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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Effect of Germination and Type III Sourdough on the Fermentation Behavior and Quality of Tarhana*

Çimlendirme ve Tip-III Ekşi Hamurun Tarhana Fermantasyonu ve Kalitesi Üzerindeki Etkisi

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Abstract

This study aimed to investigate the impact of pre-processed whole wheat flours, specifically those obtained through germination and sourdough fermentation followed by lyophilization (corresponding to Type III sourdough), on the physicochemical, biochemical, and microbiological properties of tarhana. Germinated and non-germinated whole wheat flours were subjected to Type II sourdough fermentation using *Lactobacillus paracasei* as the starter culture, then lyophilized to obtain Type III sourdough. These pre-treated sourdough powders were subsequently incorporated into tarhana formulations to assess their combined effect on product quality. Results revealed that germination significantly enhanced LAB viability after drying, yielding higher survival rates (log 5.72±0.03 to 6.20±0.06 CFU g^{-1}) compared to the non-germinated samples (log 1.75±0.19 to 1.91±0.07 CFU g^{-1}). Germination further improved the nutritional quality of tarhana samples, leading to increases in the protein solubility index (22.66%), water-soluble protein content (95.54%), and total free amino acid levels (69.90%). However, the incorporation of Type III sourdough was observed to cause a significant reduction in total free amino acids in the germinated group (p=.003). On the other hand, the effect of Type III sourdough on water-soluble protein content differed depending on the germination status. While water-soluble protein content increased in the non-germinated samples (T1 vs T2: 35.08 vs. 44.26 g kg^{-1}, p<.05), a significant decrease was observed in the germinated samples (T3 vs T4: 81.01 vs. 74.13 g kg^{-1}, p<.05). Notably, the lowest pH (3.64) and highest titratable acidity (34.96 mL) were recorded in sample T4, prepared using Type III sourdough derived from germinated whole wheat flour, highlighting the effect of the combined pre-treatment on acidification. Overall, these results suggest that germination and Type III sourdough contribute to improving the nutritional attributes and protein bioaccessibility of tarhana proteins, while also supporting the survival of beneficial lactic acid bacteria.

Keywords: Tarhana, Germination, Fermentation, Type III sourdough, Lactic acid bacteria, Bioaccessibility

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Öz

Bu çalışmada, çimlendirme ve ekşi hamur fermantasyonunu takiben uygulanan liyofilizasyon (Tip III ekşi hamur prosesi) ile elde edilen ön işlem görmüş tam buğday unlarının tarhananın fizikokimyasal, biyokimyasal ve mikrobiyolojik özellikleri üzerindeki etkileri araştırılmıştır. Çimlendirilmiş ve çimlendirilmemiş tam buğday unları, *Lactobacillus paracasei* starter kültürü ile Tip II ekşi hamur fermantasyonuna tabi tutulmuş, ardından liyofilize edilerek Tip III ekşi hamur örnekleri hazırlanmıştır. Ön işlem görmüş materyaller, ürün kalitesi üzerindeki birleşik etkilerini değerlendirmek amacıyla tarhana formülasyonlarına ilave edilmiştir. Sonuçlar, çimlendirmenin kurutma sonrasında laktik asit bakterilerinin canlılığını anlamlı düzeyde artırdığını ve çimlendirilmemiş örneklere kıyasla (log 1.75±0.19 ve 1.91±0.07 KOB g^{-1}) daha yüksek hayatta kalma oranları sağladığını (log 5.72±0.03 ve 6.20±0.06 KOB g^{-1}) ortaya koymuştur. Çimlendirme ayrıca, tarhana çeşitlerinin besinsel kalitesinde iyileşmelere yol açmış, protein çözünürlüğü indeksi, suda çözünür protein miktarı ve toplam serbest amino asit içeriğinde sırasıyla 22.66%, 95.54%ve 69.90% oranında artış sağlamıştır. Bununla birlikte, Tip III ekşi hamur kullanımı, çimlendirilmiş gruptaki örneklerin toplam serbest amino asit içeriğinde istatistiksel olarak anlamlı bir azalmaya yol açmıştır (p=.003). Öte yandan, Tip III ekşi hamurun suda çözünür protein içeriği üzerindeki etkisi çimlendirme durumuna bağlı olarak farklılık göstermiştir. Çimlendirilmemiş örneklerde suda çözünür protein içeriği artarken (T1 ve T2 sırasıyla; 35.08 ve 44.26 g kg^{-1}, p<.05), çimlendirilmiş örneklerde anlamlı bir azalma gözlenmiştir (T3 ve T4 sırasıyla; 81.01 ve 74.13 g kg^{-1}, p<.05). Dikkat çekici biçimde, en düşük pH (3.64) ve en yüksek titrasyon asitliği (34.96 mL) değerleri, çimlendirilmiş tam buğday unundan elde edilen Tip III ekşi hamur kullanılarak hazırlanan T4 örneğinde kaydedilmiştir. Bu sonuç, ön işlemlerin birlikte uygulanmasının asitlik gelişimi üzerinde belirgin bir etki yarattığını göstermektedir. Özetle, elde edilen bulgular çimlendirme ve Tip III ekşi hamurun, tarhananın besinsel özellikleri ile protein biyoerişilebilirliğini geliştirdiğini ve yararlı laktik asit bakterilerinin canlılığını desteklediğini göstermektedir.

