

BIOGENIC AMINE PRODUCTION IN HISTIDINE DECARBOXYLASE BROTH BY SELECTED LACTIC ACID BACTERIA STRAINS

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ABSTRACT

The purpose of the current study was to investigate the biogenic amine production of lactic acid bacteria (LAB) strains (*Lactobacillus pentosus*, *Lactobacillus plantarum*, *Pediococcus acidilactici*, *Lactobacillus paraplantarum*, *Lactobacillus fermentum*, *Lactococcus raffinolactis*, *Pediococcus pentosaceus* and *Leuconostoc mesenteroides*) which in fermented foodstuff. Biogenic amine production of LAB strains was monitored in histidine decarboxylase broth using HPLC method. Significant differences were observed on ammonia (AMN) and biogenic amines accumulation among LAB strains ($p < 0.05$). Although *P. acidilactici* produced lesser AMN than other LAB, it was observed that all LAB strains produced high amount of AMN. LAB strains produced all amines in amounts between 1.48 and 1187.50 mg/L. Histamine production by LAB was in range from 17.20 mg/L by *P. acidilactici* to 126.26 mg/L by *Lb. fermentum*. The lowest tyramine concentration was observed by *P. acidilactici* with value of 14.66 mg/L whilst the highest tyramine production was found by *L. raffinolactis* with value of 64.43 mg/L.

Keywords: biogenic amine, lactic acid bacteria, histamine, tyramine

BAZI LAKTİK ASİT BAKTERİ ÜYELERİNİN HİSTİDİN DEKARBOKSİLAZ SIVISINDA BİYOJEN AMİN ÜRETİMLERİ

ÖZ

Bu çalışmada fermente gıda ürünlerinden izole edilen yedi laktik asit bakteri türünün (*Lactobacillus pentosus*, *Lactobacillus plantarum*, *Pediococcus acidilactici*, *Lactobacillus paraplantarum*, *Lactobacillus fermentum*, *Lactococcus raffinolactis*, *Pediococcus pentosaceus* ve *Leuconostoc mesenteroides*) biyojen amin üretimi araştırılmıştır. LAB üyelerinin, histidin dekarboksilaz sıvısında biyojen amin üretimleri HPLC yöntemi kullanılarak belirlenmiştir. LAB üyeleri arasında amonyak (AMN) ve biyojen amin üretimi bakımından istatistiksel farklılıklar gözlenmiştir ($p < 0.05$). *P. acidilactici* diğer LAB'lardan daha az amonyak üretmesine rağmen bütün LAB suşlarının yüksek miktarda biyojen amin ürettiği gözlenmiştir. LAB suşları bütün aminleri 1.48 ila 1187.50 mg/L arasında çeşitli miktarlarda üretmiştir. En düşük histamin üretimi 17.20 mg/L ile *P. acidilactici* tarafından üretilirken en yüksek histamin üretimi 126.26 mg/L ile *Lb. fermentum* tarafından gerçekleştirilmiştir. En düşük tiramin konsantrasyonu 14.66 mg/L ile *P. acidilactici* bakterisinde belirlenirken en yüksek tiramin üretimi 64.43 mg/L ile *L. raffinolactis* bakterisinde gözlenmiştir.

Anahtar kelimeler: biyojen amin, laktik asit bakterileri, histamin, tiramin

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INTRODUCTION

The consumption of foods with high quantities of biogenic amines especially histamine and tyramine can be implicated in various pharmacological reactions or demonstrate toxicological effect on the consumer's health (Spano et al., 2010). Therefore, the formation of biogenic amines such as histamine, cadaverine and putrescine in fish is an important concern for consumers since they are responsible for histamine poisoning and are indicator for spoilage of fish (Jorgensen et al., 2000; Alberto et al., 2002; Kuley et al., 2013). Histamine, tyramine, putrescine, cadaverine, tryptamine, agmatine, spermine and spermidine are the basic biogenic amines found in fish, fish products and fermented foods such as dairy and meat products and beverage such as beer and wine (Visciano et al., 2012).

