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Impact of stale bread utilization on the quality attributes of tarhana production

Tarhana üretiminde bayat ekmek kullanımının ürün kalitesi üzerine etkileri

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ABSTRACT

The objective of this study was to utilize stale bread, of which 4.9 million pieces are wasted daily, in the production of fermented tarhana and to assess its impact on the physicochemical, microbiological, and sensory properties of the final product. Five different tarhana formulations were developed using breadcrumbs (25%, 50%, 75%, 100%) and wheat flour (100%) derived from stale bread in varying proportions. Tarhana produced with 32.74% wheat flour or breadcrumbs underwent comprehensive physicochemical, microbiological, and sensory analyses to evaluate the effects of these formulations. The study revealed no significant differences in pH, protein, fat, and moisture values among the tarhana samples ($p>0.05$). Furthermore, the data analysis of TMAB, coliforms, *S. aureus*, lactic acid bacteria, yeasts, and moulds did not indicate any notable differences among the tarhana samples ($p>0.05$). The *Lb. delbrueckii ssp. bulgaricus* and *S. thermophilus* isolates were obtained as the results of this investigation. There was no significant disparity in the quality attributes of tarhana prepared from varying ratios of stale breadcrumbs or entirely from wheat flour. It implies that the physicochemical features of tarhana were not negatively impacted by stale breadcrumbs. The results show that incorporating ≤25% stale bread instead of solely wheat flour in the production of tarhana can be feasible. Additionally, tarhana possesses a high biological value attributed to the presence of lactic acid bacteria including Lb. delbrueckii ssp. bulgaricus and S. thermophilus in samples. This presents an opportunity for incorporating stale or discarded bread into tarhana, thereby potentially increasing its economic value. Future investigations might also explore consumer acceptance and market potential for tarhana made with stale bread to assess its commercial viability. Lastly, expanding the microbiological analysis to include a broader spectrum of probiotic strains could enhance the understanding of tarhana's health benefits, thus contributing to its positioning as a functional food product.

Key Words: Tarhana, Stale Bread, Breadcrumbs, Lactic Acid Bacteria

ÖZ

Bu araştırmanın amacı; günde 4,9 milyon israf edilen bayat ekmeğin fermente tarhana üretiminde kullanılması ve bayat ekmeğin tarhananın fizikokimyasal, mikrobiyolojik ve duyusal özellikleri üzerindeki etkisinin belirlenmesidir. Bu araştırmada çeşitli oranlarda bayat ekmeklerden elde edilen galeta ununun (%25, %50, %75, %100) ve buğday unu (%100) kullanılarak beş farklı tarhana formülasyonu üretilmiştir. Çalışmada % 32,74 buğday unu ya da galeta unu ile yapılan tarhanalar kapsamlı fizikokimyasal, mikrobiyolojik ve duyusal analizlere tabi tutulmuştur. Yapılan çalışma tarhana örnekleri arasında pH, protein, yağ ve nem değerleri açısından önemli bir fark olmadığını ortaya koymuştur ($p>0.05$). Yapılan analizde TMAB, koliform, S. aureus, laktik asit bakterileri, mayalar ve küflerin veri analizi tarhana örnekleri arasında kayda değer bir farklılık göstermemiştir ($p>0.05$). *Lb. delbrueckii ssp. bulgaricus* ve *S. thermophilus* izolatları bu araştırmanın sonuçları olarak

elde edilmiştir. Farklı oranlarda bayat ekmek kırıntılarından veya tamamen buğday unundan hazırlanan tarhanaların kalite özelliklerinde önemli bir farklılık görülmemiştir. Bu da tarhananın fizikokimyasal özelliklerinin bayat ekmek kırıntılarından olumsuz etkilenmediğini göstermektedir. Sonuçlar tarhana üretiminde sadece buğday unu yerine ≤%25 oranında bayat ekmek kullanılmasının mümkün olabileceğini göstermektedir. Elde edilen tarhana *Lb. delbrueckii ssp. bulgaricus* ve *S. thermophilus* dahil olmak üzere laktik asit bakterilerinin varlığına atfedilen yüksek bir biyolojik değere sahiptir. Bu durum bayat veya atılmış ekmeğin tarhanaya dahil edilmesi için bir fırsat sunmakta ve böylece potansiyel olarak ekonomik değerini artırmaktadır. Gelecekteki araştırmalar, bayat ekmekle üretilen tarhananın tüketici kabulü ve pazar potansiyelini inceleyerek ticari uygulanabilirliğini değerlendirebilir. Ayrıca, mikrobiyolojik analizlerin kapsamının daha geniş bir probiyotik suş yelpazesini içerecek şekilde genişletilmesi, tarhananın sağlığa faydalarının daha iyi anlaşılmasına katkıda bulunabilir ve böylece fonksiyonel bir gıda ürünü olarak konumlandırılmasını destekleyebilir.

Anahtar Kelimeler: Tarhana, Bayat Ekmek, Galeta Unu, Laktik Asit Bakterileri

Introduction

Bread is not only a staple food but also a significant source of energy, protein, and essential minerals (Cappelli & Cini, 2021). With the global population steadily increasing and economic conditions shifting, the role of bread in the human diet continues to grow in importance. Per capita annual bread consumption varies significantly across countries, reflecting both cultural and dietary patterns. For instance, in Bulgaria, the average annual bread consumption per person is 95 kg, followed by Ukraine at 89 kg, Italy at 68 kg, Russia at 62 kg, France at 57 kg, Germany at 56 kg, and the United Kingdom at 32 kg (Yusufoğlu et al., 2021). In comparison, Turkey's per capita bread consumption stands at 104 kg, significantly higher than the global average. This elevated consumption underscores the central role of bread in the Turkish diet, highlighting its economic and nutritional significance within the country. Although bread plays a crucial role in global nutrition, it is also one of the most frequently wasted food products. Its affordability, versatility in culinary applications, and high satiety value contribute to its widespread consumption; however, these same attributes make it particularly susceptible to wastage (Kumar et al., 2023). In Turkey alone, it is estimated that approximately 4.9 million slices of bread are discarded on a daily basis (Yıldırım et al., 2016). Several factors contribute to this significant wastage, including inadequate storage practices, over-purchasing, the rapid staling of bread, serving bread in unsliced form, and the failure to repurpose or recycle stale bread for alternative uses (Dymchenko et al., 2023; Gökalp, 2020). The issue of bread waste is exacerbated by its short shelf life and the tendency for consumers and retailers to discard bread that has lost its freshness. Various strategies have been explored to address this issue, with one of the most effective approaches being the conversion of stale bread into new food products, thereby reducing waste while simultaneously creating value-added products. This method not only mitigates the environmental and economic impacts of bread wastage but also offers opportunities for innovation in the food industry through the development of sustainable practices and the incorporation of waste reduction into food production processes.

