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The Comparison of the Microbiological Characteristics of Wet Dough and Dry Powder Tarhana's and Evaluation of Possible Health Risks

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ABSTRACT

Tarhana is a traditional food produced by different traditional methods and the materials used in production are changing from a region to another region. The total yeast and molds, total mesophilic aerobic bacteria, *Escherichia Coli* and *enterococci* bacteria count of wet dough Kastamonu tarhana and dry powdered tarhana samples were investigated in this study. All microorganisms examined in our study were detected in one of the wet dough tarhana samples. The highest total yeast and molds, total mesophilic aerobic bacteria, *Escherichia coli* and *enterococci* bacteria counts were determined for the wet dough tarhana samples to be 2.2×10^6, 6.6×10^7, 1.2×10^6 and 1.9×10^6 cfu/g, respectively. No growth of microorganism capable of reproduction was observed in the powdered tarhana produced industrially. In addition, the *Escherichia coli* and *enterococci* bacteria were not detected for any of the dry powder tarhana samples. It was seen that the microbial load of the wet tarhana produced at home in Kastamonu was higher than the powdered tarhana. The reason for this situation was thought to be due to poor production and hygiene conditions. The presence of *Escherichia coli* and *enterococci* bacteria in samples indicates that there is possible fecal contamination of the raw materials used in wet dough tarhana production. Electron microscope images of molds obtained in our study are similar to molds producing mycotoxins. These results show that the wet dough tarhana have a greater risk for microorganism development and human health compared to dry powder tarhana.

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Introduction

Tarhana is a very popular traditional Turkish fermented cereal food and consumed in large quantities especially in rural areas. Besides that, there are some food products similar to tarhana in other countries, which are called different names such as "tahonyaltakuna" (Hungary), "talkkuna" (Finland), "trahana" (Greece), "atole" (Scotland), "kishk" (Syria, Jordan and Egypt), "kushuk" (Iraq) (Kabak and Dobson 2011; Uçar and Çakiroğlu, 2011; Özçam et al., 2014; Arslan-Tontul et al., 2018). Although various vegetables, herbs and spices are used in different proportions in the production of tarhana, cereal products such as wheat flour and yoghurt are indispensable ingredients (Özçam et al., 2014; Kaymak et al., 2019). It is usually produced at home, but it is also one of the leading commercially produced instant soups. The desired ingredients are made into dough, and this dough is fermented for 1-7 days with yeast and lactic acid bacteria (LAB). Then the fermented dough is dried under the sun (domestic production) or with a hot air dryer (industrial production) and pulverized (Kivanc and Funda, 2017; Kaymak et al., 2019). Powdered tarhana can be stored for a long time and consumed as a soup (Ibanoğlu et al., 1999; Arslan-Tontul et al., 2018). In some regions of Turkey, it can be spread as a thin layer and dried in the form of chips, and it can be consumed as a tortilla (Kaymak et al., 2019), or sometimes it can be consumed wet without drying (Arslan-Tontul et al., 2018).

Tarhana is a source of protein, vitamins and minerals resulting from its ingredients and has a very high nutritional value (Daglioglu, 2000; Özdemir et al., 2007; Kivanc and Funda, 2017). Since the drying process significantly reduces the LAB and yeast numbers, the consumption of wet tarhana is better than dried tarhana in terms of nutritional value (Erbaş et al., 2005; Arslan-Tontul et al., 2018). In the Kastamonu city, fermented tarhana dough is consumed wet without drying. This tarhana dough is fermented by occasionally mixing with dill and basil stalks for a few weeks. Then the dough is taken into storage

