Effect of Wheat Grits Level on Biogenic Amines Formation in Traditional Kurdish Fermented Food Tarsas

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Abstract:

«Biogenic amines (BA) are nitrogenous compounds produced when amino acids undergo decarboxylation or amination by microorganisms. They are found in fermented foods like sauerkraut, beer, cheese, kimchi, soy sauce, and fermented vegetables. Tarasas is a traditional fermented Kurdish dish made from turnip roots, leaves, and wheat grits. The current study aims to investigate the effect of decreasing the amount of wheat grits used in the Tarasas product on the production of biogenic amines during fermentation. The study measured pH, titratable acidity, total nitrogen, soluble nitrogen, hydrolysis degree, and determination of biogenic amines (BA), in addition to lactic acid bacteria counts, during and after fermentation of Tarasas at °٢٥C in treatments with wheat grits ratios (٪٠ ,٪٢٥ ,٪٥٠ ,٪٧٥ ,٪١٠٠) of the total weight of grits in the Tarasas product. The results were analyzed using SPSS. One-way analysis of variance and Duncan›s multiple-range test were used to test for significant differences between treatments. The results show a decrease in pH and an increase in titratable acidity (TTA). It was also observed that the degree of hydrolysis and the percentage of biogenic amines increased during the fermentation period. The growth of lactic acid bacteria (LAB) was also observed, with sharp rise levels on the ٥th day, and the growth becomes less within ١٥–٥ days of fermentation.»

Keywords: Lactic acid bacteria, pH, degree of hydrolysis, histamine, tyramine

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Introduction:

Recent trends in food security are fueling an increased interest in trace compounds that can affect human health, such as biogenic amines. Biogenic amines comprise organic bases with aliphatic (putrescine, cadaverine, spermine, and spermidine), aromatic (tyramine and 2-phenylethylamine), or heterocyclic (histamine and tryptamine) structures, which may be present in various foods (Ruiz-Capillas & Herrero, 2019). They are primarily produced by the microbial decarboxylation of amino acids, except for physiological polyamines. While biogenic amines may occur endogenously at low concentrations in non-fermented foods like fruits, vegetables, meat, milk, and fish, higher concentrations are often found in fermented foods due to contaminating microflora exhibiting amino acid decarboxylase activity (Saha, Turna, Chung & McIntyre, 2024). These compounds pose health risks, particularly to sensitive individuals, leading to symptoms such as nausea, respiratory distress, hot flashes, cold sweats, heart palpitations, headaches, red rashes, hypotension, and hypertension (Vally & Misso, 2012).

The estimation of biogenic amines, including histamine, tyramine, 2-phenylethylamine, agmatine, putrescine, and cadaverine, is crucial not only from a toxicological perspective but also as indicators of food freshness or spoilage (Alberto, Arena & De Nadra, 2004). Establishing maximum permissible levels for biogenic amines in foods is challenging due to individual responses and the presence of other amines. Proposed acceptable levels for fermented foods range from 50–100 mg/kg for histamine, 100–800 mg/kg for tyramine, and 30 mg/kg for 2-phenylethylamine (Doeun, Davaatseren & Chung, 2017). Similarly, an acceptable level of 1 g/kg for total biogenic amine content has been suggested. Some studies have reported a maximum allowable limit of 100 mg/kg of histamine (Yu et al., 2021).

Research on the types and quantities of biogenic amines in traditional foods such as Shalgam, Sunki, and Douchi has been conducted using HPLC. Understanding the correlation between grain proportions and biogenic amine production in fermented foods is essential for developing fermentation processes and ensuring food safety and quality (Gong et al., 2014).

Cereal-based fermented foods, which are staple foods in many regions including Asia, Africa, the Middle East, and parts of Europe, play a significant role in nutrition. Tarasas, a traditional fermented Kurdish food, is produced using lactic acid bacteria through spontaneous fermentation (Phiri et al., 2019). It consists of turnips, leaves, and roots mixed with cooked wheat grits. Homemade production of Tarasas involves chopping turnip roots and leaves, adding them to cooked wheat grits with salt and fermenting the mixture at 25°C for 15 days.