The interest of the food biotechnology sector and researchers towards lactic acid bacteria has increased recently since LAB have the potential to inhibit disagreeable spoilage and pathogenic bacterial growth and have an important function in reducing biogenic amine formation. However, some species of lactic acid bacteria are able to convert amino acids into amine containing compounds called biogenic amines by means of decarboxylase activity during fermentation processes of different food products such as seafood, meat, dairy products, vegetables and wine (Griswold et al., 2006; Coton et al., 2010; Özogul and Hamed 2018). Therefore, lactic acid bacteria are considered to be the major biogenic amine producer in fermented foods. Certain lactic acid bacteria strains produce histamine due to their capacity to own the histidine decarboxylase gene (Landete et al., 2005; Lucas et al., 2005; Kuley et al., 2013; Gezginc et al., 2013). Tyramine is also the most widely detected biogenic amine in cheese and fermented meat product. LAB strains such as *Enterococcus* and *Lactobacillus* are responsible for the tyramine accumulation in these products. In particular, *Enterococcus faecalis*, *Enterococcus faecium*, and *Enterococcus durans* are regarded as main tyramine-producer (Russo et al., 2010). Some LAB strains e.g. *Lactobacillus fermentum*, and *Lactobacillus paracasei* isolated from meat, cheese and sausage are able to accumulate

putrescine (Wunderlichova et al., 2014; Özogul and Hamed, 2018). Several LAB strains are commonly used as starter culture in fermented food products. They are used in foodstuff because of their inhibitory constituents against fish spoilage and food borne pathogen bacteria (Galvez et al., 2010).

Biogenic amine accumulations have been reported in seafood products such as fish salads, cold-smoked fish, fish paste and fish sauce (Leuschner and Hammes, 1999; Petaja et al., 2000; Yongsawatdigul et al., 2004; Jorgensen et al., 2000; Gonzalez-Rodriguez et al., 2002; Thapa et al., 2006; Udomsil et al., 2010; Zhong-Yi et al., 2010). Lorencova et al., (2012) reported that most of the tested LAB isolated from dairy products and beer demonstrated decarboxylase activity. Thus, it is important to determine biogenic amine producing LAB strains in these product due to providing food safety. The purpose of the current study was to investigate the biogenic amine production potential of selected LAB possess of bioprotective properties in fermented foodstuff.

MATERIALS AND METHODS

Bacterial Strains

The lactic acid bacteria used in this research were *Lb. pentosus*, *Lb. plantarum*, *P. acidilactici*, *Lb. paraplantarum*, *Lb. fermentum*, *L. raffinolactis*, *P. pentosaceus* and *Leu. mesenteroides* that were isolated from various fermented foods commercially available in market. In our previous (unpublished) study, the LAB strains were identified using biochemical (API 50 CHL) and molecular tests (polymerase chain reaction; PCR). The food sources for isolation of lactic acid bacteria were as follow; *Lb. pentosus* and *Lb. plantarum* from marinated and smoked fish, *P. acidilactici* and *Leu. mesenteroides* from Turkish traditional cheese, *Lb. paraplantarum* from butter, *Lb. fermentum* from smoked salmon, *L. raffinolactis* and *P. pentosaceus* from kefir.

Culture Media and Bacterial Extraction for Biogenic Amine Analysis

Histidine decarboxylase broth (HDB) proposed by Klausen and Huss (1987) was used to monitor biogenic amine production of *Lactobacillus* strains.

HDB was prepared using 1 g peptone, 0.5 g Lab-Lemco powder (Oxoid CM0017, Hampshire, England), 2.5 g NaCl (Merck 1.06404.1000, Darmstadt, Germany), 4.01 g L-histidine HCl (Sigma H8125, Steinheim, Germany) and 2.5 mg pyridoxal-HCl (Sigma P9130, Steinheim, Germany) in 500 mL distilled water. The pH (5.5-6.8) were adjusted according to their optimum growth pH 1 KOH (Riedel-deHaen 06005, Seelze, Germany) or 6% TCA (Riedel-deHaen 27242, Seelze, Germany). HDB was pipetted in 10 mL microbiologic tube and then autoclaved at 121 °C in 15 min prior to use. After propagation of *Lactobacillus* strains, 0.5 mL of bacterial cultures ($\sim 10^8$ cfu/mL) was removed and put into 10 mL of HDB. Samples were incubated at 30 °C for 72h. Extraction process and derivatization of biogenic amines were performed according to the method of Kuley and Ozogul (2011). For the extraction, 5 mL of the HDB including LAB strain was removed to separate microbiologic tube and then 2 mL of sulphosalicylic acid was added. They were centrifuged at 3000 g for 10 min and then filtered through a filter paper (Whatman GmbH, Dassel, Germany). Afterwards, 4 mL of bacterial supernatant was taken to derive from each of LAB strains.