containers such as glass jars and stored in the refrigerator. However, due to its high-water activity, the storage period for wet tarhana is limited to 6 months in the refrigerator (Erbaş et al., 2005), and if it is produced without complying with hygiene rules, contamination and microbial growth may threaten human health. Since the ingredients used in its production are uncooked, the most important risk factors that Tarhana may contain are lack of pasteurization, use of contaminated raw materials and poorly controlled fermentation conditions (Daglioglu et al., 2002; Uçar and Çakiroğlu, 2011). In addition, contamination of ingredients is an important source of mycotoxins at every stage of production and up to consumption (Kaymak et al., 2019). Although there are studies examining the effects of different production techniques on tarhana properties, a limited number of studies examining tarhana in terms of microbiology have been carried out (Kivanc and Funda, 2017). Moreover, microbiological changes in the fermentation process and after drying of tarhana produced in the laboratory were discussed, and the total mesophilic aerobic bacteria (TMAB), total yeast and mold (TYM) and lactic acid bacteria (LAB) counts were examined in general (Erbaş et al., 2005; Karagozlu et al., 2008; Settanni et al., 2011; Donmez, 2015; Özel, 2015; Kivanc and Funda, 2017; Arslan-Tontul et al., 2018; Kaya and Şimşek, 2020). For this reason, TMAB, TYM, *Escherichia coli* (*E. coli*) and *enterococci bacteria* (EB) count of wet dough tarhana samples from Kastamonu city and dried powder tarhana samples were investigated and samples taken from TYM colonies cultured from wet dough tarhana were examined under an electron microscope. Finally, wet dough Kastamonu tarhana and powder tarhana were compared in terms of hygiene, contamination and health risks by evaluating the results obtained from this study.

Materials and Methods

Sample Collection

Five Kastamonu wet dough tarhana samples, which are produced at home with the traditional method, were purchased from the villagers who were selling in the bazaars established in Kastamonu city centre. Three homemade powdered dry tarhanas were obtained from shops selling homemade products from different regions, and one industrially produced powdered dry tarhana sample was obtained from a chain market in the Kastamonu city. All of the samples were obtained in 1 kg jars and two pieces of each. While one of each sample pair was kept in the refrigerator at + 2-8°C until analysis, the others were kept at room temperature with their lids tightly closed for five days in order not to lose their moisture.

Sample Preparation

10 g of sample was transferred to 90 ml of 0.1% peptone water (Merck Millipore, Darmstadt, Germany) and homogenized with a blender. Serial decimal dilutions were prepared from this solution to a dilution of 10^{-6} by the addition of peptone water (Şengün et al., 2009).

Materials

Plate Count Agar (PCA) (Merck 1.05463, Darmstadt, Germany), Membrane-filter Enterococcus Selective Agar acc. to SLANETZ and BARTLEY (Merck 1.05262), Eosin Methylene-blue Lactose Sucrose Agar (EMB) (Merck 1.01347), Potato Dextrose Agar (PDA) (Merck 1.10130) were used as media in the study.

Methods

Colony counting was performed using the surface plating technique for all microbiologic assays of tarhana samples and aliquots (0.1 mL) of each serial dilution were inoculated by spreading over the agar plates. PCA was used for TMAB analysis and incubated for 48 hours at 30°C (TS EN ISO 4833-2, 2014). Membrane-filter Enterococcus Selective Agar acc. to Slanetz and Bartley was used for EB analysis and incubated at 37°C for 24 hours (Lachica and Hartman, 1968). *E. coli* was incubated for 24 hours at 35°C using EMB for assays (Nys et al., 2004). PDA was used for TYM analysis and incubated at 20°C C for five days (TS ISO 21527-2, 2014). Colonies on agar plates were counted at the end of the incubation time, and the number of colonies forming units (cfu) per g was calculated. Agar plates containing less than 300 colonies were used for colony counting (TS EN ISO 4833-2, 2014). Nuve En 120 (Turkey) incubator was used in incubation processes. Moisture contents of all tarhana samples were determined by drying 5-gram pieces of samples in a drying oven (Protech – PLF/PKD, Norway) at 130 ± 5°C for 2 hours (TS EN ISO 712, 2012). For the measurement of pH, the sample and distilled water were mixed (1:9, w/v) by vortex (Velp Sci. Classic, Italy) until a homogeneous mixture was obtained, the pH was then measured by pH meter (ISOLAB – Laborgerate GmbH, Germany). Electron microscope images of samples taken from colonies obtained from yeast-mold incubation were taken with a scanning electron microscope (SEM) (FEI Quanta FEG 250, USA) located in Kastamonu University Central Research Laboratory Application and Research Center.