Tarhana holds a significant position in the Turkish diet, recognized as a traditional fermented food product primarily made from cereal grains, and is commonly consumed across the Middle East, particularly in Turkey. In addition to its widespread consumption, tarhana is also produced on an industrial scale in Turkey, contributing to its accessibility. This nutrient-dense food, enriched with B vitamins, essential minerals, organic acids, and proteins due to its composition of cereal flours, yogurt, and various vegetables, serves as an important dietary element for both children and the elderly. Its nutritional profile, combined with its ease of preparation and extended shelf life, owing to its low water activity, has further bolstered its growing popularity (Tarakcı et al., 2004; Köse & Çağındı, 2002).

The composition of tarhana typically includes 60.9% carbohydrates, 16% protein, 10.2% moisture, 6.2% ash, 5.4% fat, 3.8% salt, and 1% crude fiber. Additionally, it is recognized as a rich source of bioavailable minerals, such as calcium

(109 mg/100 g), magnesium (78 mg/100 g), potassium (114 mg/100 g), and copper (450 mg/100 g). The fermentation process contributes to a significant increase in the bioavailability of these nutrients, as the rising acidity and phytase activity during fermentation degrade phytic acid, thereby enhancing the total mineral and protein content. The protein content of dried tarhana, when prepared with yogurt and inoculated with varying concentrations of probiotic cultures (ranging from 0.5% to 4.5%), has been reported to fluctuate between 18% and 20%. Furthermore, commercially produced tarhana has been identified as containing seven essential water-soluble vitamins, including ascorbic acid (vitamin C), niacin, pantothenic acid (vitamin B5), pyridoxine (vitamin B6), thiamine (vitamin B1), folic acid, and riboflavin (vitamin B2) (Gök, 2023).

In traditional formulations of tarhana, functional enhancements are often achieved through the incorporation of tomatoes, peppers, or their pastes. These ingredients are particularly valuable due to their biologically active compounds, such as lycopene, phenolic compounds, organic acids, and vitamins. Additionally, the inclusion of dietary fiber, pectin, oil, and protein derived from the pulp, seeds, and skin of these vegetables further augments the nutritional and functional properties of tarhana (Gök, 2023).

Tarhana is traditionally prepared by combining wheat flour, yogurt, bread yeast, onions, tomatoes, fresh red and green peppers, along with various spices. The preparation typically follows a 1:1 ratio of yogurt to flour, though variations in ingredient proportions may occur depending on regional preferences and specific formulations. Commonly added spices include mint, thyme, red chili flakes, chili powder, dill, and salt, which contribute to the distinctive flavor profile of tarhana. After mixing the ingredients, the tarhana dough is left to undergo a fermentation process that lasts between 1 to 7 days, depending on environmental conditions and desired fermentation levels. This fermentation enhances the sensory and nutritional properties of the final product by promoting the development of organic acids and increasing the bioavailability of nutrients. Following fermentation, the dough is dried either by sun-drying or by using low-temperature ovens, a method that preserves the product's quality while minimizing nutrient degradation. Once dried, the tarhana is finely ground to achieve a particle size of less than 800 µm, making it suitable for culinary use. The low pH and reduced moisture content of the dried product contribute to its extended shelf life, allowing it to be stored for long periods without the risk of microbial spoilage or quality deterioration (Değirmencioğlu et al., 2005; Kilci & Göçmen, 2014). This characteristic, combined with its ease of preparation and high nutritional value, solidifies tarhana's importance as a staple food in many households.

The objective of this study was to ascertain the quality attributes of the product obtained by incorporating flour from stale bread into tarhana mixture at specific ratios, to demonstrate the feasibility of utilising stale bread in tarhana and to identify the impact of stale bread on the physicochemical, microbiological, and sensory characteristics of tarhana. It is further emphasized that the incorporation of stale bread will not only offer nutritional and technological benefits but also generate social and economic value by reducing bread waste. Mitigating bread waste is crucial for ensuring food security, and the repurposing of stale bread will foster an economically sustainable production model. Such initiatives represent a significant step towards enhancing public awareness and minimizing waste, while simultaneously contributing to the economy.

Material and Method

Material

White wheat flour (from Ülker Bizim, İstanbul, Turkey), stale bread, yoghurt (from Pınar, Izmir, Turkey), tomato paste (from Tat Gıda Sanayi ve Ticaret A.Ş.), green pepper, onion, mint, thyme and salt were used as ingredients to prepare the

tarhana. All the ingredients were purchased from a local supermarket in Kayseri. The newly purchased bread was stored in bags under ambient conditions for two days to facilitate staling. After the two-day period, the stale bread was sliced and baked for 25 min at 100 C for drying. The dehydrated bread was then ground into fine breadcrumbs using a grinder.

Grouping of Tarhanas

The tarhana samples were categorized based on the proportion of wheat flour and/or stale breadcrumb mixtures used in their formulation. Group (A) consisted exclusively of wheat flour, whereas group (B) incorporated 25% breadcrumbs with wheat flour, group (C) included a 50% mixture of breadcrumbs and wheat flour, group (D) contained 75% breadcrumbs with wheat flour, and group (E) was composed entirely of breadcrumbs without any wheat flour. Overall, the combined wheat flour and breadcrumb mixtures accounted for 32.74% of the total tarhana formulation.