Preparation of Standard Amine Solutions for HPLC Analysis

The biogenic amine standards used in current study were purchased from Sigma Alderich (Munich, Germany). The mobile phase consisting of acetonitrile and HPLC grade water were also used for amine analysis. Ammonia chloride (296.9 mg), putrescine dihydrochloride (182.9 mg), cadaverine dihydrochloride (171.4 mg), spermidine trihydrochloride (175.3 mg), Tryptamine hydrochloride (122.8 mg), 2-phenylethylamine hydrochloride (130.1 mg), spermine tetrahydrochloride (172.0 mg), 5-hydroxytryptamine (serotonin) (133.9 mg), trimethylamine hydrochloride (161.7 mg), 3-hydroxytyramine hydrochloride (dopamine) (123.8 mg), agmatine sulphate (175.4 mg), tyramine hydrochloride (126.7 mg), and histamine dihydrochloride (165.7 mg) were dissolved in 10 mL HPLC grade water.

Derivatization Procedure

To increase the reaction with amines, a stock solution was prepared by dissolving 2% benzoyl chloride in acetonitrile. Briefly, 100 μ L was taken (4 mL for extracted bacterial culture) from each free base standard solution (Sigma Alderich, Munich, Germany) at 10 mg/mL to derive the standard amine solution and mixed with 1 mL sodium hydroxide (2 M). After that 1 mL of 2 % benzoyl chloride (dissolved in acetonitrile) was added and the solution was stirred in a vortex shaker for 1 min. The reaction mixture was left at ambient temperature to reacted for 5 min and then centrifuged for 10 min. Afterwards, the benzoylation was stopped by adding 2 mL of saturated sodium chloride solution and the solution extracted twice with 2 mL diethyl ether. The upper organic layer was transferred into a clean tube after mixing. Then, the organic layer was evaporated to dryness in stream of pure nitrogen (99.9 %, Linde Gas, Adana, Turkey). The residue was dissolved in 1 mL of acetonitrile, and 5 μ L aliquots were injected into the HPLC.

Analytical Method

The confirmation of biogenic amine production was carried out using a rapid HPLC method (Özogul, 2004) with a reversed-phase column by using a gradient elution program. For ammonia and trimethylamine (TMA) analysis, same analytic method was conducted.

Statistical Analysis

To find the average value and standard deviation, the data obtained from the three samples for each treatment was used. The significance of differences ($P < 0.05$) was determined using Duncan's multiple comparison test with SPSS version 19.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULT AND DISCUSSION

Ammonia and biogenic amine production by selected LAB isolates in HDB were presented in Table 1. Significant differences were observed on ammonia (AMN) and biogenic amines accumulation among LAB strains ($p < 0.05$). Although *P. acidilactici* produced lesser AMN than other LAB strains, it was observed that all LAB

strains produced high amount of AMN (Table 1). On the other hand, AMN production of LAB strains including *P. pentosaceus*, *Lb. fermentum*, *Lb. paraplantarum*, *Lb. pentosus* and *Lb. plantarum* were found to be statistically similar. Similar results were also obtained by Özoğul et al. (2012) who reported that LAB strains of *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis*, *Lb. plantarum* and *Streptococcus thermophilus* produced high amount of AMN in arginine decarboxylase

broth at level of 2554.86, 2349.01, 1872 and 1079 mg/L. In other study conducted by Arena et al. (1999), AMN production by *Lb. plantarum* N8 and N4 strains in arginine decarboxylase broth was reported to be over 1070 mg/L. In the current study, putrescine production of LAB strains except for *Lb. fermentum* and *Lb. paraplantarum* were detected low concentration in range of 17.80-96.24 mg/L.

Table 1. Ammonia and biogenic amines production (mg/L) of selected LAB strains in histidine decarboxylase broth (HDB)