Results and Discussion

The results of microbiological and moisture analysis of tarhana are given in Table 1. and Table 2., respectively. The microorganism examined in this study were found in one of the doughs tarhana's from Kastamonu city (sample coded with K1). In addition, TYM and TMAB growth were detected in all wet dough tarhanas. The highest TYM, TMAB, *E. coli* and EB counts were determined for the Kastamonu dough tarhana samples to be 2.2×10^6 (sample coded with K1), 6.6×10^7 (sample coded with K4), 1.2×10^6 (sample coded with K1) and 1.9×10^6 (sample coded with K1) cfu/g, respectively (Table 1). While no microorganism capable of reproduction growth ($<10^2$ cfu/g) was observed from industrially produced powdered dry tarhana, TYM and TMAB growth were observed in homemade powdered dry tarhana from Zonguldak province and only TMAB was detected at a minimum level (1×10^2 cfu/g) in homemade powdered dry tarhana originating from Afyon and Uşak. In addition, *E. coli* and EB growth was not observed ($<10^2$ cfu/g) in any of the powdered dry tarhana samples. TMAB and EB counts of tarhana samples which the tests carried out after the samples were kept at room temperature for 5 days were found to decrease compared to the results of the samples stored at + 2-8°C, possibly due to pH decreasing during storage (Erbaş, 2005). However, an increase in TYM counts was observed, possibly due to the good