Preparation of tarhana dough and powder

The method outlined by Temiz and Pirkul (1990) was employed for the preparation of tarhana. Initially, a uniform mixture was created by blending all specified ingredients namely tomato puree, diced onion, green pepper, mint, thyme, salt, and water using a blender, as detailed in Table 1. This mixture was heated over medium flame for 10 minutes. After cooling, it was combined with yogurt and thoroughly kneaded.

Table 1. Formulation and grouping of tarhana

Ingredient (g)	Sample Groups				
	A	B	C	D	E
Wheat flour	32.74	24.55	16.37	8.19	0
Stale breadcrumbs	0	8.19	16.37	24.55	32.74
Yogurt	32,74	32,74	32,74	32,74	32,74
Tomato paste	16,37	16,37	16,37	16,37	16,37
Onion	7,86	7,86	7,86	7,86	7,86
Green pepper	6,55	6,55	6,55	6,55	6,55
Salt	0,94	0,94	0,94	0,94	0,94
Water	0,94	0,94	0,94	0,94	0,94
Oregano	0,94	0,94	0,94	0,94	0,94
Mint	0,94	0,94	0,94	0,94	0,94

A: %100 wheat flour; B: %75 wheat flour+%25 breadcrumbs; C: %25 wheat flour+%75 breadcrumbs; D: %50 wheat flour+%50 breadcrumbs; E: %100 breadcrumbs

Subsequently, the mixture was divided into five separate containers. In accordance with the proportions provided in Table 1, wheat flour and/or stale breadcrumbs were added to each container and the mixtures were kneaded once more. The tarhana mixtures were then allowed to ferment at a constant temperature of 30°C for 5 days, with manual mixing performed every 12 hours throughout the fermentation period.

Following fermentation, the mixtures were dried in an incubator (Nüve KD-200, Kayseri, Turkey) at 50°C for 24 hours. The dried tarhana was then ground (IKA M 20 Universal Grinder), to a particle size of 500 µm. Finally, the tarhana samples were stored in polyethylene bags and kept in a refrigerator until required for analysis. Traditional tarhana production is given in Figure 1.

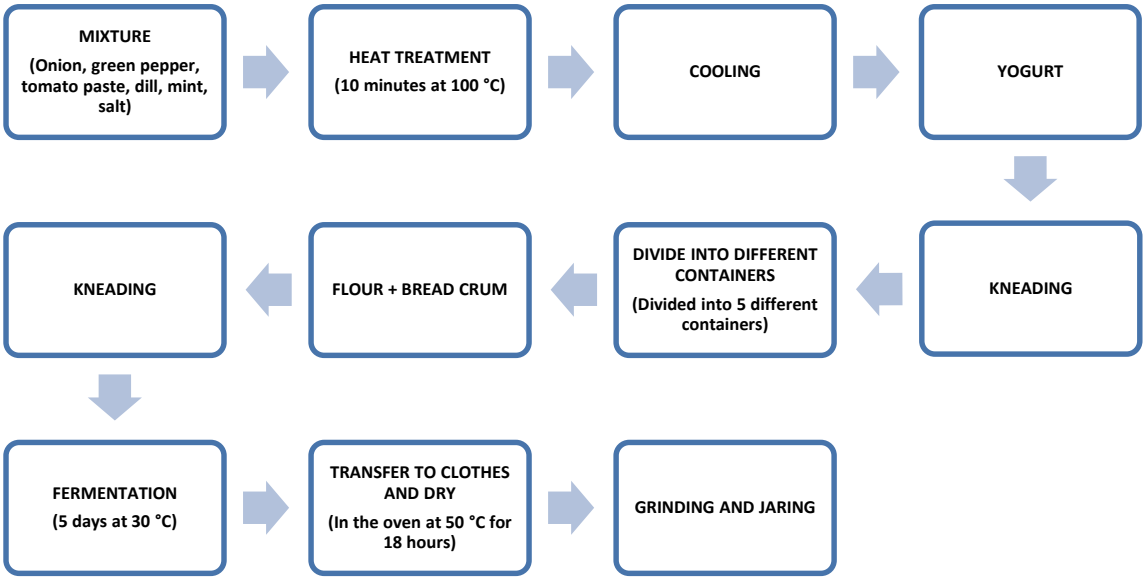

Figure 1. Flow chart of tarhana samples produced

Chemical composition

On the 1st, 3rd and 5th day of fermentation, pH and acidity analyses were carried out on tarhana dough samples. pH, water activity, acidity, moisture, ash, protein and fat analyses were carried out on tarhana powder samples. On the other hand, water activity, moisture and ash were determined in stale breadcrumbs. Moisture, ash, protein and fat were assessed according to the methods proposed by the American Association of Cereal Chemists (AACC 2000). pH was determined using a pH meter (Hanna Instruments 211) after mixing a 5 g sample with 100 ml distilled water. The acidity degree (as lactic acid) was determined by the Tarhana Standard (TS 2282) of Turkish Standards Institute (Anonymous, 1981). The water activity value of the samples was determined using a water activity meter (LabTouch, Novasina, Switzerland) with a precision of ±0.001. After 3-4 g of sample was quickly placed into the stainless steel chamber of the device, the water activity value was read directly from the display of the device and recorded.

Color measurements

Colour analyses of the tarhana dough and powder tarhana samples utilized in production were conducted via a colorimeter (Minolta CR-400, Osaka, Japan) with measurements of L*, a*, and b* values (Akarca et al., 2015).

Microbiological analysis

After homogenized with a stomacher, 10 g sample from tarhana dough (fermentation days 1, 3 and 5) and powder tarhana diluted and decimal dilutions were conducted in sterile physiological solution (90 mL). For the quantification of mesophilic aerobic bacteria, samples were incubated for 48±3 hours at 30±1 ºC on Plate Count Agar (Merck); for the quantification of lactic acid bacteria, samples were incubated for 48±72 hours at 30±1 ºC on MRS (Man Rogosa Sharpe); for the quantification of yeasts and moulds, samples were incubated for 3 to 5 days at 25 ºC on Potato Dextrose Agar (PDA); for the quantification of coliform bacteria, samples were incubated for 24 to 48 hours at 37 ºC in tubes containing Mac Conkey broth; for the determination of the presence of *Staphylococcus aureus* (*S. aureus*) were cultured for 24-48 hours at 37ºC on Baird Parker (Merck) medium supplemented with egg-yolk tellurite emulsion (Merck). After incubation, the number of bacteria, yeasts and moulds present in a 1 g sample was determined. Counts were carried out in duplicate and the results were expressed as log10 cfu/g (Stringini et al., 2009).