The current study aims to investigate the effects of adding wheat grits in different ratios on the production of biogenic amines in fermented Tarasas. By doing so, we aim to enhance the safety and nutritional profiles of fermented Tarasas while gaining insights into the complexity of biogenic amine production. **Materials and Method:**

According to the traditional Kurdish recipe, 2.0 kg of fresh turnip roots and 2.0 kg of turnip leaves were washed and cut into small sections or slices. Subsequently, 1.0 kg of wheat groats were boiled until softened, cooled at room temperature (25 ^{o}C), and then added to the mixture of turnip roots and leaf slices. Finally, 225 gm of salt was added. The treatments were placed in a clean container with a well-covered lid and stored at room temperature to start fermentation.

After 5 days of fermentation, the sample underwent phase separation, with the upper layer being removed and placed at the bottom, while the lower layer was returned to the top without agitation. The container was then sealed and left at room temperature to complete the fermentation process until it reached 15 days. At this point, the product was mixed thoroughly and became ready for further laboratory analysis.

Chemical Examination

The pH and Titratable Acidity (TTA) Determination

The pH of Tarasas treatments was measured using a pH meter (HI-2210-02 pH Bench Meter, Hanna Instruments) after calibrating the electrode meter with a fresh standard buffer at a pH of 4.0 (Daji et al., 2022). Titratable acidity was determined following the AOAC method of analysis (Lane, 1995) and expressed as a percentage of lactic acid. All measurements were conducted in triplicate.

Total Nitrogen (TA), Soluble Nitrogen (TCA-SN), and Degree of Hydrolysis

The total nitrogen was determined, and trichloroacetic acid-soluble nitrogen (TCA-SN) was determined by the Kjeldahl method as described by Tomita, Nakamura & Okada (2018). The degree of hydrolysis of nitrogen was obtained by dividing the TCA-SN result by the total nitrogen and multiplying by 100, as described by Fong et al. (2020).

Analysis of Biogenic Amines

Extraction and analysis of biogenic amine compounds in Tarasas treatments during different periods of fermentation were performed using the HPLC method (Shori & Baba, 2012).

Lactobacillus Enumeration

The enumeration of Lactobacillus in Tarasas was conducted using the standard plate-counting method. About 11 g of the sample was diluted 10-fold with 99 mL of sterile saline solution. Then, 0.1 mL of the diluted sample was inoculated onto MRS agar and M17 agar. The plates were incubated at 37 °C for 24 hours.

Statistical Analysis

Results were analyzed using the Statistical Package for the Social Sciences (SPSS, version 25, Chicago, USA). One-way analysis of variance was used to test the effect of the fermentation process on the detection of biogenic amines. Duncan's multiple range test was conducted to test the significant differences between the means of the treatments at a level of $p \leq 0.05$.

Result and Discussion:

The pH, titratable acidity, total nitrogen, soluble nitrogen, and degree of hydrolysis were assessed before the fermentation process (day zero), and subsequently after 5, 10, and 15 days for all treatments. Observations of pH and acidity changes in Tarasas during and after fermentation at 25°C indicated a pH decrease accompanied by increased acidity by the fifth day of fermentation. Additionally, acidity levels exhibited a slight rise on days 10 and 15 of fermentation (Table 1). These alterations may be attributed to microbial activity primarily responsible for converting sugars present in raw materials (such as turnip roots or grains) into various organic acids, including lactic acid, as metabolic byproducts. Consequently, this process leads to a decrease in pH and an increase in titratable acidity (Yassunaka Hata et al., 2023). Moreover, longer fermentation durations generally yield higher levels of acid production and result in lower pH levels, establishing an osmotic stress environment for microorganisms by extracting water from their cells. This phenomenon can impede the growth of numerous microorganisms, including spoilage bacteria (Park, Zhang & Kim, 2022).

Table 1: The levels of the factors affected by Lactobacillus and yoghurt in Tarasas, a Kurdish traditional food, in three periods.

