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Control of Biogenic Amines in Peanut Butter by Incorporation of Some Probiotic Bacteria (Useful impact of adding *B. infantis* and *L. plantarum* to peanut butter)

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BIOGENIC amines have been reported to present in different foods. The formation of biogenic amines is influenced by many factors, such as raw materials, microorganisms, and storage conditions. The study aimed to investigate the possibility of incorporation of probiotic bacteria (*B. infantis* 35624 and *L. plantarum* 299v) to control biogenic amines formation in peanut butter under the conditions prevailing in this product. The data showed that peanut butter might be a suitable matrix to protect probiotics during storage, and *B. infantis* had the highest survivability in all samples during incubation at different temperatures. The values of biogenic amines were decreased continuously in liquid medium relevant to the activity of probiotic bacteria cells. All liquid medium samples had high histamine decreasing ratio; 77.52% for *B. infantis* and 76.12 % for *L. plantarum* compared with other amines at pH 6.8. The data showed that the incorporation of probiotic bacteria in peanut butter, reduced biogenic amines, especially tyramine and cadaverine, during storage at different temperatures. Degradation of biogenic amines was less at 37° C comparing to 4° C and 25° C. All organoleptic properties were significant when compared to the control sample after 12 weeks of storage at 4, 25 and 37°C, respectively.

Keywords: *Probiotic bacteria; Peanut butter; Biogenic amines*; *Bifidobacterium; Cadaverine; Histamine.*

Introduction

Biogenic amines are anti-nutritional compounds formed from decarboxylation of free amino acids by microorganisms. Many developed methods have also been used to reduce the levels of these amines to allowable limits; good manufacturing practices the key to control biogenic amines (Jairath et al., 2015).

Food poisoning and foodborne illness have different origins (biological, chemical and natural toxins). One of the toxins aimed by the Food and Drug Administration (FDA) are biogenic amines (BAs), BAs in food constitute a prospect public health concern due to their physiological and toxicological effects. They are found in fluctuating concentrations in a wide range of foods, BAs formation is affected by vaious factors associated with the raw material used in the products, microorganisms, processing, and preservation conditions. In the case of microorganisms, it is required to control not only the microbial load in the final product but also the type of microbiota comprising that load (bacterial species and strain). Proteolysis happens by the vast quantities of microorganisms in these products, gives large amounts of the free amino acids, forming the substrate on which decarboxylase enzymes work and the nutrient which is needed by the microorganisms. In some cases, fermented

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products that constitute on BAs have been assigned to the bad quality of raw materials and defective processing. The final products' storage temperature had significant effects on BAs formation (Ruiz-Capillas and Herrero, 2019). Biogenic amines may play an essential role as quality and acceptability indicators in some foods (Herrero, 2008). Cadaverine, tyramine, histamine, or a combination of many amines, such BAs have been used as a quality index (Vinci and Antonelli, 2002). Biogenic amines in food are undesirable because they can have adverse effects on consumers, such as hypotension, allergic, respiratory distress, and headaches (Eom et al., 2015).

The concentration of amines formed in food depends on the type of microorganisms existing, favorable conditions for enzymatic activity, and the action of decarboxylase enzymes (Rodriguez et al., 2014; Martin et al., 2016). The availability of free amino acids in food increasing the possibility of biogenic amines' accumulation in that food, and the existence of microorganisms with decarboxylase activity on amino acids depends on food intrinsic and extrinsic parameters such as pH, the concentration of free amino acids, oxygen and temperature (Bunkova, 2010).

Temperature and starter culture are the major factors for controlling the formation of the biogenic amine in food. Various techniques can be combined to control microbial growth and enzyme activity during processing and storage (Chong et al., 2011). Contamination of peanut butter with *Salmonella* and *E. coli* 0157: H7, posing health risks to consumers. Many bacterial genera, including some foodborne pathogens, can decarboxylate amino acids and produce biogenic amine (Özogul, 2011 and Grasso et al., 2015).