Isolation of Lactic Acid Bacteria (LAB)

In the process of isolating LAB from tarhana samples, MRS (Merck) and M17 agar (Merck) were used as culture media. Cycloheximide (actidione) with a concentration of 10 ppm was supplemented

to the media to hinder the growth of yeast and moulds during the weighing process. After dilution, 0.1 ml of the samples were pipetted into petri dishes containing MRS and M17 agar and thereafter were incubated for 72 hours at a temperature of 30 °C. At the end of incubation, countable colonies were identified, marked and segregated. Two or three colonials were categorized accordingly. After ensuring their growth in MRS (Merck) and M17 broth (Merck) media, the selected colonies were purified through streaking on agar medium again (Sawadogo-Lingani et al., 2007).

Identification of LAB ısolates

The morphological features, gram reactions (under the microscope), catalase activities of the bacterial isolates were checked. Then their initial classifications were established. API 50 CHL LAB identification kits (Biomerieux, France) were used to determine the carbohydrate utilization characteristics of gram positive and catalase negative isolates (Sawadogo-Lingani et al., 2007).

Sensory evaluation

A sensory evaluation was conducted on five different tarhana soup samples prepared using tap water at a low temperature (approximately 60°C). No additives were used in order to avoid masking any inherent flavors or aromas. Panelists were asked to assess all tarhana soup samples based on several sensory attributes, including color, consistency, aroma, off-flavors, and overall acceptability. The evaluation was carried out by a panel of 10 individuals, aged between 20 and 25, from the Department of Food Engineering at Erciyes University. The students were told about the properties of the soup and asked to compare it with the tarhana soup they would like to drink in daily life. The panelists rated the samples using a 9-point hedonic scale, where 1 indicated "dislike extremely," 5 represented "neither like nor dislike," and 9 signified "like extremely" (Akarca et al., 2015; Bilgiçli, 2009). This approach allowed for a detailed assessment of the sensory properties of the tarhana soups across a range of key quality parameters.

Statistical analysis

Statistical analysis of the results was performed using SPSS V 21.0.0 statistical package software (IBM, New York, USA). The physicochemical, microbiological, and sensory properties of various samples were analysed using single factor analysis of variance. The difference between fermentation days was determined using the Tukey multiple comparison method. The data obtained from the study were tested with one-way analysis of variance (ANOVA) methods to test the significant differences between the samples, and the level of significance was set as $p<0.05$.

Results and Discussion

Analytical results of tarhana dough samples

The analysis of the acidity, pH, and colour measurements of tarhana dough samples are detailed in Table 2.

Table 2. pH, titratable acidity and colour measurement of the tarhana samples.

Chemical parameteres	Fermentation days	Samples				
		A	B	C	D	E
pH	1	4,10^{Ac}±0,01	4,07^{BAb}±0,00	4,03^{Bb}±0,02	4,05^{BAb}±0,05	4,07^{BAc}±0,01
	3	4,18^{Ab}±0,04	4,15^{Ab}±0,05	4,30^{Aa}±0,10	4,19^{Aa}±0,01	4,15^{Ab}±0,05
	5	4,27^{CBa}±0,01	4,28^{Ba}±0,02	4,22^{Da} ±0,00	4,23^{CDa}±0,02	4,34^{Aa}±0,01
Acidity degree (%)	1	5,50^{Ba}±0,50	7,25^{BAa}±0,25	7,50^{Ab}±1,00	6,50^{BAa}±1,00	5,50^{Bb}±0,00
	3	3,25^{Cb}±0,38	3,50^{Cb}±0,90	10,50^{Aa}±1,00	4,25^{Cb}±0,75	7,75^{Ba}±0,25
	5	4,00^{BAba}±1,00	2,75^{Bb}±0,25	5,00^{BAb}±1,00	4,75^{BAba}±0,25	5,50^{Ab}±1,50
L*	1	51,992^{Aa} ±0,87	44,86^{Ba}±0,71	44,25^{Ba}±0,18	40,73^{Cb}±0,63	37,93^{Aa}±0,67
	3	42,76^{Ac}±1,13	40,87^{Ac}±1,32	38,11^{Ac}±1,21	37,02^{Ac}±1,42	35,66^{Ac}±1,02
	5	40,73^{BAb}±1,84	39,13^{BAb}±1,12	36,05^{BAb}±1,47	34,73^{BAb}±1,65	32,73^{BAb}±1,84
a*	1	19,9^{Ba}±0,84	17,7^{Ba}±0,4	16,9^{Ba}±0,28	15,9^{Ba}±0,21	14,1^{Ba}±0,41
	3	20,4^{Aa}±1,09	18,8^{Aa}±1,05	17,4^{Aa}±1,11	16,2^{Aa}±0,89	15,02^{Aa}±1,09
	5	21,5^{Aa}±1,19	20,5^{Aa}±1,14	19,4^{Aa}±0,15	18,5^{Aa}±1,17	18,3^{Aa}±0,08
b*	1	33,77^{Aa}±0,42	30,18^{Aa}±0,22	28,67^{Aa}±0,69	27,17^{Aa}±0,85	25,22^{Aa}±0,11
	3	25,82^{Bb}±2,29	22,12^{Bb}±2,65	21,58^{Bb}±1,78	20,42^{Bb}±1,12	19,89^{Bb}±2,25
	5	22,37^{Bc}±1,02	20,65^{Bc}±1,41	20,37^{Bc}±1,25	19,14^{Bc}±1,14	17,87^{Bc}±1,05

A-D: Capital letters in the same row are the comparison of tarhana varieties. The same letters indicate that there is no statistical difference between the samples (p>0.05).

a-c: Small letters in the same column are the comparison of fermentation times. The same letters indicate that there is no statistical difference between the samples (p>0.05)

The pH measurements of the samples ranged from 4.03 to 4.34, with the E tarhana sample exhibiting the highest pH value (4.34). This may be attributed to the comparatively lower fermentable sugar content in stale breadcrumbs relative to wheat flour, which likely resulted in reduced acid production during fermentation. This observation is consistent with the findings of Kılıç Keskin et al. (2022), who reported a pH value of 4.28 for wheat flour-based tarhana. Such differences in pH are often associated with variations in the carbohydrate composition of the raw materials utilized in fermentation.