Ammonia and Biogen Amines	Lactic Acid Bacteria							
	<i>P. pentosaceus</i>	<i>Leu. mesenteroides</i>	<i>Lb. fermentum</i>	<i>Lb. paraplantarum</i>	<i>Lb. pentosus</i>	<i>Lb. plantarum</i>	<i>P. acidilactici</i>	<i>L. raffinolactis</i>
AMN	2413.8 ^{ax} (235.32) ^y	2067.76 ^{bx} (129.20) ^y	2358.86 ^{ax} (32.27) ^y	2355.26 ^{ax} (21.97) ^y	2376.18 ^{ax} (151.91) ^y	2166.46 ^{abx} (7.77) ^y	1490.12 ^{cx} (29.81) ^y	2008.16 ^{bx} (10.57) ^y
PUT	63.06 ^d (4.10)	20.40 ^f (0.56)	887.04 ^a (18.57)	801.46 ^b (12.36)	96.24 ^c (5.65)	43.76 ^c (2.26)	17.80 ^f (0.79)	21.46 ^f (0.70)
CAD	183.9 ^c (6.64)	128.10 ^d (1.72)	131.18 ^d (8.73)	1187.50 ^a (0.31)	931.24 ^b (35.18)	105.34 ^{de} (4.94)	89.82 ^c (3.87)	206.04 ^c (7.58)
SPD	136.46 ^b (5.06)	20.68 ^e (0.96)	220.22 ^a (7.38)	133.82 ^b (1.44)	97.28 ^c (3.84)	136.22 ^b (5.79)	36.62 ^d (2.29)	30.00 ^{de} (1.41)
TRP	34.9 ^c (1.72)	0.00 ^f	264.70 ^a (10.77)	98.44 ^b (43.33)	17.10 ^d (1.66)	4.94 ^e (1.32)	3.90 ^e (0.28)	0.00 ^f
PHEN	140.86 ^c (2.96)	92.28 ^e (1.81)	167.40 ^b (4.24)	0.00 ^g	145.72 ^c (3.39)	78.72 ^f (2.09)	237.02 ^a (1.83)	123.48 ^d (0.19)
SPN	0.00 ^d	0.00 ^d	0.00 ^d	56.62 ^b (0.02)	0.00 ^d	119.76 ^a (1.52)	43.20 ^c (1.97)	0.00 ^d
SER	140.48 ^d (1.13)	36.80 ^g (0.84)	348.42 ^c (1.66)	401.80 ^b (2.37)	414.90 ^a (1.41)	45.28 ^e (1.69)	35.94 ^g (2.40)	41.02 ^f (1.21)
TMA	75.85 ^c (0.07)	125.80 ^b (9.67)	207.68 ^a (10.86)	0.00 ^g	46.60 ^d (1.35)	11.18 ^{ef} (0.19)	1.48 ^f (0.04)	21.48 ^e (0.73)
DOP	312.32 ^b (0.22)	85.62 ^d (1.66)	85.96 ^d (3.90)	19.98 ^f (1.44)	86.68 ^d (2.54)	321.26 ^a (2.40)	78.86 ^e (2.06)	244.32 ^c (0.96)
AGM	234.90 ^a (12.30)	104.40 ^b (1.24)	10.86 ^f (0.84)	27.62 ^e (0.70)	42.98 ^d (2.51)	55.26 ^c (0.65)	21.56 ^e (0.79)	4.46 ^f (0.14)

Explanation; AMN, ammonia; PUT, putrescine; CAD, cadaverine; SPD, spermidine; TRP, tryptamine; PHEN, 2-Phenyl-ethylamine; SPN, spermine; SER, serotonin; TMA, trimethylamine; DOP, Dopamine. AGM, agmatine. x: Values represents mean, y: Values mean standard deviation. The same superscript (a-f) in the same row was not significantly different ($P>0.05$)

Cadaverine were also produced in various amounts by the tested all of the LAB strains. However, the highest cadaverine formation were observed with values 931.24 and 1187.50 mg/L by *Lb. pentosus* and *Lb. paraplantarum* respectively. Spermidine, tryptamine, 2-phenyl-ethylamine,

spermine, serotonin, dopamine, agmatine were also produced all LAB strains in various amounts ranging from 1.48 to 414 mg/L. However, spermine was not produced by *P. pentosaceus*, *Leu. mesenteroides*, *Lb. fermentum*, *Lb. pentosus*, *L. raffinolactis*. In addition, tryptamine was not

produced by *Leu. mesenteroides* and *L. raffinolactis* (Table 1). Özogul (2011) found that in HDB, three LAB strains containing *Lactococcus lactis* subsp. *cremoris* (MG 1363), *Lactococcus lactis* subsp. *lactic* (IL1403), *Lactobacillus plantarum* (FI8595) strains produced putrescine, spermidine, 2-phenyl-ethylamine, dopamine, agmatine, tyramine, trimethylamine (TMA), tryptamine and serotonin. Similarly, in the present study 2-phenylethylamine and TMA were not produced by *Lb. paraplantarum*. It has been reported that *Leu. mesenteroides*, *Lb. brevis*, *Lactococcus lactis* ssp. *cremoris*, *P. pentosaceus* isolated from fermented Uzicka sausage displayed very low concentration of biogenic amines production in vitro. Histamine, spermine and tyramine formation were observed with values 12.76-20.42 µg/mL, 5.54-14.93 µg/mL and 16.39-21.92 µg/mL by various isolates of *Leu. mesenteroides*. *Lb. brevis* produced histamine and tyramine up to 12.45 and 15.10 µg/mL (Moracanin et al., 2015). Buňková et al., (2009) demonstrated that LAB strains containing *Lc. Lactis* subsp. *cremoris*, *Strep. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* produced tyramine but did not produce other biogenic amines such as histamine, putrescine, cadaverine, agmatine,

