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The Involvement of Lactic Acid Bacteria in Modulating the Levels of Biogenic Amino Acids within Fermented Tarasas Products

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Combining turnip roots and leaves with cooked wheat grits and salt makes Tarasas, a fermented dish from Iraqi Kurdistan. The fermentation process takes 15 days, to produce a ready-to-eat dish. This study aims to identify the bacteria responsible and the role of wheat grits during the 0, 5, 10, and 15 days of fermentation for forming biogenic amines in Tarasas. The results demonstrate that pH levels during the fermentation periods decreased gradually. Microbial analysis showed an increased lactic acid bacteria count during Tarasas fermentation. The study also investigates how adding *Lactobacillus plantarum*, and yogurt starters (*Lactobacillus bulgaricus and Streptococcus thermophilus*) affects the process. The current study shows that adding yogurt starters at 3% increased biogenic amine levels, On the other side, using *Lactobacillus plantarum* as a starter at 3% reduced biogenic amine levels compared with treatments that ferment spontaneously. Additionally, decreasing the wheat grit content to 50% (of the total weight of wheat grits) was associated with a decrease in biogenic amines in the Tarasas. The sensory evaluation results also revealed that the treatments containing *Lactobacillus plantarum* were more acceptable to the consumer than the traditional treatment or the one with added yogurt.
Keywords: Biogenic amines, HPLC, histamine, *Lactobacillus plantarum*, wheat grits, fermentation.

INTRODUCTION

Tarasas is a traditional Kurdish dish enjoyed by the Kurdish people in the northern region of Iraq, particularly during the winter season. This flavorful dish incorporates turnip root and leaves, cooked wheat grits, and a touch of salt. Worldwide traditional fermented foods are desirable and prepared from different raw materials such as Shalgam (Aysun *et al*., 2017), Sunki (Tomita *et al*., 2018), and Douchi (Fong *et al*., 2020). The production of Tarasas involves a spontaneous fermentation process aided by specific lactic acid bacteria (LAB), which greatly influence its sensory characteristics. Biogenic amines (BA)s are nitrogenous compounds that are present in fermented foods as a result of amino acids decarboxylation by some microorganisms (Kim *et al*., 2020). Numerus microorganisms associated with food fermentation own amino acid decarboxylase activity. BAs are found in a broad range of foods such as wine and beer (Li *et al*., 2020), Cheese, Fishery products (Rego *et al*., 2014), soy sauces, meat products, and fermented vegetables. The consumption of foods that exceed the recommended levels of BAs can have toxicological consequences. Several parameters can impact the formation of biogenic amines. These include such as pH levels, temperature, salt concentration, the abundance and type of specific microorganism's present (such as lactic acid bacteria), and the duration of the fermentation process (Swider *et al*., 2021). Additionally, the raw materials composition used in fermentation, such as the type of grains or plant matter, can influence biogenic amine production. Monitoring and controlling these parameters are crucial in regulating the biogenic amines levels in products that are fermented. The objective of this study was to look into the role of lactic acid bacteria in the formation of biogenic amines in Tarasas product while fermentation is happening, time and effects of adding yogurt starter, *lactobacillus plantarum*, and decreasing wheat grits ratio, on forming biogenic amines, in addition to detect the dominant strain in Tarasas product. This study can improve valuable knowledge about the intricate process of biogenic amine formation in Tarasas product. This understanding will contribute to enhancing the safety and nutritional value of the product.