Trait	Treatment (% wheat ratio)	0 day	5th day	10th day	15th day
pH	T	5.80	3.30	3.20	3.10
	T^{100}	5.80	3.20	3.20	3.10
	T^{75}	5.80	3.30	3.30	3.20
	T^{50}	5.70	3.30	3.20	3.20
	T^{25}	5.80	3.20	3.20	3.10
Treatable acidity	T^{0}	0.91	1.97	1.97	2.02
	T^{100}	0.89	1.96	1.96	2.01
	T^{75}	0.90	1.96	1.96	2.01
	T^{50}	0.93	1.96	1.96	2.00
	T^{25}	0.92	1.94	1.94	2.02
Total nitrogen %	T^{0}	1.16	1.15	1.11	1.10
	T^{100}	0.82	0.81	0.77	0.75
	T^{75}	0.57	0.56	0.54	0.53
	T^{50}	0.42	0.41	0.39	0.38
	T^{25}	0.22	0.21	0.19	0.18
Soluble nitrogen %	T^{0}	0.009	0.590	0.701	0.840
	T^{100}	0.006	0.350	0.440	0.470
	T^{75}	0.003	0.196	0.210	0.291
	T^{50}	0.001	0.115	0.125	0.174
	T^{25}	0.001	0.034	0.044	0.056
Degree of hydrolysis %	T^{0}	0.83	51.50	63.22	77.04
	T^{100}	0.77	43.70	58.07	63.05
	T^{75}	0.54	35.40	40.05	55.01
	T^{50}	0.41	27.20	32.11	46.03
	T^{25}	0.22	16.10	23.012	31.51

0

The results presented in Table 1 also demonstrate a variation in the total nitrogen percentage within Tarasa's product at day zero, ranging from 1.16% to 0.22% across all treatments. This slightly declined to a range of 1.10% to 0.18% by the 15th day of fermentation. This study suggests a direct relationship between total nitrogen and the proportion of wheat grits utilized, as wheat grits represent the primary nitrogen source due to their elevated amino acid content compared to turnip roots and leaves, which contain lower nitrogen levels (Wang et al., 2021).

During fermentation, a slight decrease in nitrogen was observed, attributed to microorganisms consuming nitrogen from various ingredients, notably wheat grits. Nitrogen is crucial for microbial growth and metabolism, particularly for lactic acid bacteria (LAB) involved in Tarasa's fermentation. While wheat grits may not inherently contain abundant free nitrogen compounds, they do contain proteins that LAB can break down into peptide chains and amino acid units, serving as nitrogen sources during fermentation. Microorganisms, particularly bacteria, utilize these nitrogen-containing compounds for amino acids and other essential nutrients, thereby facilitating microbial growth and metabolic byproduct production (Thomas & Ingledew, 1990).

Moreover, the variation in nitrogen ratios in Tarasa's product may be attributed to differences in nitrogen content among its ingredients (wheat grits, turnip roots, and leaves), influenced by factors such as wheat variety, growth conditions, and processing methods. Turnip roots also contribute nitrogen through proteins, potentially at varying concentrations from wheat. The nitrogen levels in roots are influenced by factors including plant genetics, soil composition, and agricultural practices (Adamczyk et al., 2010).

Turnip leaves typically exhibit higher nitrogen content than roots, being richer in proteins and other nitrogen compounds. The nitrogen content in leaves varies depending on factors such as plant age, environmental conditions, and nutrient availability. Moreover, soluble nitrogen (SN) increased with prolonged fermentation time, as evidenced by the rise in SN-TCA levels across all Tarasas treatments. Initially, SN-TCA levels ranged from 0.009 to 0.0004 at day zero, increasing to levels between 0.88 and 0.056 by the 15th day of fermentation (Duan, 2023). Furthermore, our observations from Table 1 suggest a correlation between the decrease in SN-TCA and the reduction in wheat grits ratio in the treatments.