growth of molds even at low pH values thanks to high water activities (Gödek et al., 2021). In the literature review, only a few studies were found in which homemade tarhana was examined microbiologically (Coşkun, 1996; Soyyiğit, 2004; Uçar and Çakiroğlu, 2011; Özdemir et al., 2012); Gülbandılar et al., 2014); Hendek Ertop et al., 2019). Coşkun (1996) found the maximum number of microorganisms to be 60000 units/g and the TYM number to be between 0-52000 units/g in homemade tarhana collected from Trakya and its region. Coliform bacteria were not found in any of the 51 tarhana samples he examined. Soyyiğit (2004) also examined 27 homemade tarhana produced in Isparta and its region, and the TMAB is 1.4×10^3-2.1×10^7 cfu/g and the TYM is <10–3.3×10^7 cfu/g. Uçar and Çakıroğlu (2011) examined the home-made tarhanas obtained from the province of Ankara microbiologically and observed that 8 out of 20 samples (one at 10^2cfu/g, six at 10^3cfu/g and one at 10^4 cfu/g) contained mesophilic aerobic bacteria growth. Again, they detected 10^3-10^2 cfu/g Staphylococcus growth in two of 20 samples, yeast and mold growth between 10^2 and 10^3 cfu/g in five samples, and 10^2-10^3 cfu/g *Bacillus cereus* growth in three samples. They observed no *E. coli*, coliform or Salmonella contamination in any of the investigated samples. Özdemir, Alkan and Çon (2012) stored wet tarhana from Kastamonu city for 4 months and at the end of the storage, Lactobacillus spp. number increased by 0.96 log to 5.24 log cfu/g; Lactococcus spp. count increased by 0.74 log to 5.07 log cfu/g; TYM count increased by 0.75 log to 5.25 log cfu/g; The number of TMAB also increased by 0.40 log to 5.16 log cfu/g. The numbers of *Staphylococcus aureus*, coliform group microorganisms and *E. coli*, which were below the countable level in the post-production analyzes of the samples, were also determined below the countable level at the end of the storage period. In a study conducted in Gediz and Kütahya region, TMAB count in homemade tarhana was found in the range of 1.5×10^2-2.5×10^2 cfu/g, and *Staphylococcus aureus*, *E. coli*, *Salmonella sp*., *Shigella sp*., *Bacillus cereus*, *Enterococcus faecalis*, *Clostridium sp*. not detected (Gülbandılar et al., 2014). Hendek Ertop, Cerit and Atasoy (2019) in their study, detected 10^3-10^6 cfu/g yeast, 10^3-10^6 cfu/g aerobic LAB and 10^5- 10^6 cfu/g anaerobic LAB in wet dough Kastamonu tarhana samples. They also stated that wet tarhana carries more microbial load than dry tarhana. In the studies of Özdemir et al. and Hendek Ertop et al. unlike our study, no microbiological comparison of homemade Kastamonu wet dough tarhanas with powdered dry tarhanas was made, nor electron microscopy examination of samples taken from yeast and mold cultivation. In these two studies, EB examination is also not seen. Moreover, in our study, samples taken from PDA medium containing yeast and mold were examined by SEM and the images obtained are given in Figure 1. TYM colony color in PDA was yellow, and electron microscope images showed conidiophores and conidial heads, which resembled molds such as aspergillus and penicillium rather than yeast. In the study by Kıvanç and Funda (2017), yeasts determined during tarhana fermentation were *Kluyveromyces marxianus*, *Yarrowia lipolytica*, *Pichia membranaefaciens*, *Pichia mexicana*, *Pichia angusta*, *Debaryomyces hansenii*, *Candida sorboxylosa*, *Candida fluviatilis*, *Saccharomyces cerevisiae*. Electron microscope images of *Saccharomyces Cerevisiae* differ from the images in our study. The yeast cell is typically about five to ten micrometers in diameter and buds can be easily selected in the images as the cells proliferate through a process called budding (Karimy, et al., 2020) Electron microscope images of other yeasts (*Kluyveromyces marxianus*-Mehmood et al., 2018), (*Yarrowia lipolytica*-Apte et al., 2013), (*Pichia membranaefaciens*-Kurtzman, 2011), (*Debaryomyces hansenii*-Kreger-van Rij and Veenhuis, 1975) determined in tarhana in the study of Kıvanç and Funda are not similar to the images obtained in our study. Mycotoxins are natural toxins with low molecular weight and a wide variety of chemical structures, which are formed as a result of secondary metabolism of fungal (mold) genera such as *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria* and *Claviceps*. These products are extremely toxic, most of them carcinogens, teratogens, mutagens (Sabuncuoğlu et al., 2008). Aspergillus flavus colonies are seen on PDA agar as yellow to green\granular. This species is microscopically characterized by conidiophores that are globose vesicles bearing chains of conidia and conidial heads are typically radiate figüre (Shekhany and Rostam, 2016). The electron microscope images obtained in our study are similar to those described in the above sentence. *Penicillium* species also have conidiophores and conidia similar to those in our study (Visagie et al., 2014). There are also studies that determine aflatoxin, which can be produced by *Aspergillus* species, and ochratoxin, which can be produced by *Penicillium* species in various tarhanas (Özçam et al., 2014; Kaymak et al., 2019).