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MATERIALS AND METHODS

***Preparing Tarasas treatments*:** The process of preparing the sample involves several steps. First 4 kg of turnip roots and leaves (1:1) are washed and sliced into small sections. Next, 1 kg of cooked wheat grits after being cooled, is added to the mixture of turnip roots and leaves. Finally, 225 gm of iodine-free salt is added to the mixture. The mixture is then placed in a clean container with a well-covered lid and stored at room temperature. After 5 days of fermentation, the sample undergoes phase separation, with the upper layer being removed and placed at the bottom, while the lower layer is returned to the top without agitation. The container is then sealed and left at room temperature to complete the fermentation process for 15 days. At this point, the product is mixed thoroughly and ready for consumption. It is important to note that there is no specific information provided about the technique used to produce Tarasas, as it has been passed down from previous generations and used since ancient times. The Tarasas samples were prepared in a traditional method using three separate groups with varying formulations. Each group consisted of two ratio of wheat grits, the first group, T which is considered the traditional method mentioned above, the second group TLB (with Lactobacillus plantarum 3% of the Total weight of tarasas product), and the third group TY (with yogurt starter 3% of the total weight of Tarasas product), all groups divide in two section depending on wheat grits ratio (T, TLB, Ty) for 100% wheat grits of the total weight of wheat grits used in Tarasas product, ($T_{50,}$ $TLB_{50,}$ TY_{50}) for 50% wheat grits of the total weight of wheat grits used in Tarasas product. Tarasas sensory evaluation was performed at local markets in Sulaymaniyah in Kurdistan Iraq. Fifteen people were randomly selected and trained. Evaluation of the samples was conducted by the judges for flavor, texture, color, taste and overall acceptability by the use of a nine-point hedonic scale, where 1 was the lowest score and 9 was the highest. The likeability was expressed extremely liked (9 points), very much liked (8 points), moderately liked (7 points), slightly liked (6 points), neither liked nor disliked (5 points), slightly disliked (4 points), moderately disliked (3 points), very much disliked (2 points), and extremely disliked (1 point). 20gm coded samples were presented to the panelists. Each panelist was presented six white plastic cups and teaspoons for use in the sensory test. In order to cleanse their palate in between tastings clean water was provided to the judges to avoid carry-over effect.

Chemical examination

***Determination of pH, terrible acidity (TTA)*:** The pH meter (HI-2210-02 pH Bench Meter, Hanna co.) was used to measure the samples pH, after calibrating the electrode meter with a fresh standard buffer pH 7.0and at pH 4.0. This calibration step was performed to ensure accurate and reliable pH measurements. The procedure was conducted according to the method described by Lane (1995). And the determination of the percentage of lactic acid was according to the method described by (Wakil and Ajayi, 2013).

***Biogenic amines determination*:** Extraction and the analysis of Biogenic amines compounds in Tarasas treatments during different periods of fermentation were analyzed by using HPLC method (Shori and Baba, 2012).

***Extraction method*:** (50 mL) centrifuge tube was filled with (10 g) of the homogenized fermented Tarasas sample. Then (50 mL) of a 5% trichloroacetic acid (TCA) solution was added and mixed using a vortex mixer for 5 min. The sample was then centrifuged at 10,000 rpm for 10 min at 4°C, the supernatant was filtered through a (0.45 µm) nylon filter.

***Separation conditions*:** The filtered sample was injected into the HPLC system with a volume of (20 µl), reverse-phase C18 column (4.6 x 250 mm) with a particle size of (5 µm) was used a mobile phase consisting of (0.02 M) sodium phosphate buffer (pH 2.7) and acetonitrile in a ratio of 75:25 (v/v) was utilized. The HPLC system was run at a flow rate of 1 ml /min. Also, a fluorescence detector with an excitation wavelength of (340 nm) and an emission wavelength of (450 nm) was used, Biogenic amines were identified and quantified using calibration curves for each analyze. In data analysis the concentrations of biogenic amines in the sample were calculated using the calibration curves, and the results were expressed as mg/kg.

***Enumeration and isolation of lactic acid bacteria*:** To enumerate and isolate lactic acid bacteria, the standard plate counting method was used, 11 g of each sample was diluted 10-fold with 99 ml of sterile saline solution. 0.1 ml of the diluted sample placed onto Man-Rogosa and Sharp Agar (MRS), and onto M17 agar for the counting of Lactic acid bacteria. All plates were incubated at 37°C for 24 hours. However, to reduce the bacterial load to the permissible range for bacteria, the M17 plates were incubated at 37°C for 24 hours under microaerophilic conditions using a gas pack.

***Statistical analysis*:** All experiments were done three times. Results were analyzed by using SPSS/PASW for windows (SPSS, 2011). One-way analysis of variance was used to test (the effect of two different ratio of wheat grits) Duncan's multiple range test (Duncan, 1955) was conducted to test the significant differences between the means of the treatments at level ($p \leq 0.05$).