Anahtar Kelimeler: Tarhana, Çimlendirme, Fermantasyon, Tip III ekşi hamur, Laktik asit bakterisi, Biyoerişilebilirlik

1. Introduction

Fermentation is a long-established process that transforms organic compounds through microbial or enzymatic activity. Initially used for preservation and flavor, it now plays a vital role in improving nutritional quality, ensuring food safety, and producing bioactive compounds (Fraberger et al., 2020).

Sourdough fermentation, driven by lactic acid bacteria (LAB) and/or yeast species, enhances the nutritional, functional, technological, and sensory properties of cereal-based products (Karlıdag et al., 2022) and is typically categorized into three main types; Type I, Type II, and Type III, each differing in fermentation conditions, microbial composition, and industrial applications (Arendt et al., 2007; De Vuyst et al., 2017). Type I sourdoughs have a firm consistency (dough yield, DY < 200) and require continuous back-slopping to maintain microbial activity. Type II sourdoughs are semi-liquid (DY > 200), produced through extended fermentation, and commonly used in industrial applications. Type III sourdoughs, in contrast, are dried and mainly serve as aroma carriers and dough acidifiers.

Metabolic interactions between LAB and wheat-derived hydrolytic enzymes play a key role in sourdough fermentation (Reale et al., 2021). Cereal enzymes support LAB growth by releasing substrates, while fermentation-induced acidification (pH 4.2-5.5) enhances enzymatic activity and nutrient release from the grain matrix (Gänzle, 2013). Besides, LAB-driven secondary proteolysis breaks down oligopeptides into free amino acids, which contribute to the nutritional value and influence the sensory and rheological properties of fermented products (De Vuyst et al., 2021).

Germination is another strategy for modifying the nutritional and technological properties of grains, as it enhances hydrolytic enzyme activity, increases dietary fiber, free amino acids, antioxidants, and reduces antinutritional compounds such as phytic acid (Van Hung et al., 2015; Lemmens et al., 2018a). These biochemical changes improve the nutritional and functional qualities of wheat (Johnston et al., 2019) and may offer health benefits, including reduced gluten immunoreactivity (Donkor et al., 2012; Žilić et al., 2016).

Tarhana is a traditional fermented product made from wheat flour, yogurt, and various plant-based ingredients, followed by drying and grinding. Fermentation enhances protein digestibility (Ozdemir et al., 2007), reduces phytic acid content to improve mineral bioavailability (O'Callaghan et al., 2019), and lowers the glycemic index of dairy-cereal blends (Tsafrakidou et al., 2020). It also increases vitamin and essential amino acid levels (Molfetta et al., 2022) and enriches food matrices with bioactive compounds such as organic acids, exopolysaccharides, phenolics, and bioactive peptides (Rizzello et al., 2019; Shevade et al., 2019; Da Ros et al., 2021).

In this study, an integrated approach was adopted to enhance the nutritional quality and protein bioaccessibility of tarhana formulations. Two well-established bioprocessing strategies, specifically germination and sourdough fermentation, were applied as pre-treatments to whole wheat flour prior to its incorporation into tarhana. While both approaches have been individually reported to improve the nutritional quality of cereal-based formulations, their combined effect in tarhana production remains unexplored. To address this gap, germinated and non-germinated whole wheat flours were subjected to Type II sourdough fermentation using *Lactobacillus paracasei* as the starter culture, followed by lyophilization to obtain Type III sourdough preparations. These pre-treated materials were then incorporated into tarhana formulations and fermented for seven days at 30 ºC. The final products were characterized in terms of their physicochemical, biochemical, and microbiological properties, with a particular focus on protein/amino acid related parameters.