The observed increase in pH values during tarhana fermentation may be attributed to the higher proportion of stale breadcrumbs used. Similar findings were reported by Cankurtaran Kömürcü and Bilgiçli (2022), who demonstrated that tarhana dough made with 100% maize flour had an initial pH of 5.65, which was higher than that of traditional wheat-based tarhana. This suggests that non-wheat flours or modified flour compositions, such as those incorporating ancient grains or alternative sources like maize, can have a significant impact on the fermentation process and the final characteristics of the product. The use of stale breadcrumbs likely follows a similar trend, where the altered carbohydrate composition leads to a slower fermentation rate, thereby reducing the overall decline in pH over time.

Bozkurt and Gürbüz (2008) supported these findings by reporting that the final pH values of tarhana products generally ranged from 3.3 to 5.0, depending on the fermentation conditions and the raw materials used. This range underscores the variability in tarhana fermentation, which can be influenced by factors such as fermentation duration, the type of starter culture, and the use of different flours or carbohydrate sources. In a study on tarhana made with rice and corn bran, Aktaş and Akın (2020) observed that as the proportion of bran increased, the pH values decreased, reinforcing the idea that fiber-rich ingredients tend to enhance acid production during fermentation.

In addition to these findings, research on the role of various carbohydrate substrates in fermented products further supports the hypothesis that sugar availability plays a crucial role in pH regulation. For example, Gökmen et al. (2021) showed that fermentation using ingredients with lower fermentable sugar content leads to less acidic final products, as a result of reduced lactic acid production. This highlights the importance of ingredient selection in determining the physicochemical properties of fermented foods like tarhana.

Color is a fundamental sensory characteristic that plays a critical role in shaping consumer purchasing behavior, often serving as a key determinant of product appeal and quality perception (Akan and Özdestan, 2019). As detailed in Table 2, both the duration of fermentation and the specific composition of the tarhana samples exerted a notable influence on the L*, a*, and b* color parameters, which are commonly used to quantify lightness, red-green, and blue-yellow values, respectively. The results indicate that the effect of tarhana composition on color was statistically significant when analyzed across identical fermentation times ($p<0.05$), underscoring the importance of ingredient variation in product aesthetics. Sample A exhibited the highest L*, a*, and b* values, suggesting that this formulation maintained superior brightness and vividness of color. This could potentially enhance its marketability, as consumers often associate brighter, more vibrant colors with freshness and quality. Conversely, an increase in the proportion of stale bread crumbs in the tarhana mixture led to a marked decrease in L* values, contributing to a darker, more brownish appearance. This change in color could negatively affect consumer perception, as darker hues in food products are sometimes linked with staleness.

These findings suggest that managing the ratio of ingredients, such as stale bread crumbs, in tarhana production is crucial not only for maintaining the desired sensory attributes but also for optimizing consumer appeal.

Analytical results of powder tarhana samples

Some physicochemical properties and colour values of powdered tarhana samples are presented in Table 3.

Table 3. Chemical composition and color values of powder tarhana samples.

	Samples				
	A	B	C	D	E
pH	4,24^{c}±0,01	4,36^{a}±0,01	4,29^{bac}±0,03	4,34^{ba}±0,04	4,28^{bc}±0,04
Acidity (%LA)	5,75^{b}±0,25	4,25^{c}±0,75	8,00^{a}±0,50	6,50^{b}±0,50	5,50^{cb}±0,00
Protein (%)	11,46^{c}±0,02	11,70^{b}±0,03	11,18^{d}±0,02	11,68^{b}±0,04	12,02^{a}±0,05
Fat (%)	5,75^{a}±0,95	4,48^{a}±0,42	4,35^{a}±0,04	4,65^{a}±0,23	4,36^{a}±1,00
Ash (%)	4,48^{d}±0,06	5,51^{b}±0,02	5,15^{c}±0,08	6,04^{a}±0,01	6,09^{a}±0,02
Moisture (%)	9,48^{a}±0,16	9,22^{ba}±0,04	9,24^{a}±0,07	9,22^{ba}±0,11	8,97^{b}±0,09
Water Activity (a_w)	0,55^{a}±0,01	0,53^{b}±0,01	0,49^{c}±0,01	0,46^{d}±0,01	0,45^{d}±0,00
L*	59,22^{a}±0,48	55,98^{b}±0,64	48,71^{c}±0,44	44,03^{d}±1,21	44,71^{d}±0,74
a*	20,7^{a}±0,19	19,4^{c}±0,48	20,5^{ba}±0,59	19,7^{bc}±0,81	20,0^{bac}±0,34
b*	35,21^{a}±0,42	35,29^{a}±0,54	34,47^{a}±0,96	29,71^{b}±1,07	34,43^{a}±0,80

a-d: Small letters in the same row are the comparison of powdered tarhana varieties.
The same letters indicate that there is no statistical difference between the samples ($p>0,05$).

The statistical results indicate that pH, acidity, ash, protein, moisture and water activity of the powdered tarhana samples were found to be statistically significant ($p<0.05$), whereas fat values were insignificant ($p>0.05$). The findings also suggest that the chemical properties of the tarhana samples were not negatively impacted by stale breadcrumbs.

The moisture content of powdered tarhana, produced with varying proportions of stale breadcrumbs, ranged from 8.97% to 9.48%. These values align with the standards set by the Turkish Standards Institute, which stipulates that the moisture content of powdered tarhana should not exceed 10% (TSE No: 2282, 2004). The reduction in moisture content with the inclusion of stale breadcrumbs is a significant observation. As the proportion of stale breadcrumbs increased, there was a corresponding decline in the moisture content of the tarhana samples, with the most pronounced reduction observed when stale breadcrumbs constituted 100% of the formulation.