RESULTS AND DISCUSSION

***pH and titratable acidity (TTA)*:** The changes in pH and titratable acidity (TTA) values during different periods of fermentation in (0, 5, 10, and 15 days) at 25ºC with two ratios of wheat grits (100, 50 %) were measured. During the fermentation process, there was a notable decline in pH levels, dropping from 5.7-5.8 in unfermented Tarasas to 3.2-3.1 on the fifth day of fermentation. Additionally, there was a significant increase in acidity, rising from 1.02-1.19 to 1.96-

2.08 during the same period. This trend continued as the pH further decreased to 3.1-3.2 and acidity slightly increased to 1.96-2.10 at the fifteenth day of fermentation, as depicted in Table 1.

Terrible acidity. Different superscripts values in a column are significantly different (P<0.05).

At the beginning of fermentation, the pH of the raw material is typically higher because of sugars and other organic compounds presence. As fermentation progresses, micro-organisms such as bacteria metabolize sugars into various byproducts, including organic acids, then conversion of sugars to organic acids leads to an increase in acidity, which is often measured as titratable acidity (Slizewska and Chlebicz-Wojcik, 2020), and during fermentation, lactic acid bacteria are commonly involved. These microorganisms produce organic acids as metabolic byproducts. Lactic acid is a common acid produced by lactic acid bacteria in fermented food, also the fermentation duration plays a significant role as fermentation progresses, the activity of microorganisms increases, leading to more significant production of organic acids. This continued production leads to titratable acidity and pH changes. And different microbial species may dominate the fermentation process, leading to changes in the type and quantity of organic acids produced. This can influence the overall acidity and pH of the Tarasas product. Some factors such as temperature, oxygen availability, and initial raw material composition can also influence the fermentation process and, consequently, the pH and titratable acidity (Muyzer, 1999). Also decreasing pH, and increasing in acidity, during fermentation plays a significant role in spoilage and pathogenic microorganisms controlling the growth of (Oh *et al*., 2020). This is a crucial aspect of the fermentation process that not only helps preserve the Tarasas but also contributes to the final product's unique taste, texture, and nutritional benefits.

***Detection and enumeration of lactic acid bacteria*:** Lactic acid bacteria from Tarasas were enumerated on MRS (De Man–Rogosa–Sharpe agar) and M17 agar at different periods of fermentation in (0, 5, 10, and 15 days) at 25ºC for all treatments, the log (CFU/g) of lactic acid bacteria (LAB) determined on MRS and M17 agar is shown in Table 2. Generally, MRS obtained LAB counts is more than the LAB in M17, and the highest count of lactic acid bacteria (LAB) in the treatment TY_{100} which was 8.97 log (CFU /g) on MRS

Table 1. The changes in pH and TTA during different periods of fermentation (0, 5, 10, and 15) days at 25ºC (Mean±S.E).

Treatment	Zero day		5th day		10th day		15th day	
	pH	TT%A	pH	TT%A	pH	TT%A	pH	TTA%
T_{100}	5.8±0.12a	1.07±0.01a	3.3±0.12a	1.98±0.01b	3.20±0.12a	1.97±0.01a	3.1±0.12a	1.96±0.01a
TLB_{100}	5.8±0.12a	1.11±0.01a	3.2±0.12a	2.06±0.01a	3.20±0.12a	2.07±0.01b	3.2±0.12a	2.08±0.01b
TY_{100}	5.7±0.12a	1.14±0.01a	3.3±0.12a	2.08±0.01a	3.17±0.12a	2.09±0.01b	3.2±0.12a	2.10±0.01b
T_{50}	5.7±0.12a	1.02±0.01a	3.3±0.12a	1.96±0.01b	3.30±0.12a	1.96±0.01a	3.2±0.12a	1.99±0.01a
TLB_{50}	5.8±0.12a	1.04±0.01a	3.3±0.12a	2.05±0.01a	3.30±0.12a	2.06±0.01b	3.2±0.12a	2.08±0.01b
TY_{50}	5.7±0.12a	1.19±0.01a	3.2±0.12a	2.07±0.01a	3.20±0.12a	2.09±0.01b	3.1±0.12a	2.09±0.01b

T_{100}= Traditional method with100% wheat grits, TLB= Traditional method with 100% wheat grits and 3% *Lactobacillus plantarum*, TY_{100}= Traditional method with 100% wheat grits and 3% yogurt starter. T_{50}= Traditional method with50% wheat grits, TLB_{50}= Traditional method with 50% wheat grits and 3% *Lactobacillus plantarum*, TY_{50}= Traditional method with 100% wheat grits and 3% yogurt starter. TTA= Terrible acidity. Different superscripts values in a column are significantly different (P<0.05).