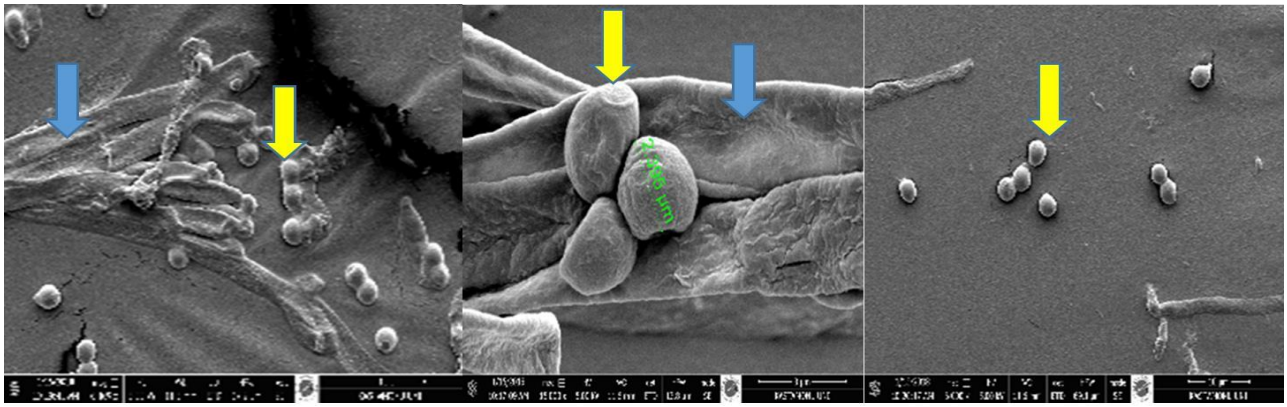

Figure 1. Scanning electron micrographs showing molds on PDA. The blue arrows mean shriveled conidiophore. The yellow arrows mean conidial heads or conidiospore.

Table 1. The microbiological analysis results of this study.

	Sample Code	TYM		TMAB	
		Cfu/g	Cfu/g on DW	Cfu/g	Cfu/g on DW
Stored at +4-8 C	K1	2.2×10^6	4×10^6	2.8×10^6	5.1×10^6
	K2	7.2×10^5	1.4×10^6	4.4×10^7	8.5×10^7
	K3	1.1×10^5	2×10^5	1.8×10^6	3.3×10^6
	K4	1×10^5	1.8×10^5	6.6×10^7	1.2×10^8
	K5	7.3×10^5	1.4×10^6	1.2×10^6	2.2×10^6
	Z	3.5×10^5	4×10^5	2.5×10^5	2.9×10^5
	U	$<10^2$	$<10^2$	1×10^2	1×10^2
	A	$<10^2$	$<10^2$	1×10^2	1×10^2
	I	$<10^2$	$<10^2$	$<10^2$	$<10^2$
Strored at room temperatature for 5 days	K1	1.9×10^6	3.5×10^6	2.5×10^6	4.5×10^6
	K2	9.5×10^6	1.8×10^7	1×10^7	1.9×10^7
	K3	1.7×10^6	3.1×10^6	3.7×10^5	6.8×10^5
	K4	3.4×10^6	6.2×10^6	2.7×10^7	5×10^7
	K5	1.6×10^6	3×10^6	1×10^7	1.9×10^7
	Z	4.6×10^5	5.3×10^5	1×10^2	1×10^2
	U	$<10^2$	$<10^2$	$<10^2$	$<10^2$
	A	$<10^2$	$<10^2$	$<10^2$	$<10^2$
	I	$<10^2$	$<10^2$	$<10^2$	$<10^2$
	Sample Code	E. coli		EB	
		Cfu/g	Cfu/g on DW	Cfu/g	Cfu/g on DW
Stored at +4-8 C	K1	1.2×10^6	2.2×10^6	1.9×10^6	3.5×10^6
	K2	$<10^2$	$<10^2$	4.9×10^5	9.5×10^5
	K3	$<10^2$	$<10^2$	$<10^2$	$<10^2$
	K4	$<10^2$	$<10^2$	$<10^2$	$<10^2$
	K5	$<10^2$	$<10^2$	$<10^2$	$<10^2$
	Z	$<10^2$	$<10^2$	$<10^2$	$<10^2$
	U	$<10^2$	$<10^2$	$<10^2$	$<10^2$
	A	$<10^2$	$<10^2$	$<10^2$	$<10^2$
	I	$<10^2$	$<10^2$	$<10^2$	$<10^2$
Strored at room temperatature for 5 days	K1	1.3×10^6	2.4×10^6	1.7×10^5	3.1×10^5
	K2	$<10^2$	$<10^2$	1.2×10^5	2.3×10^5
	K3	$<10^2$	$<10^2$	$<10^2$	$<10^2$
	K4	$<10^2$	$<10^2$	$<10^2$	$<10^2$
	K5	$<10^2$	$<10^2$	$<10^2$	$<10^2$
	Z	$<10^2$	$<10^2$	$<10^2$	$<10^2$
	U	$<10^2$	$<10^2$	$<10^2$	$<10^2$
	A	$<10^2$	$<10^2$	$<10^2$	$<10^2$
	I	$<10^2$	$<10^2$	$<10^2$	$<10^2$

Cfu/g: Colony forming unit/gram sample, DW: Dry weight, K: Dough tarhana from Kastamonu, Z: Powder tarhana from Zonguldak, U: Powder tarhana from Uşak, A: Powder tarhana from Afyon, I: Industrially produced packaged powder tarhana

Table 2. Average moisture content of tarhana samples.