This decrease in moisture content can be attributed to the reduced water-holding capacity of stale bread compared to fresh bread or other traditional tarhana ingredients. Stale bread, having undergone partial dehydration over time, inherently contains less moisture, which is

reflected in the overall lower moisture levels of the final tarhana product. This phenomenon is critical for the shelf stability of tarhana, as lower moisture content is generally associated with extended shelf life and reduced microbial activity, thus enhancing product preservation.

The results of this study are consistent with prior research on tarhana formulations, which similarly reported a decline in moisture content when stale breadcrumbs or other dehydrated components were used (Cankurtaran Kömürcü and Bilgiçli, 2022; Aktaş and Akın, 2020). These findings support the notion that ingredient selection, particularly the use of stale bread, plays a crucial role in determining the physical properties of tarhana, specifically its moisture content.

In the present study, the protein content of powdered tarhana samples ranged from 11.18% to 12.08%. These values are comparatively lower than those reported by Kılıç Keskin et al. (2022), who documented protein levels ranging from 16.46% to 31.90% in tarhana produced using cereal and legume flours. The notable difference in protein content can be largely attributed to the use of legume flours, which are recognized for their significantly higher protein concentrations compared to cereal flours. As highlighted in the literature, legume flours, such as chickpea or lentil flour, are rich sources of protein, thus contributing to elevated protein levels in food products incorporating these ingredients (Binou et al., 2020). This suggests that the specific type of flour utilized in tarhana formulation plays a decisive role in determining its overall protein content.

Furthermore, Cankurtaran Kömürcü and Bilgiçli (2022) investigated the protein content of tarhana samples made with ancient wheat flours and found values between 13.05% and 14.95%. These findings highlight the influence of wheat type on the nutritional profile of tarhana, as ancient wheat varieties have been shown to possess higher protein and ash content compared to modern wheat (Hammed and Simsek, 2014). The elevated protein content in ancient wheat is often linked to its genetic composition and lower levels of refinement, which may preserve more of its nutritional integrity.

The powdered tarhana samples obtained in our study had a varying total ash content ranging between 4.48% to 6.09%. Table 3 depicts that the mean ash content of powdered tarhana samples made from stale bread crumbs was higher compared to that of all wheat flour tarhanas. The inclusion of stale breadcrumbs resulted in a significant rise of ash content in powdered tarhana. Kılıç Keskin et al. also found similar findings (Kılıç Keskin et al., 2022).

In the tarhana sample made with 100% wheat flour, the ash content was determined to be 4.48%. An increase in ash content was observed across all tarhana samples as the proportion of stale breadcrumbs increased. The highest ash content was recorded in Sample E, with a value of 6.09%. This increase can be attributed to the fact that the ash content of stale breadcrumbs is higher than that of wheat flour. Similarly, Akan and Özdestan-Ocak (2019) reported an increase in dry matter and ash content with the increasing addition of grape seed extract, while Erol and Özdestan Ocak (2020) documented a decrease in dry matter content and an increase in ash content. Likewise, Aktaş and Akın (2020) examined the effect of corn flour substitution on tarhana quality and found that as corn flour substitution increased, dry matter content decreased while ash content increased.

Additionally, the dry matter and ash contents of homemade or commercially produced tarhana are consistent with the findings of this study, ranging between 82.99% to 92.06% for dry matter and 1.03% to 8.79% for ash content (Bilgiçli, 2009; Erol and Özdestan Ocak, 2020). These similarities indicate that the variations in dry matter and ash content observed in this study align with the typical composition of tarhana across different production methods.

The fat content of the powdered tarhana samples in our study ranged from 4.35% to 5.75%. Statistical analysis revealed that the inclusion of stale breadcrumbs did not exert a significant effect on the fat content of the tarhana samples ($p>0.05$),

indicating that the fat composition remained relatively stable despite variations in the formulation. This stability in fat content can likely be attributed to the fact that stale bread, typically composed of carbohydrates and minimal fat, does not substantially alter the lipid profile of the final product when used in place of wheat flour.

Our findings are consistent with those of previous studies. For instance, Cankurtaran Kömürcü and Bilgiçli (2022) similarly reported no significant changes in fat content when alternative ingredients were incorporated into tarhana formulations. Likewise, Aktaş and Akın (2020) observed minimal variation in fat content when evaluating the effect of different flour substitutions on tarhana quality. These studies reinforce the notion that the fat content of tarhana is largely influenced by its core ingredients, such as dairy products or oils, rather than the carbohydrate-based components like bread or flour.

The color attributes of the powdered tarhana samples, as measured by L*, a*, and b* values, ranged from 44.03 to 59.22 for L*, 19.4 to 20.7 for a*, and 29.71 to 35.29 for b*. These results indicate a notable variation in the color profiles of the tarhana samples, with a discernible difference between those made with stale breadcrumbs and those made with wheat flour. Specifically, tarhana formulations incorporating stale breadcrumbs exhibited lower average values for L*, a*, and b*, suggesting a darker and less vibrant color compared to tarhana made solely from wheat flour (see Table 3).

In particular, Group A tarhana displayed higher L* (lightness) and a* (red-green) values, which implies a lighter and more reddish hue. Conversely, Group B tarhana, while also exhibiting a higher b* (yellow-blue) value than other groups, had a color profile similar to Group A but with greater b* values. This phenomenon can be attributed to the inherent darker color of stale breadcrumbs compared to wheat flour, which affects the overall color characteristics of the tarhana product.

The observed variations in color measurements are consistent with findings from Gularte et al. (2011), who noted that the color of flour components significantly influences the final color of food products. The presence of darker ingredients, such as stale breadcrumbs, directly impacts the lightness and color intensity of the tarhana, reflecting the sensory characteristics that consumers may perceive.

Microbiological counts of tarhana doughs and powder tarhanas

Microbiological analysis findings for tarhana dough and powdered tarhana are provided in Table 4 and Table 5.