Table 2. Log (CFU /g) of LAB on MRS, M17 during (0, 5, 10, and 15) days of fermentation for different treatments (Mean±S.E).

Treatment	Zero day		5th day		10th day		15th day	
	MRS agar LAB No. (CFU /g)	M17 agar LAB No. (CFU /g)	MRS agar LAB NO. (CFU /g)	M17 agar LAB No. (CFU /g)	MRS agar LAB No. (CFU /g)	M17 agar LAB No. (CFU /g)	MRS agar LAB No. (CFU /g)	M17agar LAB No. (CFU /g)
T_{100}	6.12±1.01b	6.22±0.03a	8.88±0.01b	8.06±0.01ab	8.91±0.01b	8.17±0.01a	8.96±0.02ab	8.19±0.01a
TLB_{100}	7.90±0.01a	6.83±0.04a	9.18±0.01a	8.32±0.01a	9.40±0.01a	8.37±0.01a	9.46±0.01a	8.44±0.01a
TY_{100}	7.82±0.02a	6.75±0.01a	8.90±0.00b	8.19±0.01ab	8.93±0.01b	8.26±0.01a	8.97±0.00ab	8.31±0.01a
T_{50}	6.02±1.01b	6.13±0.06a	8.71±0.01b	7.92±0.01b	8.82±0.01b	7.98±0.01a	8.84±0.01b	8.06±0.01a
TLB_{50}	7.75±0.01a	6.68±0.02a	9.06±0.01a	8.16±0.01a	9.32±0.01a	8.25±0.01a	9.45±0.01a	8.34±0.01a
TY_{50}	6.73±0.02b	6.64±0.02a	8.74±0.00b	7.98±0.01ab	8.84±0.01b	8.05±0.01a	8.87±0.01b	8.15±0.01a

T_{100}= Traditional method with100% wheat grits, TLB= Traditional method with 100% wheat grits and 3% *Lactobacillus plantarum*, TY_{100}= Traditional method with 100% wheat grits and 3% yogurt starter. T_{50}= Traditional method with50% wheat grits, TLB_{50}= Traditional method with 50% wheat grits and 3% *Lactobacillus plantarum*, TY_{50}= Traditional method with 100% wheat grits and 3% yogurt starter. TTA= Terrible acidity. Different superscripts values in a column are significantly different (P<0.05).

agar, while the highest growth for the TLB_{100} on M17 agar which is 8.44 log (CFU /g) M17, on 15th day of fermentation. And the lowest growth of LAB in the treatment T_{50} was 6.02 log (CFU /g) on MRS and 6.13 log (CFU /g) on M17 respectively.

The findings unequivocally demonstrated that, at each fermentation time point, the treatments incorporating *Lactobacillus plantarum* starter and yogurt starter exhibited higher bacterial counts compared to the two treatments relying on spontaneous fermentation. This observation is inherently logical since the addition of starter lactic acid bacteria to the fermentation medium is directly linked to bacterial proliferation, Furthermore, the result a detrimental effect on bacterial growth when 50% of wheat grits were introduced. Notably, the bacterial count decreased throughout the fermentation periods. This phenomenon can be attributed to the richness of wheat grits in amino acids, vitamins, and carbohydrates—crucial elements for bacterial growth. The presence of these components in the fermentation medium has a pivotal role in influencing bacterial proliferation, thereby explaining the observed decline in bacterial numbers (Zhang *et al*., 2022). Also, the highest LAB number on MRS medium and M17 is noticed in all treatments at all period of fermentation (0, 5, 10, and 15) day of fermentation. As fermentation starts the initial count of LAB is low, and increases rapidly during the spontaneous fermentation period between zero and five days of fermentation, followed by low increasing. The activity of LAB increased due to the pH level decreasing at different fermentation periods. The ideal pH range for Lactobacillus species is 4.0 to 7.0, but specific pH requirements may vary among different species (Adesulu-Dahunsi *et al*., 2022). Lactococcus prefers a pH range of 5.5 to 6.8, with optimal growth at around 6.5. The results agreed with another study that found a relationship between decreasing pH levels and increased abundance of LAB during fermentation periods (Slizewska and Chlebicz-Wojcik 2020). Furthermore, a higher increase in the quantity of LAB during fermentation stages (5, 10, and 15 days) was observed in this study, agreed with (Bukvicki *et al*., 2020). The main bacteria found in fermented foods is Lactobacillus, along with some types of Lactococcus and streptococcus, and leuconostoc. The decrease in pH levels during the fermentation of cabbage from 6.0 to 3.4 over a 5-day fermentation period is the result of the LAB activity, specifically *Lactobacillus plantarum* and *Lactococcus lactis*. In addition to wheat, grits can be considered as a nutrients source for LAB, providing carbohydrates and other essential components for its growth and metabolism. By decreasing the ratio of wheat grits in the fermentation process, the available nutrients for LAB may be reduced, leading to slower growth and decreased activity of the bacteria. The decrease in wheat grits ratio can have a negative impact on LAB growth and activity, affecting the process of fermentation and potentially reducing the probiotic properties of the resulting product, there for adding 100 % wheat grits to Tarasas gives the highly growth to LAB. LAB produces lactic acid as a metabolic byproduct during fermentation. The acidification of the environment created by LAB can have a positive impact on 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. Both yogurt starter and Lactobacillus plantarum are commonly used in the fermentation process of various foods, and they contribute to the development of distinct flavors, textures, and nutritional profiles. Yogurt starter typically contains LAB, such as *Streptococcus thermophilus* and *Lactobacillus bulgaricus,* and the fermentation by *L. plantarum* produces lactic acid, creating an acidic environment that can act as a natural preservative, extending the shelf life of the fermented product (Capozzi *et al*., 2012).