Sample Code	Moisture content % (w/w) ± SD
K1	45.28 ± 0.02
K2	48.40 ± 0.01
K3	45.69 ± 0.02
K4	45.45 ± 0.03
K5	46.50 ± 0.00
Z	12.55 ± 0.03
U	10.58 ± 0.02
A	4.73 ± 0.04
I	5.44 ± 0.03

SD: Standart deviation, K: Dough tarhana from Kastamonu, Z: Powder tarhana from Zonguldak, U: Powder tarhana from Uşak, A: Powder tarhana from Afyon, I: Industrially produced packaged powder tarhana

It is accepted that food products with high TMAB and TYM counts are not produced and preserved under hygienic conditions. The limits of TMAB and TYM counts are specified in the TS 2282 Tarhana standard (2004), and all of the wet dough tarhanas obtained from Kastamonu city and powdered dry tarhana from Zonguldak city, which we examined in our study, do not comply with this standard. *E. coli* is one of the facultative anaerobic bacteria that persist in the flora of the large intestine (Turgut, 2021). Therefore, the presence of *E. coli* in water and food

indicates possible fecal contamination, and the presence of corresponding intestinal pathogens causes various infectious diseases (Arslan and Özdemir, 2013). *Enterococci* are the dominant flora of the digestive system of humans and animals, but are also found in soil, surface waters, plants, vegetables and insects. *Enterococci* are among the most common nosocomial pathogens, especially *Enterococcus faecium* and *Enterococcus faecalis* are opportunistic pathogens, in addition to bacteremia and endocarditis, it has been reported that it can cause infections in the urinary system and tissues such as the central nervous system. It has been stated that resistance to some antibiotics such as vancomycin and their various virulence factors play an important role in the pathogenicity mechanisms of *enterococci* (Isleroglu et al., 2008). The growth of both *E. coli* and EB in the K1 coded wet dough tarhana sample, and the EB growth in the K2 coded wet dough tarhana sample, is an indication that these tarhanas were contaminated by feces. The reason for this contamination may be the mixing of sewage into the water used in tarhana production, or the use of vegetables and spices grown by irrigation with sewage-contaminated water. These results show that the wet dough tarhanas from Kastamonu city (especially K1 and K2 coded samples) are produced under poor production conditions and hygienic rules are not followed. In addition, the moisture content (Table 2.) of wet dough tarhanas and powder tarhanas originating from Zonguldak and Uşak are not in accordance with the TS 2282 Tarhana standard (2004) (moisture limit 10%, m/m), and the high-water activity of these tarhanas has been seen as the main reason for their microorganism growth susceptibility. The reason why the powdered tarhana of Zonguldak contains higher amount of moisture than other powdered tarhana may be that the drying efficiency is poor due to the low number of sunny days and humid air in the Black Sea region. Although the humidity in Uşak tarhana is above 10%, the lack of significant microorganism growth is thought to be due to the high amount of spices it contains, especially the hot chili pepper. Zonguldak tarhana contains almost no spices, as supportive evidence.

In our study, it was seen that the microbial load of the wet tarhana produced at home in Kastamonu was higher than the powdered tarhana, and this was thought to be due to poor production and hygiene conditions. In fact, the presence of samples with *E. coli* and EB indicates that there is a possible fecal contamination of the raw materials used in tarhana production. These results shown that the wet dough tarhana samples have a greater risk of microorganism development and human health. Electron microscope images of molds obtained in our study are similar to molds producing mycotoxins, and their identification is planned with a further study. In addition, it is thought that people who produce at home should be informed about the proper and correct production and hygiene conditions with the cooperation of authorized institutions and organizations in terms of public health.

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Conflict of Interest

The author declares that there is no conflict of interest.

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