Table 4. Microbiological Analysis Results of DoughTarhana Samples (microbial number log CFU/g)* (Results of total mesophilic aerobic bacteria (TMAB), coliform, *S.aureus*, lactic acid bacteria, yeast and mold analyzes in samples)

Microorganism Type	Fermentation days	Samples				
		A	B	C	D	E
TMAB	1	6,09^{Aa}±0,10	5,17^{Ba}±0,00	5,95^{Aa}±0,08	5,96^{Aa}±0,06	4,69^{Cb}±0,00
	3	4,69^{Cc}±0,00	4,58^{Cb}±0,13	5,11^{BCa}±0,48	5,52^{BAb}±0,07	5,93^{Aa}±0,08
	5	5,46^{BACb}±0,08	5,00^{Cba}±0,31	5,18^{BCa}±0,36	5,70^{BAba}±0,04	5,94^{Aa}±0,10
Yeast	1	3,63^{Aba}± 0,67	3,03^{Ab}±0,19	3,63^{Ab}±0,67	3,03^{Ab}±0,19	4,19^{Ab}±0,11
	3	3,29^{Ab}±0,30	3,47^{Ab}±0,36	4,06^{Ab}±0,30	4,00^{Ab}±0,17	4,22^{Ab}±0,02
	5	5,06^{Aa}±0,03	6,06^{Aa}±0,30	6,06^{Aa}±0,30	5,73^{Aa}±0,76	5,86^{Aa}±0,61
Mold	1	<1^{Aa}	<1^{Aa}	<1^{Aa}	<1^{Aa}	<1^{Aa}
	3	<1^{Aa}	<1^{Aa}	<1^{Aa}	<1^{Aa}	<1^{Aa}
	5	<1^{Aa}	<1^{Aa}	<1^{Aa}	<1^{Aa}	<1^{Aa}
Coliform	1	<1^{Aa}	<1^{Aa}	<1^{Aa}	<1^{Aa}	<1^{Aa}
	3	<1^{Aa}	<1^{Aa}	<1^{Aa}	<1^{Aa}	<1^{Aa}
	5	<1^{Aa}	<1^{Aa}	<1^{Aa}	<1^{Aa}	<1^{Aa}
S. aureus	1	<1^{Ba}	<1^{Ba}	2,59^{Aa}±0,15	2,69^{Aa}±0,06	<1^{Ba}
	3	<1^{Aa}	<1^{Aa}	<1^{Ab}	<1^{Ab}	<1^{Aa}
	5	<1A^{a}	<1^{Aa}	<1^{Ab}	<1^{Ab}	<1^{Aa}
MRS	1	7,48^{Aa}±0,05	7,54^{Aa}±0,03	7,11^{Ba}±0,06	7,53^{Aa}±0,02	7,46^{Aa}±0,02
	3	6,44^{Ab}±0,08	6,48^{Ab}±0,02	6,05^{Bc}±0,03	6,01^{Bc}±0,01	5,24^{Cc}±0,10
	5	6,07^{Cc}±0,06	5,74^{Dc}±0,03	6,44^{Ab}±0,01	6,18^{CBb}±0,01	6,29^{Bb}±0,01
M17	1	6,19^{Aa}±0,27	4,96^{Bc}±0,02	4,93^{Bc}±0,08	7,06^{Aa}±0,25	4,58^{Bb}±0,07
	3	6,54^{Aa}±0,01	6,39^{Bb}±0,02	6,42^{Ba}±0,03	6,30^{Ca}±0,01	6,27^{Ca}±0,00
	5	6,38^{BAa}±0,01	6,56^{Aa}±0,01	5,65^{Cb}±0,02	5,95^{BCa}±0,21	6,30^{BAa}±0,01

*: mean ±standard deviation.

A-D: Capital letters in the same row are the comparison of tarhana varieties. The same letters indicate that there is no statistical difference between the samples (p>0.05).

a-c: Small letters in the same column are the comparison of fermentation times. The same letters indicate that there is no statistical difference between the samples (p>0.05).

Table 5. Microbiological Properties of Powder Tarhana Samples (Microbial number: log CFU/g)*

Samples	TMAB	Yeast	Mold	*S. aureus*	Coliform	Lactic Acid	
						MRS agar	M17 agar
A$_P$	4,08^{c}± 0,26	3,00^{b}± 0,17	<1^{a}	<1^{a}	<1^{a}	5,86^{bc}± 0,02	4,60^{a}± 0,06
B$_P$	4,69^{b}± 0,00	3,90^{a}± 0,15	<1^{a}	<1^{a}	<1^{a}	5,60^{d}± 0,05	3,77^{c}± 0,01
C$_P$	4,69^{b}± 0,00	3,86^{a}± 0,09	<1^{a}	<1^{a}	<1^{a}	5,99^{ba}± 0,01	4,37^{b}± 0,04
D$_P$	5,74^{a}± 0,03	3,90^{a}± 0,31	<1^{a}	<1^{a}	<1^{a}	5,83^{c}± 0,03	3,32^{b}± 0,04
E$_P$	4,65^{b}± 0,08	4,22^{a}± 0,07	<1^{a}	<1^{a}	<1^{a}	6,11^{a}± 0,02	4,57^{a}± 0,04

*: mean ± standard deviation

a-d: Small letters in the same row are comparison tarhana samples. The same letters indicate that there is no statistical difference between the samples (p>0.05).

The total counts of bacteria, yeast and lactic acid bacteria in tarhana dough samples on the 1st, 3rd and 5th day of fermentation were found to be in accordance with the standards in all samples. TMAB loads varied between 4.08 and 6.09 log CFU/g in all tarhana samples, including fermentation. There was no presence of coliforms or mold growth in any of the samples. All samples showed yeast detection between 3.00 and 6.06 log CFU/g. Yeast counts increased during fermentation in all samples of tarhana dough (p<0.05). Only 2 samples contained *S. aureus*. Furthermore, *S. aureus* did not develop in the later stages of fermentation nor in the powdered samples. These findings are in line with previous research on the fermentation of tarhana dough samples (Tasdelen and Şimşek 2021).

Upon examining the tarhana samples, it was observed that the progression of the fermentation process led to an increase in pH levels. This phenomenon may be attributed to the consumption or neutralization of acidic compounds produced during fermentation (Özdemir et al., 2018). Notably, the increase in pH

became more pronounced with the higher proportion of stale bread used in the tarhana formulation, suggesting that buffering compounds present in the bread influenced the fermentation environment by reducing acidity and consequently raising the pH.