***Biogenic amines detection*:** The level of biogenic amines in (mg/kg), which are made with different ratios of wheat grits (100, 50 % of the total wheat grits weight in tarasas product) after days (5^{th}, 10^{th}, and 15^{th}) of fermentation are shown in table 3,4,5, respectively. All the biogenic amines concerning (Histamine, Agmatin, B-phynylathyamine, Putricne, Cadaverine, Tyramine, Spermidine, and spermine) were estimated.

Biogenic amines were quantified across eight types during fermentation intervals spanning 5, 10, and 15 days for all Tarasas treatments. Tables 3,4 and 5, containing the results, reveals a consistent upward trend in the percentage of biogenic amines throughout the fermentation periods. Notably, treatments enriched with yogurt exhibited the highest percentage, contrasting with a significant decrease in the treatments containing the *Lactobacillus plantarum* starter compared to spontaneously fermented ones. Studies have suggested that *Lactobacillus plantarum* bacteria can potentially reduce biogenic amine percentages through biogenic amine analysis (Gezgin *et al*., 2013), and enzymes produced by *Lactobacillus plantarum* responsible for the breakdown of biogenic amines. Conversely, an addition of a yogurt starter has a pronounced effect in elevating biogenic amines in fermented products. That may be due to During fermentation of Tarasas with yogurt starter, starter cultures, usually consisting of *Lactobacillus delbrueckii* and *Streptococcus thermophilus* subsp. *bulgaricus*, contribute to the proteins breaking down into peptides and amino acids. This process may influence the availability of substrates for biogenic amine formation by different strains of LAB within a yogurt starter, and most of the *S. thermophilus* isolates are able to form biogenic amines, particularly tyramine, and histamine, which is a key consideration in the process of strains as starter cultures selection (Fernández and Zúñiga *et al*., 2006). Moreover, the percentage of added grains emerges as a substantial factor influencing biogenic amine formation. Samples with half the amount of wheat grains demonstrated a notable reduction in biogenic amines. This underscores the

Table 3. The levels of biogenic amines (mg/kg) among Tarasas treatments on the 5th day of fermentation (Mean±S.E).

Treat.	Histamine	Agmatin	B-phynyla-thyamine	Putricne	Cadaverine	Tyramine	Spermidine	Spermine	Total amount of BA
T_{100}	73.90±0.01a	68.00±0.01a	51.20±0.01a	54.90±0.01a	61.50±0.01a	49.30±0.01a	52.10±0.01a	49.90±0.01a	460.8±0.01ab
TLB_{100}	67.00±0.01e	58.80±0.01e	48.10±0.01e	48.50±0.01de	42.60±0.01d	39.10±0.01de	42.60±0.01cd	36.80±0.01cd	383.5±0.01cd
TY_{100}	77.50±0.01	69.10±0.01	55.30±0.01	56.50±0.01	64.40±0.01	52.30±0.01	54.40±0.01	51.30±0.01	480.8±0.01
T_{50}	56.30±0.01c	48.30±0.01c	42.47±0.01b	40.20±0.01c	54.10±0.01b	35.70±0.01c	38.00±0.01c	41.10±0.01b	356.1±0.01c
TLB_{50}	48.10±0.01b	39.80±0.01b	38.40±0.01bc	33.60±0.01b	32.10±0.01b	29.40±0.01b	30.30±0.01b	29.10±0.01bc	280.8±0.01b
TY_{50}	58.60±0.01	49.40±0.01	44.60±0.01	43.60±0.01	55.70±0.01	37.20±0.01	42.43±0.01	42.50±±0.01	374.0±0.01

