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Investigation of Traditional Shir Berenj Safety in Khouzestan Province: Microbiological and Chemical Quality Assessment

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ABSTRACT

Background: Consumption of artisanal foods, such as Shir Berenj, is common in Iran. Nevertheless, little evidence can be found about the safety of traditional foods in the literature. This study aimed to investigate the microbiological and chemical quality of Shir Berenj samples in Khouzestan province.
Methods: Shir Berenj samples were collected from Ahvaz, Shoushtar, Andimeshk, and Dezful. They were put in an ice pack and transferred to the laboratory. Microbiological (total count, psychrophile count, mold and yeast, *S. aureus*, *Salmonella*, *Enterobacteriaceae*, and *E. coli*) and chemical (fat content, pH, and dry matter) tests were carried out to determine the safety and quality of samples.
Results: According to Iran standards, 96% of Shir Berenj samples were rejected. The data showed that microbial contamination was the most critical problem for the safety of Shir Berenj samples. Merely 70 % of samples were contaminated with *Enterobacteriaceae* beyond the standard limit. Moreover, 50 % of samples were contaminated with *Escherichia coli*, indicating poor hygienic conditions.
Conclusion: Surveillance organizations should be more watchful about the safety of Shir Berenj. We suggest that appropriate approaches should be used to improve the knowledge of food handlers involved in producing Shir Berenj.

1. Introduction

Shir Berenj is popular artisanal food in Khouzestan, Iran, and is consumed widely throughout the province. It is made with rice and milk and served either cold or warm. Traditional foods, such as Shir Berenj are popular due to their nutritional value [1-3]. The manufacturing process of traditional Shir Berenj is shown in Fig 1. Briefly, rice is rinsed and placed in a large container. Then milk and a small amount of salt are added and heated for about an

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hour. During heating, an adequate amount of milk is added, and the contents of the container are agitated simultaneously to achieve a desired viscosity and texture.

Despite all nutritional and health benefits, Shir Berenj is highly prone to spoilage and putrefaction. Two main components of Shir Berenj, including milk and rice, are an excellent environment for the growth of microorganisms [4]. Foods containing milk ingredients at suitable conditions, such as room temperature, facilitate the growth of microorganisms. Many microorganisms, including mold, yeast, and pathogens, can grow quickly in these foods. Many pathogens identified in milk and dairy products, such as Staphylococcus aureus, Escherichia coli (E. coli), Salmonella, etc., can seriously threaten public health [5]. *S. aureus* can be identified in the nose and skin of approximately 25% of individuals [6] and can be a source of contamination in milk and dairy products, such as Shir Berenj. *S. aureus* can cause food poisoning, and symptoms, including vomiting and nausea, can be observed 30 to 8 h after consuming contaminated food [6]. *E. coli* is an essential index of food safety, indicating fecal contamination. Symptoms such as diarrhea (often bloody), vomiting, and severe stomach cramps are seen five to seven days after ingestion of contaminated food. Although the response to the disease can differ in each person, in some cases, Hemolytic Uremic Syndrome (HUS) can be observed as potentially life-threatening [7]. *Salmonella* is another important bacterium that can grow in foods without unusual taste and smell. Symptoms often begin 6 h to 6 days after consuming contaminated food, including severe diarrhea, bloody stool, vomiting, and dehydration [8]. Chemical quality analyses are critical to ensure foods' safety, encompassing various tests, such as fat content, pH, and dry matter. Compliance of chemical quality indices with standards is crucial to ensure the consumers' health. On the other hand, incompatible chemical tests can be regarded as adulteration [9]. According to the Iranian Standards Institute (ISIRI 14681) [10], fat content, pH, and dry matter analysis must be carried out to ensure the quality and safety of Shir Berenj samples. For example, low amounts of dry matter refer to adulteration.

Although traditional Shir Berenj is consumed in high amounts, no evidence can be found in the literature about the safety and hygiene of this product. Therefore, this study aims to investigate the microbiological safety (total count, psychrophile count, mold and yeast, *S. aureus*, *Salmonella*, *Enterobacteriaceae*, and *E. coli*) and chemical quality (fat content, pH, and dry matter) of Shir Berenj samples in major cities of Khouzestan, Iran, including Ahvaz, Shoushtar, Dezful, and Andimeshk.