spermidine and spermine, which is in no agreement with this study since all LAB used in the present study produced these biogenic amines. In another research conducted by Thapa et al., (2006), it was reported that none of strains of *Lc. lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactic*, *Lb. plantarum*, *Leu. mesenteroides* and *P. pentosaceus* isolated from traditionally processed fish products produced biogenic amines.

In this research, low amount of histamine production was detected in all of monitored LAB bacteria. Histamine production by LAB strains were the lowest by *P. acidilactici* (17.20 mg/L) and the highest by *Lb. fermentum* (126.26 mg/L). *P. acidilactici* and *Leu. mesenteroides* produced histamine at very low concentration with value of 17.20 and 47.8 mg/L (Figure 1.). Gezginc et al. (2013) found that most of 58 isolates of *Streptococcus thermophilus* isolated from home-made natural yogurt formed low amounts of histamine (1–50 mg/L) from histidine amino acids. Histamine production by *Lb. plantarum* strains grown in MRS broth supplemented with L-histidine monohydrochloride was also reported as below the 250 mg/L by Alan et al. (2018).

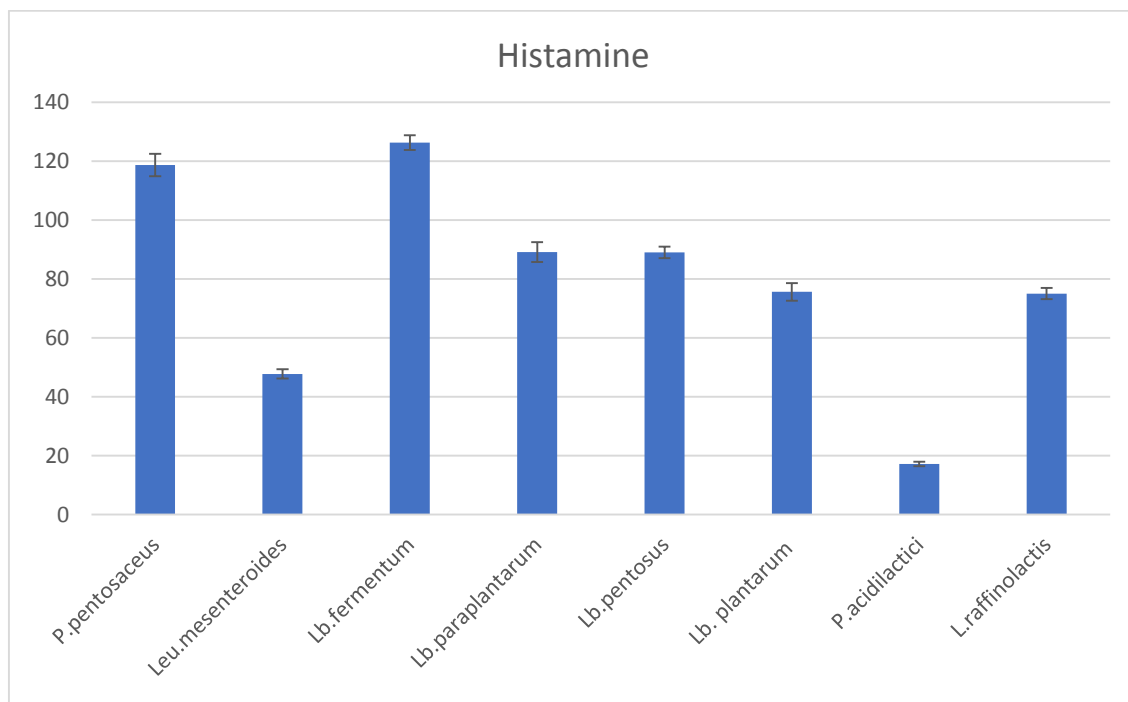


Figure.1 Histamine production of LAB strains in histidine decarboxylase broth.