Treat. = Treatment, T_{100}= Traditional method with100% wheat grits, TLB= Traditional method with 100% wheat grits and 3% *Lactobacillus plantarum*, TY_{100}= Traditional method with 100% wheat grits and 3% yogurt starter. T_{50}= Traditional method with50% wheat grits, TLB_{50}= Traditional method with 50% wheat grits and 3% *Lactobacillus plantarum*, TY_{50}= Traditional method with 100% wheat grits and 3% yogurt starter. TTA= Terrible acidity. Different superscripts values in a column are significantly different (P<0.05).

Table 4. The levels of biogenic amines (mg/kg) among Tarasas treatments on the 10th day of fermentation (Mean±S.E).

Treat.	Histamine	Agmatin	B-phynyla-thyamine	Putricne	Cadaverine	Tyramine	Spermidine	Spermine	Total amount of BA
T_{100}	86.77±0.01a	70.40±0.02a	75.10±0.01a	63.50±0.01a	91.80±0.01a	59.30±0.01a	69.50±0.01a	56.70±0.01a	574.2±0.01
TLB_{100}	73.47±0.01bc	63.40±0.01bc	64.90±0.01b	55.50±0.01c	49.30±0.01c	56.80±0.01c	53.43±0.01bc	44.60±0.01bc	461.4±0.01c
TY_{100}	88.70±0.01a	72.60±0.01a	78.90±0.01b	66.00±0.01b	92.90±0.01b	60.47±0.01b	71.50±0.01b	58.90±0.01bc	589.9±0.01bc
T_{50}	73.80±0.12b	57.80±0.12bc	48.57±0.12d	56.87±0.12b	83.90±0.12b	47.00±0.12c	44.90±0.12c	44.90±0.12b	456.7±0.46c
TLB_{50}	62.97±0.01b	54.50±0.01b	47.70±0.01b	46.40±0.01c	39.0±00.01c	38.60±0.01c	37.90±0.01c	34.30±0.01c	361.3±0.01c
TY_{50}	76.60±0.01b	58.70±0.01b	51.20±0.01b	59.77±0.01b	88.70±0.01b	48.30±0.01b	46.10±0.01b	46.90±0.01bc	476.2±0.01bc

Treat. = Treatment, T_{100}= Traditional method with100% wheat grits, TLB= Traditional method with 100% wheat grits and 3% *Lactobacillus plantarum*, TY_{100}= Traditional method with 100% wheat grits and 3% yogurt starter. T_{50}= Traditional method with50% wheat grits, TLB_{50}= Traditional method with 50% wheat grits and 3% *Lactobacillus plantarum*, TY_{50}= Traditional method with 100% wheat grits and 3% yogurt starter. TTA= Terrible acidity. Different superscripts values in a column are significantly different (P<0.05).

Table 5. The levels of biogenic amines (mg/kg) among Tarasas treatments on the 15th day of fermentation (Mean±S.E).

Treat.	Histamine	Agmatin	B-phynyla-thyamine	Putricne	Cadaverine	Tyramine	Spermidine	Spermine	Total amount of BA
T_{100}	107.0±0.01a	74.40±0.01a	84.70±0.01a	87.03±0.01a	110.2±0.01a	73.60±0.02a	76.30±0.01a	65.80±0.01a	679.2±0.01a
TLB_{100}	97.07±0.01e	69.60±0.01e	73.40±0.01d	74.30±0.01c	63.47±0.01c	64.80±0.01c	61.40±0.01d	56.80±0.01d	560.8±0.01d
TY_{100}	109.3±0.01b	75.50±0.01b	87.30±0.01b	89.27±0.01b	112.6±0.01a	74.70±0.01a	77.30±0.01a	68.60±0.01a	694.5±0.01a
T_{50}	99.00±0.01b	64.00±0.11bc	71.40±0.01bc	79.50±0.01b	102.4±0.01ab	56.60±0.01c	55.90±0.01c	50.00±0.01c	579.0±0.01c
TLB_{50}	83.30±0.01c	57.8±0.01c	60.40±0.01c	65.70±0.01b	53.90±0.01b	42.40±0.01b	65.50±0.01b	39.70±0.01b	468.7±0.01b
TY_{50}	101.4±0.01b	65.6±0.01b	74.30±0.01b	81.8±0.01b	105.3±0.01b	58.40±0.01b	60.00±0.01c	52.10±0.01c	573.7±0.01c