2. Materials and Methods

2.1 Sampling

50 Shir Berenj artisanal samples were purchased randomly from vendors of Khouzestan (Ahvaz, Shoushtar, Dezful, Andimeshk), southwest of Iran, from May 2020 to October 2020 (one sample from each vendor). Shir Berenj samples were transferred aseptically to the laboratory in an ice pack and placed at 4 °C until analysis. All analyses were carried out in the FDA laboratory of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

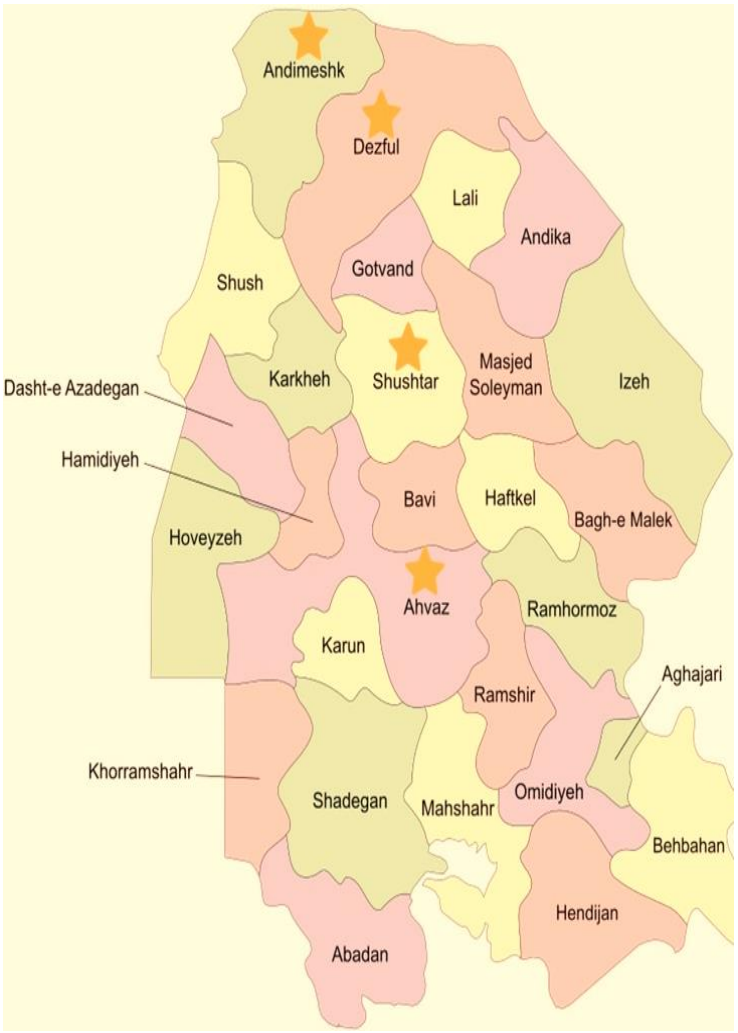

Figure 1: The geographical location of sampling in Khouzestan province, Iran.

2.2 Microbial analysis

10 g of homogenized sample was added with 90 mL ringer solution (Oxoid, Hampshire, UK), and 0.1 dilutions were obtained. Serial dilutions were made using ringer solution and exploited for all microbial tests. Total mesophilic and psychrotrophic counts were performed using plate count agar (QUELAB, Montreal, Canada). For total mesophilic count, plates were placed at 37 °C and 6 °C for psychrophilic count [11]. *S. aureus* was enumerated using Giolitti-Cantoni broth (QUELAB, Montreal, Canada) and placed in an incubator at 37 °C. After 24 h, a loopful was transferred to Baird-Parker agar (QUELAB, Montreal, Canada) and incubated at 37 °C for 48 h