2. Materials and Methods

2.1. Biological samples

Lactobacillus paracasei used for Type II sourdough fermentation was obtained from the culture stock of the Food Engineering Department at Kahramanmaras Sutcu Imam University (KSU). The wheat variety (ZDEB-103), used for flour production, was supplied by the Agricultural Biotechnology Department, KSU, Turkiye, from samples harvested in September 2022.

2.2. Preparation of tarhana

Wheat Flours

Wheat flour preparation was conducted as described by Montemurro et al. (2019) with slight modifications. Briefly, grains were rinsed to remove impurities and surface-sterilized with 1.25% sodium hypochlorite solution (1:3 dilution with sterile distilled water containing 0.01% Tween 20) for 20 minutes. Residual NaOCl was removed via thorough rinsing. Sterilization efficacy was confirmed by blotting grains tightly on 1/10 strength tryptic soy agar. Half of the grains were dried at 50 ºC (10-14% moisture) to produce non-germinated flour. The remaining grains were soaked (1:3 w/w) for 24 hours at 20 ºC with periodic agitation and germinated at 21.5 ºC and 85% humidity (*Figure 1*). Germination ended on day 4 when sprout length reached 3/4 of the grain. Grains were dried, de-rooted, milled (0.75 mm), and stored at 4 ºC until use.

Figure 1. Visual representation of wheat grains on the first (Day 0) and fourth days (Day 4) of germination

Type II Sourdough Fermentation

Lactobacillus paracasei was cultivated in MRS broth at 37 ºC and activated through two consecutive 24-hour incubations. Two mililiters of active culture were transferred to 38 mL of fresh broth and incubated to reach OD_{620}=1 (7.1x10^8 CFU mL^{-1}). Liquid sourdough (dough yield: 450) was prepared by mixing 1670 mL of sterile distilled water, 80 mL of culture suspension (5.6x10^{10} CFU), and 500 g whole wheat flour (germinated or non-germinated). Fermentation was conducted in Erlenmeyer flasks on an orbital shaker at 37 ºC for 24 hours, followed by freeze-drying yielding Type III sourdough powder.

Yogurt Production

Cow's milk was sourced from the KSU Animal Production Application and Research Center. Raw milk was pasteurized at 85 ºC for 30 minutes, cooled to 42 ºC, and inoculated with a commercial yogurt starter (Vivo, Ukraine). Fermentation proceeded at 42 ºC and was terminated at pH 4.6. Yogurt was stored at +4 ºC for 48 hours before use.

Vegetable Pasteurization

Tomatoes, red peppers, onions, and fresh mint were washed, chopped, and homogenized using a high-speed blender (Arzum AR1008, Türkiye). The mixture was pasteurized (65 ºC for 30 minutes) according to the procedure outlined by Erbas et al. (2005).

Table 1. Experimental model used for the production of different tarhana varieties*

Tarhana/dough code	Flour type	Pre-treatment applied to flour	Flour code
T1**	Non-germinated whole wheat flour	None	NG
T2	Non-germinated whole wheat flour	Type III sourdough process	NG-S
T3	Germinated whole wheat flour	None	G
T4	Germinated whole wheat flour	Type III sourdough process	G-S

*Experimental model was conducted in duplicate
**Base formulation used as control sample

Fermentation of Tarhana Dough

Tarhana samples were produced by spontaneous fermentation following the experimental design outlined in *Table 1*. Experimental model consisted of four tarhana dough formulations (T1-T4) derived from combinations of flour type (germinated or non-germinated) and pre-treatment (with or without Type III sourdough process).

Tarhana doughs were prepared according to the proportions specified in *Table 2*. Flour, yogurt, pasteurized vegetables, and salt were mixed (Arzum AR1008, Turkiye) in a polypropylene container until a homogeneous mixture was obtained. Fermentation proceeded at 30 ºC for seven days, followed by drying at 45 ºC until the doughs reached approximately 10% moisture content. Dried samples were then ground (Bosch TSM6A013B, Germany), sieved to 500 µm, and stored in resealable bags at 4 ºC. All tarhana formulations were produced in two independent batches.