Treat. = Treatment, T_{100}= Traditional method with100% wheat grits, TLB= Traditional method with 100% wheat grits and 3% *Lactobacillus plantarum*, TY_{100}= Traditional method with 100% wheat grits and 3% yogurt starter. T_{50}= Traditional method with50% wheat grits, TLB_{50}= Traditional method with 50% wheat grits and 3% *Lactobacillus plantarum*, TY_{50}= Traditional method with 100% wheat grits and 3% yogurt starter. TTA= Titratable acidity. Different superscripts values in a column are significantly different (P<0.05).

direct impact of adding wheat grits to the Tarasas product, serving as a raw material for biogenic amines, on the composition of these compounds, that may be because of amino acids presence like histidine, tyrosine, and tryptophan present in wheat can be decarboxylated by LAB bacteria to produce biogenic amines such as tyramine, histamine, and tryptamine (Capozzi *et al.*, 2012) and increased wheat grits provide more nutrients that play a significant role in providing essential nourishment for LAB during Tarasas fermentation.

Microorganisms, such as certain strains of LAB bacteria that during their growth and fermentation process of Tarasas, have the ability to generate biogenic amines as by-products of metabolism. More nutrients from increased wheat grits can result in higher microbial activity, leading to increased production of biogenic amines. Beside other factors such as time, ingredients ratio, include the concentration of salt that affects the total lactic acid bacteria (Tomita *et al.*, 2018), the ratio of wheat grits, and pH. Changes in these factors can affect the metabolic activities and growth of LAB, potentially

leading to increased biogenic amine production. And pH level plays a significant role in the amino acid decarboxylase activity. In fact, the activity of this enzyme is higher when the pH is within the range of (4 to 5.5). It is noteworthy that the 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. The biogenic amines concentrations were affected by the store period, which increased during fermentation (Fong *et al*., 2020). 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. 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. 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) sees that histamine levels in fish products should not exceed 50 parts per million (ppm) to minimize the risk of scombroid fish poisoning (Senanayake *et al*., 2023).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, respectively. Additionally, it is suggested that the upper limit for human consumption of histamine in foods should be around 100 mg/kg, and Kieliszek *et al.* (2021) mentioned that histamine levels above 500 mg/kg or tyramine levels above 1000 mg / kg are regarded as dangerous and toxic for human health (Senanayake *et al*., 2023). Upper limits of histamine have been established in different countries in wine 8 mg/L (France), 5 mg/L (Finland), 3.5 mg/L (Netherlands), 6 mg/L (Belgium), 2 mg/L (Germany), and 10 mg/L (Australia and Switzerland). 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. Not long ago the EFSA (European Food Safety Authority) Panel on Biological Hazards (BIOHAZ) performed a biogenic amines qualitative risk assessment in fermented foods. Using scientific literature obtained data, the BIOHAZ Panel determined that the BA accumulation in fermented foods is a complex process that multiple factors and their interactions affect it, the combination of which are variable, numerous and product-specific. Henceforth, risk mitigation options, which are based on controlling those factors/interactions, could not be ranked and considered individually. The results of the sensory analysis as shown in table 6 shows that the use of *lactobacillus plantarum* starter had a positive effect on the sensory properties, compared to other treatments. That is because *lactobacillus plantarum* is known for its diverse metabolic activities, contributing to various aroma production and flavor compounds at the time of fermentation. The specific strain of *L. plantarum* used in the fermentation process may produce unique and desirable compounds that positively influence the sensory characteristics of the Tarasas, in addition to have possesses enzymatic activities, and produce volatile compounds may be distinct from those produced by other microorganisms in treatments with yogurt or the control treatment, resulting in a more diverse and appealing sensory profile through its metabolic processes, that can impact the biochemical composition, texture and mouthfeel of the Tarasas, providing a smoother and more pleasing sensory experience (Smith and Johnson, 2014). Lastly, the combination of microbial interactions in mixed cultures can result in synergistic effects that positively influence sensory attributes. The specific combination of L. plantarum with other microorganisms in the Tarasas may create a unique and desirable flavor profile.