All LAB strains produced not only histamine but also produce tyramine in HDB. Tyramine production by LAB strains were range from 14.66 to 64.43 mg/L. The lowest concentration of tyramine was produced by *P. acidilactici* with value of 14.66 mg/L whilst the highest concentration of that was produced by *L. raffinolactis* with value of 64.43 mg/L (Figure 2). *P. acidilactici* produced particularly low quantities both histamine (17.20 mg/L) and tyramine (14.66 mg/L). Forty-seven isolates of *S. thermophilus* from home-made natural yogurt were reported to produce tyramine at low concentration (1-100 mg/mL), while the other ten isolates were reported to produce tyramine at

medium concentration (101-500 mg/mL). Furthermore, most of the *S. thermophilus* isolates were reported to produce low amount of histamine (1-50 mg/mL) (Gezginc et al., 2013). It has been also reported that *Lactobacillus casei* (isolated from different fermented food products) produced high quantities of histamine (1820.9 mg/L) and tyramine (5486.99 mg/L) in MRS broth enrichment with histidine and tyrosine amino acids while *Lactobacillus delbrueckii* (isolated from different fermented food products) produced only histamine (459.1 mg/L) in MRS broth enrichment with histidine (Deepika Priyadarshani et al., 2011).

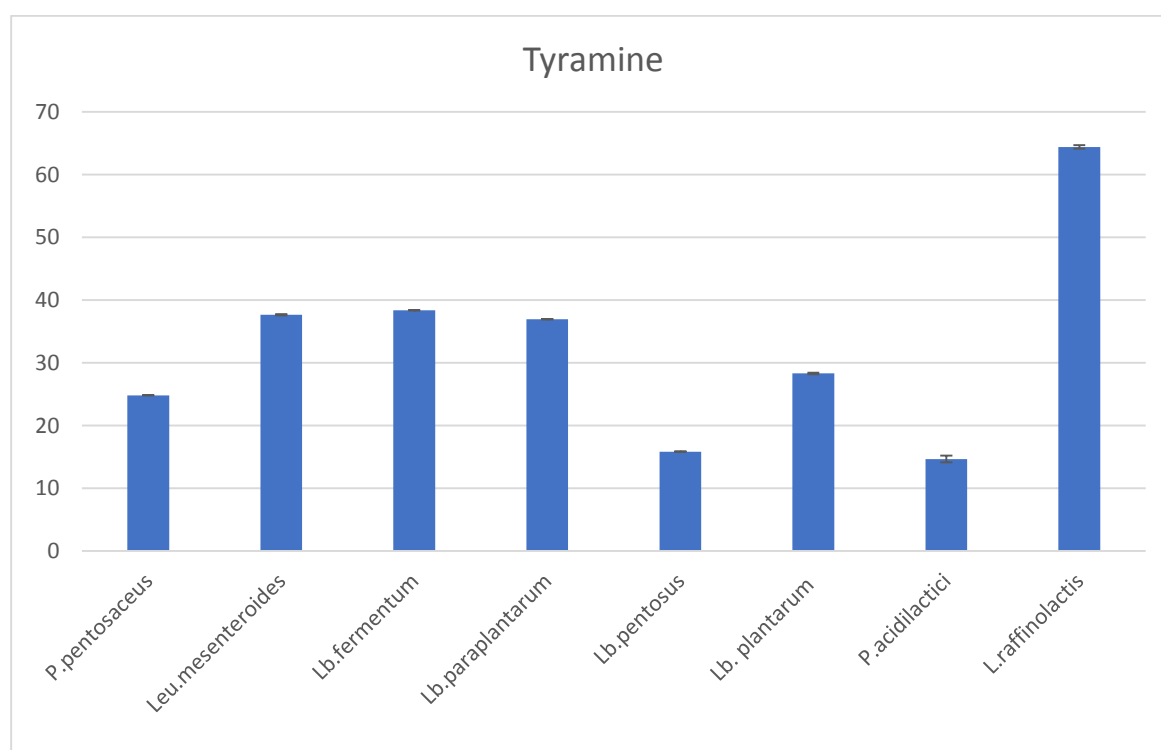


Figure 2. Tyramine production of LAB strains in histidine decarboxylase broth

CONCLUSION

The results of current study demonstrated that all LAB strains tested had the capability to produce twelve biogenic amines mainly cadaverine, serotonin, dopamine, agmatine and spermidine in HDB. They formed histamine and tyramine at low concentrations, which are the most important amines for human health. Our results emphasize the potential use of LAB as a bio-protective agent in the food industry. Further research should be

done to understand their exact antimicrobial mechanism in food system.

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