Table 2. Formulations of four different tarhana dough varieties (591 g total dough weight for each variety)

	Tarhana code			
Ingredient (g)	T1	T2	T3	T4
NG	210			
NG-S		210		
G			210	
G-S				210
Yogurt	150			
Tomato	80			
Onion	50			
Red pepper	80			
Fresh mint	12			
Salt	9			

2.3. Physicochemical analysis

Moisture (Method 44-19), protein (Method 46-12), fat (Method 30-25), and ash (Method 08-01) contents were determined according to the American Association of Cereal Chemists (AACC) International Standard Methods (AACC, 2010). Water activity (a_w) of dried tarhana samples was measured in triplicate using a calibrated AquaLab 4TE device (USA).

2.4. pH and total titratable acidity

To monitor changes in pH and total titratable acidity (TTA) during fermentation, samples were collected at days 0 (initial), 1, 3, 5, and 7. The pH of tarhana dough and dried tarhana was measured following the method of Ibanoglu et al. (1999) using a calibrated pH meter (Eutech Instruments, CyberScan pH 300, Singapore), TTA was determined in accordance with TS 2282 (TSE, 2004). Shortly, 10 g of liquid dough was mixed with 50 mL of 67% neutralized ethyl alcohol, shaken, and filtered. Then, 10 mL of the filtrate was diluted with 200 mL of distilled water and titrated with 0.1 N NaOH. The volume of NaOH used was multiplied by 5 to calculate acidity.

2.5. Microbial count

Microbiological enumeration was performed according to the method described by Reale et al. (2021). Lactic acid bacteria (LAB) and total yeast-mold counts were assessed on days 1 and 7 of fermentation in tarhana dough, and after the drying process in powdered tarhana. For analysis, 10 g of tarhana dough or powder was homogenized in 90 mL of sterile physiological saline (8.5 g L^{-1} NaCl) for 2 minutes. LAB were enumerated using the spread plate method on MRS agar (Condalab, Spain) supplemented with cycloheximide (4 mg 100 mL^{-1}). Anaerobic conditions were maintained by overlaying plates with an additional MRS agar layer, and plates were incubated at 37 ºC for 48 hours. Yeast-mold counts were determined using Yeast Extract-Peptone-Dextrose (YPD) agar supplemented with 50 mg/L streptomycin. Plates were incubated at 28 ºC for 72 hours after spread plating.

2.6. Extraction of water-soluble nitrogenous compounds

Extraction of nitrogenous compounds from tarhana samples was performed according to the method developed by Kowalska et al. (2021). Briefly, 5 g of tarhana was mixed with 20 mL of 1% trichloroacetic acid (TCA) solution and 5 mL of 70% (v/v) ethanol in a polypropylene tube. Extraction was carried out at 4 ºC, 400 rpm for 60 minutes using an orbital shaker. Following extraction, samples were centrifuged at 6250xg for 12 minutes at 4 ºC. The resulting supernatants were filtered through a 0.45 µm polyvinylidene fluoride (PVDF) membrane and collected for further analysis.

2.7. Total free amino acid content

Total free amino acid (TFAA) content in the water-soluble nitrogenous extracts of tarhana samples was determined using the trinitrobenzenesulfonic acid (TNBS) method described by Adler-Nissen (1979) with slight modifications. A 0.01% (v/v) TNBS solution was prepared in 0.2 M sodium phosphate buffer (pH 8) using TNBS reagent (Sigma, P2297, A.B.D.). A standard calibration curve was generated using L-leucine solutions (0.5-3 mM) in 10 mM HCl. For the assay, 50 µL of either L-leucine standard or tarhana extract was mixed with 200 µL of TNBS reagent in Eppendorf tubes and vortexed. The mixture was incubated at 50 ºC for 60 minutes. Following incubation, 1 mL of 0.2 M HCl was added to stop the reaction. Absorbance was measured at 340 nm using a UV-1800 spectrophotometer (Shimadzu, Japan), and results were expressed as grams of L-leucine equivalent amino acids per kilogram of tarhana.

2.8. Water-soluble protein content

Water-soluble protein (WSP) content in the nitrogenous extracts of tarhana samples was determined using the bicinchoninic acid (BCA) assay, following the protocol proposed by Dallas et al. (2015). The BCA working reagent was prepared by mixing reagent A and reagent B from the BCA protein assay kit (Thermo Fisher, A.B.D.) at a 1:50 volume ratio. Subsequently, 200 µL of the prepared reagent was added to wells, followed by 25 µL of the test sample. Plate was incubated at 37 ºC for 30 minutes. After incubation, absorbance was measured at 562 nm using a microplate reader (Moleculer Devices, Spectramax 384 Plus, A.B.D.). For sample and reagent blanks, 0.1 M sodium acetate buffer (pH 4.7) and the nitrogenous extraction solvent (1% TCA and ethanol solution) were used, respectively. Absorbance values were converted into protein concentrations using a linear regression equation derived from a calibration curve prepared with bovine serum albumin (BSA, 2 mg mL^{1}) supplied in the kit.

