and to a lesser extent in other countries of the Middle East and central Asia [1]. This dairy-based drink is usually manufactured by mixing water, salt, and yoghurt, and acidified by fermentative bacteria including *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. Chemical properties of Doogh consist of non-fat milk solids (minimum 3.2% w/w), milk fat (maximum 50% of total dry non-fat in the final product), salt (0.2-1.0% w/w), and natural or natural identical flavoring compounds with a maximum pH of 4.5. Carbon dioxide

Abstract

Doogh is an Iranian traditional dairy-based drink product formally identified as Iranian national drink. This fermented acidified drink has high nutritional value and pleasant organoleptic properties. Samples (n= 120) collected from both industrial and traditional Doogh retail stores in Hamadan in 2015 were analyzed for total solids (%), pH, titratable acidity, salt (%), mold and yeasts count (cfu/ml) according to the Iranian National Standards (no. 2453). Results showed that 100% and 24% of the traditional and industrial Doogh samples had fungal contamination over than 100 cfu/ml, respectively. Moreover, the mean values of titratable acidity, pH, salt, and total solids showed a significant between-group difference. According to this study, employing hygiene programs regarding the production processes is highly recommended.

Keywords: Chemical, Contamination, Dairy, Fungal, Hamadan

(minimum 0.4% w/w) may be artificially or naturally (through fermentation) added to the product [1,2]. Doogh have many salutary effects, including improving lactose digestion in lactose intolerance individuals, lowering serum cholesterol levels, and stimulating the immune system [3].

Nowadays, due to the increased public awareness about the harmful effects of some carbonated beverages, the consumption of this product has increased by about 40% [4]. One of the main problems in manufacturing plants is fungal contamination of dairy fermented products. Therefore, it causes undesirable features such as bitter taste, swelling, decreased shelf life, and lowered consumer acceptance [5,6]. Molds present in air, water, soil, walls,

floors, ceilings, raw ingredients, packaging materials, and plant equipment; thus, unsuitable sanitizing programs increase the risk of fungal contamination in dairy products.

Control of fungal contamination in dairy products puts emphasis on good hygiene practices during production processes like packaging atmosphere and good air-filtration systems that lead to the reduction of the mold spores level in dairy plants [7]. Because of low pH, cultured milk products such as fermented milk, yogurt, drinking yogurt, cottage cheese, cream cheese, etc. provide ideal media for the proliferation of molds and yeasts. These microorganisms are able to grow in a wide range of pH accordingly; they are normally able to spoil dairy products with a low pH. On the other hand, some microorganisms belonging to the bacterial families (i.e., Bacillaceae, Enterobacteriaceae, and Pseudomonaceae) cannot proliferate in these conditions [8]. In a study conducted by Sperber and Doyle, it was shown that galactose, which is fermented by yeasts such as *Saccharomyces cerevisiae* and *Hansenula anomala*, has an important role in the spoilage of yogurt [9]. It is now well established from a variety of studies that Acid-tolerant, psychrotrophic, fermentative yeasts may be able to grow in yoghurt and cause blowing due to CO₂ production. Species of *Candida*, *Saccharomyces*, *Pichia*, *Rhodotorula*, *Kluyveromyces*, and *Torulopsis* have been isolated from blown packs of yoghurt [10]. Furthermore, growth of species of *Mucor*, *Aspergillus*, *Rhizopus*, *Alternaria*, and *Penicillium* can occur in the product during retail storage, leading to the production of mycelia mats or buttons and visible spoilage. There are a large number of published studies describing the role of mold spores in the contamination of yoghurt by airborne dust particles, packaging, and added ingredients as well as through poor hygiene [11].