The increase in soluble nitrogen and heightened metabolic activity of microorganisms during Tarasas fermentation facilitate the synthesis and release of nitrogen-containing compounds, which are attributable to various microbial and biochemical processes inherent to Tarasas fermentation. Fluctuations in pH levels during fermentation can impact enzymatic activities and SN-TCA levels. Additionally, these pH fluctuations can influence the production of SN-TCA (Calsamiglia, Ferret & Devant, 2002).

The degree of hydrolysis (DH), calculated as (SN-TCA/total nitrogen) × 100, showed a significant increase with prolonged fermentation time across all treatments. At day zero, DH values ranged from 0.86% to 0.2%, escalating to 77.04% to 29.3% after 15 days of fermentation (Table 1). The primary disparities in DH observed at equivalent fermentation times stem from variations in wheat grit levels in the product. DH demonstrates a direct correlation with the level of wheat grits. Increased bacterial growth enhances metabolic activity, thereby promoting the breakdown of large proteins through the secretion of proteolytic enzymes, which convert them into peptides. An increase in the degree of decomposition reflects the success of the fermentation process (Kieliszek et al., 2021).

Biogenic amines detection:

The concentrations of biogenic amines (in mg/kg) generated with varying concentrations of wheat grits after the 5th, 10th, and 15th days of fermentation are detailed in Tables 2, 3, and 4, respectively. The estimation encompassed all biogenic amines, including histamine, agmatine, β-phenylethylamine, putrescine, cadaverine, tyramine, spermidine, and spermine.

“The objective of the current study was to assess the levels of biogenic amines in the traditional food Tarasas using varying levels of wheat grits. Biogenic amines were evaluated in the raw materials employed (turnip leaves and roots) as well as in wheat grits before mixing and fermentation. The results indicated that turnip leaves and roots contained 0.96 µg/kg of β-phenylethylamine, 0.076 µg/kg of cadaverine, and 0.89 µg/kg of histamine. In contrast, wheat grits contained 0.8 µg/kg of β-phenylethylamine, 0.086 µg/kg of cadaverine, 0.44 µg/kg of histamine, 0.8 µg/kg of putrescine, 1.04 µg/kg of agmatine, 0.66 µg/kg of tyramine, 0.92 µg/kg of spermidine, and 0.5 µg/kg of spermine

The data presented in Tables 2, 3, and 4 illustrate a consistent increase in the percentage of biogenic amines with prolonged fermentation time. For instance, the concentrations of histamine and tyramine escalated from 73.9 mg/kg and 49.3 mg/kg after 5 days of fermentation to 86.77 mg/kg and 59.3 mg/kg after 10 days, respectively, eventually reaching 107 mg/kg and 73.6 mg/kg after 15 days. These findings indicate a progressive rise in the levels of biogenic amines, including histamine, tyramine, and other identified compounds, throughout the fermentation period.

Table 2: The levels of biogenic amines (mg /kg) among the treatments on the 5th day of fermentation.

Tr.	Histamine	Agmatine	B-phenylethylamine	Putricne	Cadaverine	Tyramine	Spermidine	Spermine	The total amount of BA
T_{100}	73.90±0.01 a	68.00±0.01 a	51.20±0.01 a	54.90±0.01 a	61.50±0.01 a	49.30±0.01 a	52.10±0.01 a	49.90±0.01 a	460.8±0.01
T_{75}	64.70±0.01 b	56.57±0.01 b	43.60±0.01 b	51.60±0.01 ab	58.50±0.01 ab	44.60±0.01 b	49.90±0.01 ab	42.30±0.01 b	411.77±0.01
T_{50}	56.30±0.01 c	48.30±0.01 c	42.47±0.01 b	40.20±0.01 c	54.10±0.01 b	35.70±0.01 c	38.00±0.01 c	41.10±0.01 b	356.17±0.01
T_{25}	41.50±0.01 d	31.80±0.01 d	37.50±0.01 c	33.70±0.01 d	42.00±0.01 c	33.10±0.01 c	30.50±0.01 cd	35.60±0.01 c	285.7±0.01
T_0	19.60±0.01 e	26.30±0.01 e	33.30±0.01 e	29.90±0.01 e	25.70±0.01 d	22.30±0.01 d	25.70±0.01 d	28.70±0.01 d	211.5±0.01

Tr.=Treatment, T_{100}=100% wheat grits, T_{75}= 75% wheat grits, T_{50}=50% wheat grits, T_{25}=25% wheat grits, T_0=0% wheat grits, Values with different superscripts in a column are significantly different ($P<0.05$).