[12, 13]. Shiny black colonies were confirmed as *S. aureus*. Coagulase-positive *S. aureus* was detected using brain-heart infusion broth (QUELAB, Montreal, Canada); subsequently, 0.1 mL of this media was mixed with rabbit plasma fibrinogen (0.3 mL) in a tube. Clot formation was considered a coagulase-positive *S. aureus* [13]. *E. coli* tests were carried out according to the MPN method. Briefly, lauryl sulphate tryptose broth (QUELAB, Montreal, Canada) was utilized to investigate gas formation. A loopful tube with gas formation was cultivated in *E. coli* broth (QUELAB, Montreal, Canada) and investigated for gas formation. Eosin Methylene Blue agar (QUELAB, Montreal, Canada) was used, and shiny metallic colonies were selected for IMViC analysis [13, 14].The mold and yeast enumeration were carried out using Yeast extract Glucose Chloramphenicol (YGC) agar, and plates were placed at 25 °C for three days [15]. For analysis of Enterobacteriaceae, a 1 mL sample was inoculated into 15 mL of molten (45 °C) violet red bile glucose agar (VRBGA, QUELAB, Montreal, Canada). After setting, an overlay of the molten medium was added. Plates were placed in an incubator at 30 °C for one day, and the large purple colonies were enumerated [16]. To detect *Salmonella* in Shir Berenj, 25 g of sample was cultured in 225 mL of pre-enrichment medium (Buffered Peptone Water) and placed in an incubator at 35–37 °C for 20 h. Next, it was transferred to Selenite Broth (QUELAB, Montreal, Canada) and Muller–Kauffmann Tetrathionate Broth Base (QUELAB, Montreal, Canada) and placed at 35 °C for 48 h and at 43 °C for 24 h, respectively. Subsequently, Xylose Lysine Deoxycholate Agar (QUELAB, Montreal, Canada), Brilliant Green Phenol Red Agar (QUELAB, Montreal, Canada), and Salmonella Shigella Agar (QUELAB, Montreal, Canada) were used (24 h at 35–37 °C) to detect *Salmonella*. Suspected colonies were analyzed by API 10 S identification strips [17].

2.3 Chemical analyses

pH was assessed using a digital pH meter (Sartorius, USA) calibrated with buffers (pH = 4 and 7). 2 g of sample was added to a dish and placed in a water bath at 90 °C to obtain a constant weight. Dry matter was calculated using the following formula: constant weight/primary weight. Fat content (%) was measured using the Gerber method [18].

3. Results and Discussion

The results of the microbiological examination of Shir Berenj samples are shown in Tables 1 and 2. Overall, the mean values for the total count, psychrophilic count, mold, and yeast were 8.2×10^4 cfu/g, 4.6×10^4 cfu/g, and 1.2×10^3 cfu/g, respectively. Samples from Shushtar had the highest values in total count and psychrophilic count. However, in the case of mold and yeast, the most contaminated samples were observed in Andimeshk. No S. aureus and Salmonella were detected in samples from all cities. However, in the case of *E. coli*, 50% of Shir Berenj samples were contaminated with the bacterium, and the most contaminated samples were found in Andimeshk (83.3%). Microbial assessment for Enterobacteriaceae indicated that 70% of samples were contaminated with more than 1100 cfu/g number of bacteria, and the worst results were observed in Shoushtar (85.7%). With regard to ISIRI, only 10^2 cfu/g is allowed for total mesophilic count [10]. The results of our study revealed that the mean value for the total count was significantly higher than this limit ($p < 0.05$). Microbial contamination of ready-to-eat foods has been reported widely in the literature. Secim et al. (2014) conducted a study and investigated 80 samples of traditional milky desserts (containing milk and rice) for their microbiological quality [19]. They reported that 59 out of 80 samples (74%) were rejected due to high values of the total mesophilic count. High amounts of total mesophilic bacteria in traditional foods such as Shir Berenj may be due to a lack of good manufacturing practices (GMP) and poor hygienic conditions in producing these foods. According to ISIRI, another microbiological criterion for Shir Berenj is mold and yeast count set at 10^2 cfu/g [10]. The data obtained by this study showed that mold and yeast count was significantly higher than the limitation defined by ISIRI 14681. Other studies have reported high amounts of mold and yeast. Secim et al. (2014) asserted that 56% of traditional milky desserts were rejected because of high mold and yeast values [19]. High amounts of mold and yeast indicate poor manufacturing hygienic conditions and improper cold chain during storage and distribution. Moreover, high values of mold and yeast in Shir Berenj samples could be airborne – especially with regard to the air pollution of Khouzestan province. In the case of Enterobacteriaceae, no maximum limit was set by ISIRI 14681. However, the contamination of Shir Berenj samples was high. The results of our study revealed that 70% of samples were contained >1100 cfu/g Enterobacteriaceae. This contamination reached about 85% in Shoushtar. Although Enterobacteriaceae was high in samples, no Salmonella was found. In a similar study, Igbinosa et al. (2020) [20] investigated microbial contamination of ready-to-eat foods and reported high Enterobacteriaceae contamination. The current study found no contamination for *S. aureus* and Salmonella. It could be due to the dominance of other microorganisms in the samples, such as Pseudomonas. Similar results were reported in traditional Doogh by Zadeh-Dabbagh et al. (2021) [18]. However, in another study by Colak et al. (2007), Salmonella was detected in Tulum cheese samples of Turkey, which was incompatible with our study [21]. According to ISIRI 14681, no *E. coli* must be found in Shir Berenj [10]. In this study, *E. coli* were positive in 50% of samples, indicating the rejection of half of the samples merely based on this criterion. Afshari et al. (2019) investigated the microbiological quality of ready-to-eat Olivier salads in Mashhad [22]. They reported that 23.07 % of samples were contaminated with E. coli. Overall, contaminated Shir Berenj and similar food products with E. coli point at poor personal hygiene and contamination of raw food used to produce Shir Berenj, such as milk and/or rice. This could even be caused