2.9. Protein solubility index

Protein solubility is a key physicochemical property that reflects the ability of proteins to dissolve in aqueous solutions. It is largely influenced by structural modifications within the protein matrix, the extent of proteolysis, and protein-water interactions. The protein solubility index (PSI) of tarhana samples was calculated according to the method described by He et al. (2019), as the ratio of water-soluble protein (mg mL^{-1}) to total protein content (mg mL^{-1}). Protein solubility was determined as described in *Equation 1*.

$$\text{Protein solubility index} = \frac{WSP\ in\ the\ nitrogenous\ extracts}{Total\ nitrogenous\ matter\ in\ extracts} \times 100 \qquad \text{(Eq. 1).}$$

2.10. Statistical analysis

Statistical analyses were performed using JMP software (Version 16.2, SAS Institute Inc., Cary, NC, USA) (JMP, 2022). According to the experimental model described in *Table 1*, four tarhana dough formulations (T1-T4) were prepared by combining two flour types (germinated or non-germinated) and pre-treatment conditions (with or without Type III sourdough process). Two-way analyses of variance (ANOVA) were conducted to examine the main and interaction effects of flour type and pre-treatment at each fermentation day. Data presented in *Table 5* were analyzed using one-way ANOVA to compare the effects of dried tarhana varieties on different physicochemical parameters. Post-hoc mean comparisons were performed using Tukey-Kramer HSD test and Student's *t*-tests at a significance level of 5% (*p*<.05).

3. Results and Discussion

3.1. Total titratable acidity and pH

TTA content and pH levels were quantified at fermentation initiation (day 0) and subsequently on days 1, 3, 5, and 7, expressed in terms of base consumption (mL) (*Table 3*).

Table 3. Acidity profiles of tarhana dough varieties throughout the fermentation period*

pH			*Fermentation day*				
Flour type	**Pre-treatment**	Dough code	0	1	3	5	7
Non-germinated	None	T1	4.93^{bA} ±0.008	4.34^{bB} ±0.009	4.07^{bC} ±0.005	4.06^{bC} ±0.018	4.05^{bC} ±0.019
	Sourdough process	T2	3.72^{cB} ±0.015	3.74^{cB} ±0.018	3.80^{cA} ±0.012	3.80^{cA} ±0.009	3.80^{cA} ±0.018
Germinated	None	T3	4.99^{aA} ±0.009	4.46^{aB} ±0.014	4.31^{aC} ±0.005	4.20^{aD} ±0.005	4.20^{aD} ±0.012
	Sourdough process	T4	3.57^{dB} ±0.017	3.62^{dA} ±0.008	3.64^{dA} ±0.025	3.63^{dA} ±0.005	3.64^{dA} ±0.009

TTA			*Fermentation day*				
Flour type	**Pre-treatment**	Dough code	0	1	3	5	7
Non-germinated	None	T1	7.25^{cD} ±0.060	11.74^{dC} ±0.082	16.14^{cB} ±0.122	16.84^{cA} ±0.156	16.86^{cA} ±0.172
	Sourdough process	T2	23.67^{bB} ±0.069	24.09^{bA} ±0.237	23.81^{bB} ±0.064	24.16^{bA} ±0.073	24.13^{bA} ±0.053
Germinated	None	T3	7.22^{cD} ±0.020	12.19^{cC} ±0.070	15.41^{dB} ±0.117	16.73^{cA} ±0.091	16.77^{cA} ±0.058
	Sourdough process	T4	35.23^{aA} ±0.012	34.32^{aC} ±0.166	34.33^{aC} ±0.104	34.93^{aB} ±0.123	34.96^{aA} ±0.185

*Mean comparisons were performed using two-way ANOVA followed by Tukey-Kramer HSD test. Data are expressed as means±standard deviations ($n=4$). Lowercase letters (a-d) indicate significant differences ($p<.05$) between columns. Uppercase letters (A-D) indicate significant differences ($p<.05$) between rows