Table 3: The levels of biogenic amines (mg /kg) among the treatments on the 10th day of fermentation.

Tr.	Histamine	Agmatine	B-phenylethylamine	Putricne	Cadaverine	Tyramine	Spermidine	Spermine	The total amount of BA
T_{100}	86.77±0.01 a	70.480±0.01 a	75.10±0.01 a	63.50±0.01 a	91.80±0.01 a	59.30±0.01 a	69.50±0.01 a	56.70±0.01 a	573.15±0.01
T_{75}	76.90±0.01 b	63.70±0.01 b	59.50±0.01 b	59.30±0.33 b	90.10±0.01 a	51.60±0.12 b	59.90±0.12 b	49.30±0.01 b	510.3±0.01
T_{50}	73.80±0.01 bc	57.80±0.01 c	48.57±0.01 c	56.87±0.02 bc	83.90±0.01 b	47.00±0.22 c	44.90±0.11 c	44.90±0.01 c	457.74±0.01
T_{25}	61.30±0.12 c	52.60±0.01 cd	42.47±0.01 d	53.05±0.01 c	76.20±0.2 c	40.30±0.01 d	40.80±0.12 c	41.40±0.01 c	407.72±0.01
T_0	34.10±0.01 d	45.30±0.01 d	40.00±0.02 d	41.28±0.11 d	34.90±0.01 d	35.8±0.12 e	31.00±0.01 d	35.00±0.01 d	297.38±0.01

Tr.=Treatment, T_{100}=100% wheat grits, T_{75}= 75% wheat grits, T_{50}=50% wheat grits, T_{25}=25% wheat grits, T_0=0% wheat grits, Values with different superscripts in a column are significantly different ($P<0.05$).

Table 4: The levels of biogenic amines (mg /kg) among the treatments on the 15th day of fermentation.

Tr.	Histamine	Agmatine	B-phenylethylamine	Putricne	Cadaverine	Tyramine	Spermidine	Spermine	The total amount of BA
T_{100}	107.00±0.01 a	74.40±0.01 a	84.70±0.01 a	87.03±0.01 a	110.20±0.01 a	73.60±0.02 a	76.30±0.01 a	65.80±0.01 a	679.03±0.01
T_{75}	103.30±0.01 a	68.00±0.21 b	77.00±0.01 b	82.80±0.12 ab	107.30±0.01 a	65.10±0.12 b	64.80±0.01 b	58.60±0.01 b	626.9±0.01
T_{50}	99.00±0.01 b	64.00±0.11 bc	71.40±0.01 bc	79.50±0.12 b	102.40±0.01 ab	56.60±0.02 c	55.90±0.01 c	50.00±0.01 c	578.8±0.01
T_{25}	86.30±001 c	58.60±0.01 c	62.47±0.01 c	77.47±0.12 b	93.10±0.01 b	51.80±0.01 d	45.60±0.01 d	45.80±0.01 d	521.14±0.01
T_0	47.60±0.01 d	51.30±0.01 d	57.30±0.01 d	68.60±0.01 c	40.60±0.01 c	47.10±0.01 d	35.60±0.01 e	38.00±0.01 e	386.1±0.01

Tr.=Treatment, T_{100}=100% wheat grits, T_{75}= 75% wheat grits, T_{50}=50% wheat grits, T_{25}=25% wheat grits, T_0=0% wheat grits, Values with different superscripts in a column are significantly different ($P<0.05$).

The decrease in the percentage of wheat grits is noted to have a significant impact on the reduction in the rate of biogenic amine formation. This suggests a direct relationship between the percentage of wheat grits and biogenic amine formation, potentially due to the presence of amino acids such as histidine, tyrosine, and tryptophan in wheat. These amino acids can be decarboxylated by LAB bacteria, leading to the production of biogenic amines such as histamine, tyramine, and tryptamine (Molina-Gutierrez et al., 2022). Increased wheat grits provide more nutrients, which play a vital role in providing essential nourishment for LAB during Tarasas fermentation.