by using contaminated water in the production process of Shir Berenj. The mean values for pH, fat, and dry matter were 6.42, 6.09 %, and 22.79 %, respectively. The highest amount of pH was recorded for Andimeshk samples (6.66). Shoushtar samples exhibited the highest values of fat (7.07 %), and Ahvaz samples showed the highest amounts for dry matter analysis, followed by Shoushtar samples (23.41 % and 23.40 %, respectively).

Table 1: Mean values and standard deviation for the total count, psychrophilic count, and mold and yeast (log10 cfu/g)

Location	Criteria	Total count	Psychrophilic count	Mold and yeast
Ahvaz	Number of samples	30	30	30
	Mean ± SD	4.64 ± 4.73^{A}	4.51 ± 4.56^{A}	3.00 ± 3.10^{A}
Shoushtar	Number of samples	7	7	7
	Mean ± SD	5.34 ± 5.41^{B}	5.04 ± 5.16^{B}	3.00 ± 3.33^{A}
Dezful	Number of samples	7	7	7
	Mean ± SD	5.08 ± 5.33^{B}	4.60 ± 4.74^{A}	3.23 ± 3.42^{A}
Andimeshk	Number of samples	6	6	6
	Mean ± SD	5.08 ± 5.33^{B}	4.60 ± 4.74^{A}	3.23 ± 3.42^{A}
Total	Number of samples	50	50	50
	Mean ± SD	5.08 ± 5.33	4.60 ± 4.74	3.23 ± 3.42

*SD: Standard Deviation. According to the ISIRI 14681, the maximum limit for the total count and mold and yeast is set at 4 and 2 log10 cfu/g, respectively.

Another quality indicator for Shir Berenj, is chemical analysis. In this study, pH, fat, and dry matter were investigated. ISIRI 14681 set pH between 6.3-6.8 [10], and according to Table 3, all samples were within the range of standard. Moreover, ISIRI has proposed a minimum of 3% fat for Shir Berenj [10]. The mean values for fat content samples in all cities were higher than the limitation set by ISIRI 14681, which shows an ideal condition of Shir Berenj samples in this criterion. High fat content in Shir Berenj produced in Khouzestan province may be due to applying buffalo milk. In the case of dry matter, samples collected from Andimeshk demonstrated the lowest mean value, which was lower than the standard set by ISIRI (22%) [10]. Nevertheless, all other cities were higher than the limit set by ISIRI. Overall, small issues were observed in chemical quality analysis of Shir Berenj samples and major problems were detected in microbiological tests. Many factors can cause microbial contamination of Shir Berenj samples. For example, storing raw materials in inappropriate conditions, poor quality of raw materials, poor personal hygiene, and undesirable environmental conditions such as air pollution. Microbial contamination of Shir Berenj samples is important and could be conducive to harmful effects on consumers' health.