Pre-treatment had significantly affected pH throughout fermentation ($p<.0001$), whereas the effect of flour type was significant at all sampling points except on day 1 and 7. A significant interaction between flour type and pre-treatment was also observed at all sampling days ($p<.0001$). A similar trend was observed for TTA, where both flour type and pre-treatment showed significant effects at all sampling days ($p<.0001$). The interaction between these two factors was also significant ($p<.0001$). In T1 and T3 doughs, pH gradually decreased and TTA increased until the fifth day of fermentation, reflecting active LAB metabolism and organic acid accumulation (Erbas et al., 2005), thereafter, both parameters stabilized. These observations are in line with previous reports. For instance, Ozdemir et al. (2018) reported an initial average pH of 4.52 and acidity of 8.69 mL in five tarhana doughs, which progressed to pH 3.79 and 21.30 mL TTA by day 5, then remained stable. In contrast, T2 and T4 exhibited relatively stable pH and TTA values throughout fermentation. The nearly stable TTA values likely reflect the presence of organic acids introduced during pre-fermentation, which may have suppressed LAB activity and limited further acid production during tarhana dough fermentation. The slight pH increase observed on day 3 in both samples could be attributed to the buffering capacity of protein-peptide complexes formed during pre-fermentation. (Arslan-Tontul et al., 2018). Comparison of T2 and T4 revealed that the lower pH and higher TTA observed in T4 may result from the inclusion of germinated wheat flour, which likely enhanced enzymatic activity and promoted acid production through interaction with sourdough microbiota (Reale et al., 2021).

3.2. Microbial counts

LAB and total yeast-mold counts were assessed in tarhana doughs on the first and last days of fermentation and in dried tarhana samples (*Table 4*).

Table 4. LAB and total yeast-mold counts (log CFU g^{-1}) in dough and dried tarhana samples*

			Dough samples				**Dried samples**	
			Day 1**		Day 7			
Flour type	**Pre-treatment**	Code	LAB	Yeast-Mold	LAB	Yeast-Mold	LAB	Yeast-Mold
Non-germinated	None	T1	7.94^{aA} ±0.042	2.42 ±0.24	3.48^{cB} ±0.035	<2	1.75^{cC} ±0.190	<2
	Sourdough process	T2	4.65^{dA} ±0.127	<2	3.07^{dB} ±0.056	<2	1.91^{cC} ±0.070	<2
Germinated	None	T3	7.50^{bA} ±0.021	2.06 ±0.31	5.92^{aC} ±0.021	<2	6.20^{aB} ±0.063	<2
	Sourdough process	T4	5.84^{cA} ±0.106	<2	5.65^{bA} ±0.042	<2	5.72^{bA} ±0.028	<2

*Results are presented as the mean of duplicate measurements and two parallel counts

**Data are expressed as means±standard deviations (n=2). Lowercase letters (a-d) within the same column, uppercase letters (A-C) between LAB columns in the same row indicate significant differences (p<.05; Tukey-Kramer HSD)

On day 7 of fermentation, LAB counts were significantly lower in all tarhana samples compared to day 1. This decline is likely attributable to acid stress resulting from the accumulation of organic acids under prolonged fermentation, as well as nutrient depletion that limited bacterial metabolism and survival. By day 7, the incorporation of Type III sourdough further reduced LAB counts in both germinated and non-germinated groups. Nevertheless, LAB populations remained significantly higher in germinated samples compared to their non-germinated counterparts (p<.0001). This effect may be explained by the increased availability of nutrients such as free amino acids and short-chain sugars generated during germination, which likely supported LAB survival (Nelson et al., 2013). A similar trend was observed in dried samples, with significantly higher LAB counts in T3 and T4 than in T1 and T2 (p<.0001). This likely reflects the presence of sugars, soluble fibers, and protein fractions released during germination, which may have acted as natural protectants against heat and dehydration stress. Supporting this, Tan et al. (2018) reported that dehydration protectants improve microbial resistance to thermal and osmotic stress, while Oldenhof et al. (2005) demonstrated that sucrose and maltodextrin enhance LAB survival during drying.

After drying, LAB counts in T1 and T2 tarhana samples were consistent with values reported in previous studies. For instance, Herken and Çon (2014) reported LAB counts of log 2.0 CFU g^{-1} in dried tarhana, whereas Daglıoglu et al. (2002) and Erbas et al. (2005) recorded values of log 3.85 CFU g^{-1} and log 1.74 CFU g^{-1}, respectively. In contrast, markedly higher LAB survival rate was observed in T3 and T4, indicating the protective effect of germination.