Certain strains of LAB bacteria can generate biogenic amines as by-products of metabolism during their growth and fermentation process of Tarasas (Slizewska & Chlebicz-Wojcik, 2020). Enhanced microbial activity resulting from increased nutrients from wheat grits can lead to elevated production of biogenic amines. Factors such as time, ingredient ratios, with wheat grits being particularly important, and pH can also influence the growth and metabolic activities of LAB, potentially leading to increased biogenic amine production (Li et al., 2023; Bukvicki et al., 2020).

The pH level plays a significant role in the activity of amino acid decarboxylase, with higher enzyme activity observed within the pH range of 4 to 5.5 (Zapasnik, Sokolowska & Bryla, 2022). It is important to note that the specific effects of increasing wheat grits on biogenic amine production can vary depending on the type and strain of LAB present during fermentation, the specific fermentation process used, and the overall quality and handling of the wheat grits. Furthermore, the concentrations of biogenic amines are influenced by the storage period, which increases during fermentation (Wu et al., 2022).

The allowable ratio for consuming biogenic amines varies depending on the specific amine and individual health conditions, and the country and specific regulations in place (Christensen et al., 2022). In the European Union, the allowable histamine level in fishery products is 100 mg/kg, except for certain specific products such as tuna, mackerel, and sardines which have a higher limit of 200 mg/kg (Zdziobek, Jodlowski & Strzelec, 2023). While in the United States, the allowable histamine level in finfish and shellfish is 50 mg/100g. The U.S. Food and Drug Administration (FDA) recommends that histamine levels in fish products should not exceed 50 parts per million (ppm) to minimize the risk of scombroid fish poisoning (Bintsis & Papademas, 2022).

When it comes to the potential toxicity of biogenic amines, studies have suggested that consumption of 100-800 mg/kg of tyramine and 30 mg/kg of β-phenylethylamine in foods can be considered toxic doses. Additionally, it is suggested that the upper limit for human consumption of histamine in foods should be around 100 mg/kg (Bintsis & Papademas, 2022), and Body et al. (2021) mentioned that histamine levels above 500 mg/kg or tyramine levels above 1000 mg/kg are considered toxic and dangerous for human health (Qin et al., 2022). Different countries have established upper limits of histamine in wine: 2 mg/L (Germany), 3.5 mg/L (Netherlands), 5 mg/L (Finland), 6 mg/L (Belgium), 8 mg/L (France), and 10 mg/L (Australia and Switzerland) (Akamine, Mansoldo & Vermelho, 2023). EFSA suggested that the upper limit for human consumption of histamine in fermented vegetables is 92 mg/kg, tyramine is 91 mg/kg, putrescine 549 mg/kg, cadaverine 94 mg/kg, Phenylethylamine <5 mg/kg, and suggest the upper limit of the sum of biogenic amines are 747 mg/kg. Generally, it is recommended to consume biogenic amines in moderation to avoid any potential health risks.

Detection of Lactic acid bacteria during fermentation:

The enumeration of Lactic Acid Bacteria (LAB) in Tarasas treatments was conducted using MRS and M17 media at various fermentation intervals (0, 5, 10, and 15 days) at 25°C, as depicted in Table 5. The log (CFU/g) of LAB was determined on both MRS and M17 media. Generally, the LAB counts obtained from MRS media exceeded those from M17. At the onset of fermentation, the LAB count in T100 was 6.36 log (CFU/g) on day 0, which increased to 8.96 log (CFU/g) by the 15th day. The growth of LAB on the M17 medium exhibited a count of 6.22 (CFU/g) in T100 on day 0, rising to 7.7 (CFU/g) by the 15th day of fermentation.