Table 6. Shows the Results of Sensory Evaluation of the treatments Formulated Tarasas.

Treatment	T_{100}	TLB_{100}	TY_{100}	T_{50}	TLB_{50}
Color	6.38b	6.56b	6.36b	5.81b	6.26b
Taste	6.36	6.63b	6.64b	5.50b	7.30b
Flavor	6.48	6.51b	6.38b	5.78c	6.40a
Texture	6.18	6.48b	6.32b	5.41b	6.19a
Overall acceptability	6.31	6.68	5.93b	5.92c	6.63b

T_{100}= Traditional method with100% wheat grits, TLB= Traditional method with 100% wheat grits and 3% *Lactobacillus plantarum*, TY_{100}= Traditional method with 100% wheat grits and 3% yogurt starter. T_{50}= Traditional method with50% wheat grits, TLB_{50}= Traditional method with 50% wheat grits and 3% *Lactobacillus plantarum*, TY_{50}= Traditional method with 100% wheat grits and 3% yogurt starter. TTA= Terrible acidity. Different superscripts values in a column are significantly different ($P<0.05$).

In this investigation, a subtle increase in biogenic amines was observed between the 10th and 15th day of fermentation, attributed to the activity of Lactobacillus plantarum. This bacterium demonstrated its capacity to degrade biogenic amines, resulting in a reduction in their levels within the fermented food (Islam *et al*., 2023). Lactobacillus plantarum, recognized as a beneficial bacteria variant, contributes to lowering biogenic amine levels in specific foods and fermented products (Al-kaabi and Chelab, 2024). Biogenic amines stem from the breakdown of amino acids in food, facilitated by enzymes or bacteria. While biogenic amines are generally safe for consumption within normal limits, elevated levels of certain amines like histamine, tyramine, and putrescine can elicit adverse reactions in some individuals. These reactions span from migraines and flushing to more severe symptoms such as hypertension or, in rare cases,

anaphylaxis (Capozzi *et al*., 2012).Lactobacillus plantarum achieves a reduction in biogenic amine levels by utilizing amino acids and catalyzing their breakdown through diverse enzymatic reactions, thwarting the accumulation of these amines. One mechanism employed by Lactobacillus plantarum involves the production of enzymes like histamine deaminase and tyrosine decarboxylase. These enzymes selectively target and break down histamine and tyramine, respectively. Furthermore, Lactobacillus plantarum competes with other bacteria for nutrients, diminishing the availability of amino acids in the environment (Gammone *et al*., 2019). Given that biogenic amines result from amino acid breakdown; the decreased availability leads to a reduction in amine production. Moreover, Lactobacillus plantarum can alter the pH (acidity) of the surrounding environment, establishing conditions unfavorable for the growth of bacteria that produce biogenic amines. By impeding the growth of these amine-producing bacteria, Lactobacillus plantarum effectively diminishes the levels of biogenic amines in food (Sun *et al*., 2023). The inclusion of Lactobacillus plantarum in fermented or cultured food products proves instrumental in lowering biogenic amine levels through enzymatic degradation, nutrient competition, and pH modification, thereby enhancing the safety and quality of the food.

***Conclusion*:** In conclusion, our findings emphasize the potential for tailored fermentation processes, utilizing specific bacterial strains and ingredient compositions, to influence biogenic amine levels in fermented foods. This knowledge contributes to the ongoing efforts in developing fermented products that not only boast desirable sensory characteristics but also align with safety and health consideration.

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***Conflict of interest*:** The authors declare no conflict of interest.

***Ethical statement*:** This article is not including any investigations involving human participants or animals conducted by any of the contributors.

***Availability of data*:** data include raw materials can be obtained for EIC of journal, if requested.

***Consent for publication*:** All authors provide their consent for the publication of this research article in JGIAS.

***SDG's addressed*:** Zero Hunger, Good Health and Well-being, Responsible Consumption and Production

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