Microbiological analysis of bottled commercial and homemade ayran sold in Ankara and Kars in Turkey revealed the presence of very high numbers of total coliforms, yeasts, and molds. Bottled ayran was found to be of higher quality

than homemade ayran samples that this contamination could be due to the lack of satisfactory sanitary conditions and quality control during manufacturing the ayran [12]. Another study was conducted in Mashhad to determine microbial condition (i.e., total aerobic bacteria, coliforms, psychrophilic bacteria, Lactic Acid Bacteria (LAB), and yeast) of three dairy plants revealing that the quality of Doogh depends on quality of raw milk, effectiveness of heat treatment, microbiological quality of added ingredients, packaging materials, cleanliness of surfaces coming into contact with the Doogh, and efficiency of the plant sterilization [5]. The goal of this study was to evaluate fungal contamination and chemical analysis of industrial and traditional Doogh samples marketed in Hamadan, Iran.

Method

Sample preparation: Based on Iranian national standard (no. 2453), totally 120 Doogh samples consisted of 100 industrial samples from four commercial brands (25 samples per each) and 20 samples from traditional Doogh purchased from different areas of Hamadan city during 2014 were collected. After sampling, all the samples were transferred to the laboratory under refrigerated conditions and analyzed immediately.

Chemical analysis: Each sample was mixed to ensure homogeneity and then analyzed for pH value and titratable acidity. To measure pH value, first the pH meter was calibrated and then pH values of all samples were measured at room temperature using a digital pH meter (Hanna, Italy). To determine the titratable acidity, 10 ml of samples were mixed with equal volume of distilled water. 3-4 drops of 0.5% phenolphthalein indicator was added and finally, the content was titrated with 0.1 N NaOH solution [13]. Total solids and salt content were measured according to the method of Iranian National Standard no. 637.

Microbiological analysis: To enumerate mold and yeasts, all samples were diluted tenfold serial dilution by 0.1% peptone water and then,

100 µl from each dilution (10-1, 10-2, and 10-3) was poured on Sabouraud Chloramphenicol agar plates (Quelab Company, Canada) and inoculated with a sterile bent glass rod. Plates were incubated at 25°C for 3 to 5 days and then, they were examined for the presence of yeast and mold colonies. Finally, plates containing 15-150 colonies were counted. The number of colony-forming units in samples was calculated using below formula:

Yeast and Mold count (cfu/ml)=number of colonies/(volume plated×dilution factor)

Statistical analysis: Data management and analysis were performed using SPSS-20. In order to compare means, one-way ANOVA was run. In cases with a meaningful difference in means, Tukey test was performed to compare means between the groups. Differences with 95% confidence level were considered significant ($p < 0.05$).

Results

The results of chemical and microbiological

analysis of Doogh samples are presented in Table 1 and 2, respectively. One-way ANOVA showed that the mean values of acidity, pH, total solids, and salts between the samples had statistically significant differences ($p = 0.000$, $F = 265.05$, $F = 504.81$, $F = 116.4$, $F = 254.55$, respectively). Moreover, Tukey test indicated that the mean values of acidity and salt were significantly higher in traditional samples than industrial samples ($p = 0.000$). On the other hand, the traditional samples had no statistically significant differences regarding pH and total solids than B samples ($p > 0.05$). According to the Iranian national standard for Doogh, maximum acceptable limits of fungal contamination should be 100 cfu/g [1]. As can be seen from Table 2, 100% and 24% of the traditional and industrial Doogh samples had contamination level higher than the standard level. Mostly, among industrial Doogh samples, 32% of A samples were contaminated with molds and 64% of D samples were contaminated with yeasts.