3.3. Physicochemical analyses

The protein, fat, moisture, and ash contents of tarhana samples are presented in *Table 5*. Among the samples, T3 exhibited the highest protein content (17.73%), which was statistically significant compared to the other formulations. Similarly, the highest fat content was recorded in T3 (4.40%), whereas the lowest was observed in T2 (2.94%). In terms of moisture content, T4 had the highest level (10.94%), while all other samples remained below 10%. The ash content ranged between 5.87% and 6.26%, with no statistically significant differences among the samples.

The physicochemical properties observed in this study were generally consistent with previously reported values. Tamer et al. (2007) analyzed 21 tarhana samples and reported TTA values ranging from 1.7 to 40.7 mL. In the present study, the TTA of the control sample (T1) was 34.98 mL, aligning with this range, while T2, T3, and T4 exhibited higher values. The highest acidity was recorded in T4 (63.90 mL), followed by T2 (45.17 mL), indicating that flour type significantly influences acidity profile. In the same study, the average protein, fat, moisture, and ash contents were reported as 14.93%, 5.10%, 11.68%, and 4.56%, respectively, which were broadly comparable to the values obtained in this study. However, the ash content remained higher than the levels reported by Sensoy and Tarakci (2023), likely reflecting differences in formulation and raw material composition.

Table 5. Physicochemical properties of dried tarhana samples*

Code	Protein%**	Fat%	Moisture%	a_w (25 °C±0.04)	Ash%	pH	TTA
T1	16.30^{b}	3.83^{a}	7.98^{b}	0.42^{a}	5.93^{a}	4.18^{b}	34.98^{d}
	±0.033	±0.107	±0.021	±0.006	±.0.157	±0.030	±0.615
T2	15.74^{bc}	2.94^{b}	8.65^{b}	0.40^{ab}	5.60^{a}	3.82^{c}	45.17^{b}
	±0.666	±0.106	±0.573	±0.024	±0.001	±0.009	±0.681
T3	17.73^{a}	4.40^{a}	9.16^{ab}	0.37^{b}	6.26^{a}	4.27^{a}	41.86^{c}
	±0.348	±0.498	±0.695	±0.001	±0.325	±0.018	±0.243
T4	15.04^{c}	3.98^{a}	10.94^{a}	0.38^{b}	5.87^{a}	3.67^{d}	63.90^{a}
	±0.243	±0.278	±0.371	±0.007	±0.304	±0.015	±0.082

*Results are calculated on a dry matter basis
**Data are expressed as means±standard deviations (minumum *n*=3). Different letters within the same column indicate significant differences (*p*<.05; Tukey-Kramer HSD)

Water activity values ranged from 0.37 and 0.42, well below the microbial growth threshold, thereby suggesting an extended shelf life. Compared to the control (T1), germinated samples (T3 and T4) exhibited significantly lower a_w values ($p<.01$), likely due to the increased levels of soluble sugars and protein fractions generated during germination, as these compounds readily bind water molecules through hydrogen bonding.

3.4. Total free amino acid

TFAA contents of tarhana samples are presented in *Figure 2*.

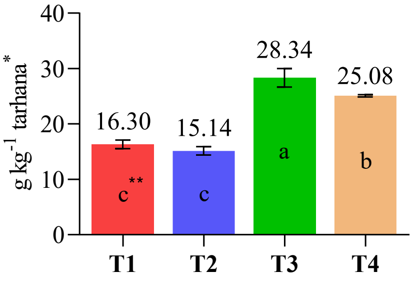

Figure 2. TFAA contents of dried tarhana samples

*Results are calculated on a dry matter basis
**Error bars represent standard deviations (*n*=4). Bars with different letters differ significantly (*p*<.01; Tukey-Kramer HSD)

Both flour type and pre-treatment significantly affected TFAA content ($p<.001$ and $p=.0009$, respectively), while their interaction was not significant. Germinated flour type significantly increased TFAA levels, $t(12) = 21.91$, $p<.001$, as samples T3 and T4 exhibited higher TFAA content than those prepared with non-germinated flour. In contrast, T2 exhibited lower TFAA levels than T1, and a similar decline was observed in T4 relative to T3, suggesting that the incorporation of Type III sourdough reduced TFAA levels. This reduction is likely attributable to the microbial utilization of free amino acids during Type III sourdough fermentation.

Akan and Ocak (2019) reported a TFAA content of 1.5 g 100 g^{-1} in traditional wheat tarhana. In the present study, T1 and T2 exhibited similar values, while T3 and T4, produced with germinated wheat flour, showed significantly higher TFAA levels, suggesting an enhancing effect of germination (Lemmens et al., 2018b).