The observed overall decrease in acidity further supports this hypothesis. Organic acids such as lactic acid, which are primarily responsible for increasing acidity during fermentation, are produced in significant amounts. However, as fermentation progresses and the drying process takes place, a portion of these acids either dissipates or becomes inactive, leading to a reduction in acidity (Şimşek et al., 2017). The more pronounced decrease in acidity with the increasing proportion of stale bread crumbs suggests that the components of the bread may inhibit or slow down acid production during fermentation.

The reduction in lactic acid bacteria (LAB) can be directly linked to the progression of fermentation and the drying process. Although LAB play a crucial role during fermentation, their survival rates decrease due to the high temperatures and low humidity conditions during drying. The decline in LAB populations observed after the tarhana is transformed into powder form indicates the bacteria's sensitivity to the drying process (Sengun et al., 2009). This finding underscores the need for careful optimization of drying parameters in order to preserve the probiotic characteristics of fermented foods like tarhana.

LAB diversity in tarhana dough and powder tarhana samples

LAB colonies were counted on both MRS and M17 media followed by purification, after which identification tests commenced. While the API 50 CHL LAB identification tests and classical biochemical identification tests revealed differences in some biochemical properties among the 20 different samples, they yielded only two isolates. The *Lb. delbrueckii ssp. bulgaricus* and *S. thermophilus* isolates were obtained as the results of this investigation. There were no other lactic acid bacteria to be found. It is believed that the heat treatment applied to the raw materials during the boiling process for tarhana production has an adverse impact on the growth of other lactic acid bacteria. Furthermore, only the bacteria found in the yogurt added to the mixture post-boiling were isolated. Ilango and colleagues reported that *L. plantarum, L. brevis, L. casei, L. bulgaricus, S. thermophilus* and *L. fermentum* species were isolated from tarhana (Ilango and Antony, 2021). In this regard, our investigation produced akin findings to the existing literature.

Sensory analysis of tarhana soups: The sensory analysis outcomes for the powdered tarhana soup samples utilized in the study are depicted in Table 6.

Table 6. Sensory analysis results of tarhana soup samples.

Features	Samples				
	A_S	B_S	C_S	D_S	E_S
Color	$8,40^{a}$± 0,89	$7,40^{a}$± 0,55	$5,80^{b}$± 0,84	$4,60^{cb}$± 0,55	$3,60^{c}$± 0,55
Taste	$7,80^{a}$±0,84	$5,40^{b}$± 0,89	$4,40^{cb}$±0,89	$3,60^{cd}$± 0,89	$2,40^{d}$±0,55
Smell	$8,00^{a}$± 0,71	$7,60^{a}$±0,55	$6,20^{b}$±0,84	$4,60^{c}$±0,55	$2,80^{d}$±0,84
Consistency	$8,40^{a}$± 0,55	$7,40^{a}$±0,55	$5,60^{b}$±0,55	$4,00^{c}$±0,71	$3,00^{c}$±0,71
Foreign taste and smell	$3,20^{c}$±0,45	$4,60^{b}$±0,55	$5,00^{b}$±0,71	$6,60^{a}$±0,55	$7,60^{a}$±0,55
General acceptability	$8,60^{a}$±0,55	$7,40^{a}$± 0,89	$5,40^{b}$±0,55	$4,40^{b}$±0,55	$2,80^{c}$±0,84

*: mean ± standard deviation

$^{a-d}$: Lower case letters in the same row are comparisons of tarhana samples. Different letters indicate a statistical difference between the samples ($p<0.05$).

The statistical analysis indicated a significant effect ($p<0.05$) of stale breadcrumbs on the colour,

taste, aroma, texture, foreign taste and odour, and general taste attributes of the samples. Based on the results of the sensory analysis, sample A, which represents the control group and uses 100% wheat flour, obtained the highest score in overall appreciation. The sample labelled B, with a wheat flour to stale breadcrumbs ratio of 75:25, came in second place. The remaining examples are identified as C, D, and E. The E sample, which used 100% stale breadcrumbs, displayed the lowest score. These findings demonstrate that tarhana composed of 25% stale breadcrumbs provides a viable alternative to tarhana produced solely from white wheat flour.

The pH, protein, fat and moisture values of all tarhana samples were similar, indicating that the physicochemical properties of tarhana were not adversely affected by stale breadcrumbs. The protein content of tarhana was low because the stale breadcrumbs were produced from modern wheat, as expected. The addition of stale breadcrumbs led to a noteworthy increase in ash content in powdered tarhana. With increased stale breadcrumbs in the tarhana, the brightness decreased and the colour darkened. It was found that the colour values of the tarhana obtained were within TSE standards. The bacterial, yeast, and lactic acid bacterial counts in the tarhana dough samples on the 1st, 3rd and 5th day of fermentation were deemed to conform to the prescribed standards across all samples. Moreover, based on the results of the microbiological analysis, adding stale breadcrumbs to tarhana did not affect yoghurt bacteria and fermentation factors in tarhana adversely. It is crucial to detect *Lb. delbrueckii ssp. bulgaricus* and *S. thermophilus* isolates in the produced tarhanas. After conducting a thorough sensory analysis, it has been determined that tarhana production can incorporate breadcrumbs obtained from stale bread at a ratio of no more than 25%.

Conclusions

This study demonstrated that the quality characteristics of tarhana prepared with varying proportions of stale bread did not differ significantly from those made with 100% wheat flour. These findings suggest that stale bread can be effectively utilized in tarhana production, offering an opportunity to reduce costs without compromising product quality. Furthermore, the presence of specific lactic acid bacteria supports the potential development of a probiotic tarhana soup. Additionally, the long-term stability of the probiotic properties in tarhana was not assessed. Future research should address these limitations by exploring the economic implications of stale bread usage in more detail, investigating a broader spectrum of lactic acid bacteria, and evaluating the potential health benefits and shelf-life stability of probiotic tarhana.

Declarations

Conflict of Interest: The authors declare that there is no conflict of interest between them.

Author Contribution: All authors contributed equally

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