Table 1 Chemical properties of the examined samples

Sample type	Sample (N)	Acidity (%)			pH			Total Solids (%)			Salt (%)		
		Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD
A	25	0.46	0.67	0.51±0.08 ^{ab}	4.20	4.25	4.22±0.01 ^a	0.29	0.32	0.30±0.009 ^a	0.31	0.37	0.34±0.015 ^a
B	25	0.50	0.55	0.51±0.51 ^a	3.20	3.52	3.46±0.09 ^b	0.23	0.26	0.24±0.009 ^b	0.29	0.32	0.30±0.01 ^b
C	25	0.66	0.70	0.67±0.67 ^c	3.75	3.81	3.78±0.02 ^c	0.33	0.39	0.36±0.014 ^c	0.29	0.33	0.30±0.012 ^b
D	25	0.40	0.50	0.44±0.03 ^b	4.20	4.27	4.23±0.02 ^a	0.25	0.30	0.28±0.014 ^d	0.28	0.33	0.31±0.012 ^b
E	20	0.92	1.67	1.26±0.2 ^d	3.03	3.65	3.39±3.17 ^b	0.17	0.35	0.23±0.053 ^b	0.40	0.61	0.50±0.057 ^c

* a, b, c, d; industrial samples, E; traditional samples

In each columns, results with the same letters had no significant difference ($p > 0.05$).

Table 2 Results of mold and yeast counts in the examined samples

Sample type	Sample (N)	Positive samples N(%)	Mould and yeast count ($\log_{10} \pm SD$)
A	25	8(32)	2.56±0.25
B	25	0	<1
C	25	0	<1
D	25	16(64)	3.7±0.02
E	20	20(100)	5.09±0.72

Discussion

Due to its nutritional value and health properties, Doogh has a special status among dairy products in Iran. This study was conducted to evaluate the quality of Iranian industrial and traditional Doogh. In line

with those of previous studies, the results indicated high fungal contamination in study samples while chemical quality was in accordance with Iranian National Standard. Noori and Keshavarzian in a study on the

physicochemical properties of four Doogh samples showed that the mean values of pH, acidity, and salt content were 3.53, 0.81, and 0.72%, respectively [14]. Gulmez et al. in a similar study evaluated the microbiological quality of bottled and homemade ayran samples and indicated the presence of very high number of total coliforms, yeast, and molds. Moreover, they indicated that bottled ayran had higher quality than homemade ayran [12]. El-Diasty et al., in mycological examination of raw milk and yoghurt samples, reported that 80 and 50% of raw milk and yoghurt samples were contaminated, respectively [15]. Furthermore, Sangatash et al. showed that microbiological quality of Doogh depends on the quality of raw milk, the sufficient temperature during processing, microbiological quality of added ingredients, packaging materials, food contact surfaces, and control of sterilization in the plant [5]. Moreira et al. conducted a study on the fungal contamination of yoghurt samples and identified high levels of contamination by some species of yeasts like *Debaryomyces hansenii*, *Saccharomyces cerevisiae*, *Mrakia frigida*, *Hansenula* spp., *Candida parapsilosis*, *Debaryomyces castellii*, *Candida maltose*, and some species of filamentous fungi like *Monilia* and *Penicillium* species, which could be due to inadequate refrigeration of products [16]. In addition, the presence of fungi in examined yoghurt samples was demonstrated by Eman et al. that could be because of unsuitable preheating process during manufacturing and using undesirable sterilized plastic containers or inefficient cooling during storage [17]. Study of Fallahi and Madani in Isfahan revealed that 13.4% of Doogh samples had more fungal contamination than acceptable standard limits and main contaminant fungi were allocated to *Penicillium* spp. [18].

Conclusion

This project was undertaken to determine fungal contamination and some chemical analysis in industrial and traditional Doogh samples marketed in Hamadan, Iran. The

most obvious finding to emerge from this study is that all traditional Doogh samples and 24% of industrial Doogh samples had fungal contamination while both traditional and industrial ones were in accordance with Iranian national standards. Further research should focus on determining the level of fungal contamination in places of production until sale markets in order to investigate the importance of distributing product. Considering the high fungal contamination of Doogh, it is recommended to apply good manufacturing practice (GMP) and HACCP program during production processes.

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Contribution

Study design: AAS

Data collection and analysis: NS, AAS

Manuscript preparation: AAS, MRP, NS

Conflict of Interest

"The authors declare that they have no competing interests.

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