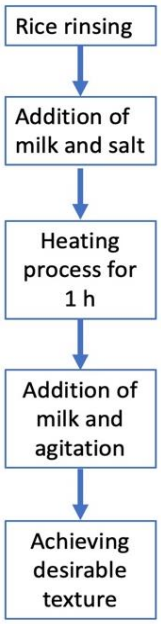

Figure 2: The traditional method for production of Shir Berenj

4. Conclusion

The data obtained by this study revealed that 96% of Shir Berenj samples were rejected by ISIRI standards [10]. The most crucial challenges were microbial quality of Shir Berenj samples, such as total mesophilic count, mold and yeast, *Enterobacteriaceae*, and *E. coli*. High microbial contamination can be due to several reasons, such as poor hygienic conditions. Minor issues were observed in chemical quality indices. Overall, related surveillance organizations should be more vigilant about the quality of Shir Berenj. We suggest that appropriate approaches should be used to improve the knowledge of food handlers involved in producing Shir Berenj.

Authors' Contributions

Mahsa Marashi: Data acquisition; analysis of data; writing the manuscript; Approval of the final version of the manuscript. Abdolazim Behfar: Study concept; designed the study; writing the manuscript; approval of the final version of the manuscript. Mohammad Hashemi: Study concept; analysis and interpretation of data; approval of the final version of the manuscript. Mehdi Safdarian: designed the study; data analysis; writing the manuscript; approval of the final version of the manuscript. Seyyed Mohammad Ali Noori: Study concept; designed the study; get funding; work supervision; writing the manuscript; approval of the final version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Table 2: Results of analysis for S. aureus, E. coli, Enterobacteriaceae, and Salmonella

	Staphylococcus aureus				Escherichia coli				Enterobacteriaceae				Salmonella			
	Negative		Positive		Negative		Positive		< 3		> 1100		Negative		Positive	
Location	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Ahvaz	30	100	0	0	14	46.8	16	53.1	9	30.6	21	69.3	30	100	0	0
Shoushtar	7	100	0	0	3	42.9	4	57.1	1	14.3	6	85.7	7	100	0	0
Dezful	7	100	0	0	3	42.9	4	57.1	2	28.6	5	71.4	7	100	0	0
Andimeshk	6	100	0	0	5	83.3	1	16.7	3	50	3	50	6	100	0	0
Total	50	100	0	0	25	50	25	50	15	30	35	70	50	100	0	0

* According to the ISIRI 14681, the maximum limitation for Enterobacteriaceae is set at 1 log10 cfu/g and S. aureus and Salmonella must be negative.

Table 3: Mean values and standard deviation for chemical analysis, including pH, fat, and dry matter

Location	Criteria	pH	Fat (%)	Dry matter (%)
Ahvaz	Number of samples	30	30	30
	Mean ± SD	6.32 ± 0.14^{A}	5.76 ± 1.78^{A}	23.41 ± 2.96^{A}
Shoushtar	Number of samples	7	7	7
	Mean ± SD	6.58 ± 0.09^{A}	7.07 ± 1.48^{B}	23.40 ± 3.17^{A}
Dezful	Number of samples	7	7	7
	Mean ± SD	6.46 ± 0.25^{A}	6.71 ± 3.20^{C}	22.46 ± 2.50^{B}
Andimeshk	Number of samples	6	6	6
	Mean ± SD	6.66 ± 0.23^{A}	4.66 ± 1.96^{D}	20.49 ± 1.62^{C}
Total	Number of samples	50	50	50
	Mean ± SD	6.42 ± 0.28	6.09 ± 2.12	22.79 ± 6.49

*SD: Standard Deviation. According to the ISIRI 14681: pH should be in the range of 6.3-6.8, fat content should be at least 3%, and dry matter should be at least 22%.

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