LAB counts significantly decreased in treatments T75–T0 during fermentation compared with treatment T100. The reduction in the percentage of wheat grits in the fermentation substrate resulted in decreased availability of nutrients for the bacteria. Consequently, the growth rate of LAB may decline, leading to a reduction in their population. This phenomenon is likely attributed to the limited availability of essential nutrients required for bacterial growth and metabolism.

Additionally, the decrease in the percentage of wheat grits may alter the overall composition of the fermentation substrate, affecting factors such as pH, moisture content, and the presence of other microorganisms. These changes can further influence the growth and activity of LAB during fermentation, consistently exhibiting the highest LAB count on both MRS and M17 media throughout the fermentation period compared to other treatments.

The initial count of Lactic Acid Bacteria (LAB) at the onset of fermentation was low, undergoing rapid proliferation during the spontaneous fermentation period between day zero and day five, followed by a slower increase thereafter. The activity of LAB significantly escalated alongside the observed decline in pH levels during various fermentation periods. The optimal pH range for Lactobacillus species typically falls between 4.0 to 7.0, though specific pH requirements may vary among different strains. Conversely, Lactococcus species prefer a pH range of 5.5 to 6.8, with optimal growth occurring around 6.5. These findings are consistent with previous studies that have identified a positive correlation between decreasing pH levels and increased LAB abundance during fermentation periods (Barcenilla et al., 2022). Furthermore, this study observed a higher increase in LAB quantity during fermentation stages (5, 10, and 15 days), which corroborates findings by Lane (1991).

It has been documented that Lactobacillus is the predominant bacteria found in fermented foods, alongside certain strains of Lactococcus, Streptococcus, and Leuconostoc (Muyzer, 1999). Additionally, in agreement with Chan et al. (2021), the decrease in pH levels observed during the fermentation of cabbage, from 6.0 to 3.4 over 5 days, can be attributed to the activity of LAB, specifically Lactobacillus plantarum and Lactococcus lactis. LABs produce lactic acid as a metabolic byproduct during fermentation, leading to acidification of the environment. This acidic environment can positively influence the activity of hydrolytic enzymes. Many enzymes involved in hydrolysis reactions are pH-sensitive, and the acidic conditions created by LAB can optimize the activity of these enzymes.

Table 5: Log10 (CFU/g) of LAB on MRS, M17 during (0, 5, 10, 15) days
of fermentation for different treatments.

Tr.	**MRS media**				**M17 media**			
	Zero-day	**5th day**	**10th day**	**15th day**	**Zero-day**	**5th day**	**10th day**	**15th day**
T_{100}	6.36±0.12 a	8.88±0.01 a	8.91±0.01 a	8.96±0.01 a	6.22±0.01 ab	7.56±0.01 a	7.67±0.01 a	7.70±0.01 a
T_{75}	6.29±0.03 b	8.84±0.01 ab	8.87±0.01 b	8.89±0.01 b	6.19±0.01 b	7.45±0.03 bc	7.60±0.01 b	7.66±0.01 b
T_{50}	6.15±0.01 c	8.71±0.01 bc	8.83±0.01 c	8.85±0.01 c	5.63±0.01 g	7.40±0.01 c	7.48±0.33 c	7.56±0.01 d
T_{25}	6.08±0.02 d	8.63±0.21 c	8.75±0.11 d	8.78±0.23 d	5.35±0.01 d	7.32±0.01 e	7.41±0.01 e	7.47±0.01 f
T_0	5.96±0.01 e	8.57±0.01 d	8.67±0.02 e	8.74±0.01 e	5.24±0.01 a	7.25±0.01 g	7.32±0.02 g	7.40±0.01 fg

Conclusion:

In conclusion, the observed increase in biogenic amines in the Tarasas product due to the presence of wheat grits can be attributed to specific compounds, such as histidine and tyrosine, which serve as precursors for biogenic amine formation. These compounds undergo enzymatic conversion by lactic acid bacteria during consumption, resulting in elevated biogenic amine levels. This elevation poses potential health risks, as high concentrations of biogenic amines have been linked to adverse effects such as allergic reactions and migraines. Therefore, it is imperative to carefully assess the impact of wheat grits on biogenic amine levels and the associated health risks before consumption.

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