3.5. Water-soluble protein

WSP contents of extracts obtained from dried tarhana samples are presented in *Figure 3*.

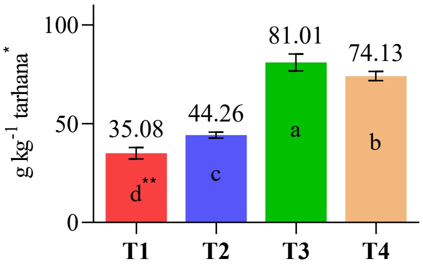

Figure 3. WSP contents of dried tarhana samples

*Results are calculated on a dry matter basis
**Error bars represent standard deviations (*n*=6). Bars with different letters differ significantly (*p*<.01; Tukey-Kramer HSD)

Flour type and its interaction with pre-treatment had significant effects on WSP content (*p*<.0001), while pre-treatment alone was not significant. WSP levels were significantly higher in T3 and T4, which were produced with germinated wheat flour, *t*(20) = 31.68, *p*<.0001, compared to samples produced with non-germinated flour. The effect of Type III sourdough on WSP content, however, varied depending on the presence of germination status. Specifically, WSP increased significantly in T2 compared with T1 (*p*=.0001), but decreased in T4 relative to T3 (*p*=.0031). The observed changes in WSP content may be attributed to biochemical interactions between the dough microbiota and cereal-derived enzymes (Wehrle et al., 1999; Loponen et al., 2007).

3.6. Protein solubility index

The results indicate that germination and the incorporation of Type III sourdough had significant effects on protein solubility (*Figure 4*).

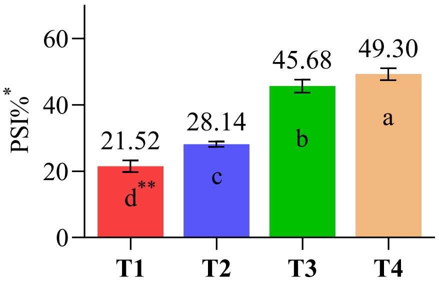

Figure 4. PSI% of dried tarhana samples

*Results are calculated on a dry matter basis
**Error bars represent standard deviations (*n*=6). Bars with different letters differ significantly (*p*<.01; Tukey-Kramer HSD)

Both flour type and pre-treatment significantly affected protein solubility (*p*<.0001), and a significant interaction between these factors was also observed (*p*<.05). Tarhana samples produced with germinated wheat flour (T3 and T4) exhibited significantly higher protein solubility, *t*(20) = 33.63, *p*<.0001 (45.68% and 49.30%, respectively), compared to non-germinated samples T1 (21.52%) and T2 (28.14%), indicating that germination enhances protein-water interactions. Moreover, Type III sourdough incorporation enhanced protein solubility in both germinated (*p*=.0058) and non-germinated (*p*<.0001) samples.

4. Conclusions

This study demonstrated that wheat germination and the incorporation of Type III sourdough, significantly influenced the microbiological, physicochemical, and nutritional properties of tarhana. Germinated wheat flour enhanced protein solubility, WSP, and TFAA content, while also supporting LAB survival during tarhana fermentation and after drying, suggesting a protective role of germination against dehydration stress. Despite lowering TFAA levels, Type III sourdough, in combination with germination, enhanced protein solubility index. While this study employed a controlled pre-fermentation system with a single LAB strain to ensure reproducibility within the Type III sourdough preparation process, future research could expand on this approach by incorporating mixed cultures to better represent the natural microbial diversity of traditional tarhana fermentation. Overall, the combined application of germination and Type III sourdough proved to be an effective strategy for improving the nutritional quality and microbial stability of tarhana. Future research should explore probiotic fortification. Since germination supported higher LAB survival in the present study, probiotic-enriched tarhana formulations could also retain high viable counts of probiotic microorganisms, thereby offering potential as a functional food.

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Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

There is no conflict of interest between the article authors.

Authorship Contribution Statement

Concept: Yuksel, D., Comertpay, S., Inanc, A. L.; Design: Yuksel, D., Comertpay, S., Inanc, A. L.; Data Collection or Processing: Yuksel, D., Comertpay, S., Inanc, A. L.; Statistical Analyses: Yuksel, D., Comertpay, S., Inanc, A. L.; Literature Search: Yuksel, D., Comertpay, S., Inanc, A. L.; Writing, Review and Editing: Yuksel, D., Comertpay, S., Inanc, A. L.

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