Developing methods include the use of diamine oxidase enzymes to degrade biogenic amines and/or use bacteria (starter cultures) that possess the enzymes which cause biogenic amines degradation. Probiotic Bifidobacteria and lactic acid bacteria can be combined into dry food matrices and other dietary supplements to extend shelf-life up to 2 years at ambient humidity and temperature. More probiotics are being consumed by many populations, such as people with allergic reactions, infants, and those suffering from immune systems problems (Fenster et al., 2019).

Brenner and Chey (2009) reported that *B. infantis* 35642 is probiotic with unique abilities to reduce intestinal inflammation and useful for the treatment of irritable bowel syndrome (IBS). *Lactobacillus plantarum* 299v is a probiotic strain relief of the abdominal symptoms with inhibition against several pathogenic bacteria (Ducrotté et al., 2012).

The aim of the present study is to investigate the possibility of the incorporation of probiotic bacteria (*B. infantis* and *L. plantarum*) in peanut butter to control biogenic amines content under the storage conditions of this product.

Materials and Methods

Bifidobacterium infantis 35624, and *Lactobacillus plantarum* 299v were purchased from DVS, Chr Hansen's lab Denmark. All media used in the microbiological analysis were obtained from Merck (Germany). All BA standards and all reagents were analytical grade and purchased from Sigma – Aldrich (Munich, Germany). Peanuts were obtained from a local market at Ismailia Governorate, Egypt (2018 season), shelled manually, and roasted at 160°C for 60 minutes, according to Gaballa (2005).

Degradation of biogenic amine by probiotic strains in a liquid medium

The degradation activity of the probiotic strains was measured by incubating strains at 30° C for 24 hr in MRS broth containing 25 ppm of each biogenic amine (histamine, tryptamine, putrescine, cadaverine and tyramine) at different pH (5.8, 6.8 and 7.8 pH)

Preparation of peanut butter

Peanut butter was prepared using the following formula, roasted peanut (92%), NaCl (1%), peanut oil (3%), dextrose (2%) and glycerol (2%), the roasted peanut was mixed with all ingredients into sterile kitchen mixer to produce homogenous peanut butter. The mixture was divided into four portions (A, B, C, and D) and pasteurized at 70° C for 15 min, then cooled. The portion (A) was left without any inoculation as control, the portion (B) was inoculated with *Bifidobacterium infants*, the portion (C) was carried out with the inoculation of *Lactobacillus plantarum*, while portion (D) inoculated with *Lactobacillus plantarum* plus *Bifidobacterium infants*. Probiotics dissolved in 500ml of cold water were added to achieve a final concentration of 10^8 CFU/g peanut butter for each bacterial strain. After adjusting pH to 6.8, all samples were packed in sterile glass jar containers with screw covers and stored at 4, 25, and 37°C for 12 weeks.

Samples from each treatment were taken at specified time intervals throughout storage for bacteriological, chemical, and sensory evaluation.

Enumeration of probiotic bacteria

The initial population counts of Bifidobacterium and Lactobacillus species were confirmed after the inoculation and during the storage period. For bacterial enumeration, 10g of peanut butter samples were homogenized in 90ml 0.1% sterile peptone at 37°C using a homogenizer, and serial dilution up to 106 was prepared from the original dilution. *Lactobacillus plantarum* was enumerated on MRS (de Man, Rogosa and Sharpe) agar and Bifidobacterium counts were enumerated on MRS modified with NPNL (Neomycin sulfate, Paromomycin sulfate, Nalidixic acid, and Lithium chloride). Bifidobacterium and Lactobacillus species were incubated under anaerobic condition (BBL anaerobic jar containing Gas generating Kit, oxid) at 30°C for 48 hr.

Chemical analysis

pH value was determined using pH meter (PTI-15, Aqua chemical Co., England)

Biogenic amine analysis

Determination of biogenic amines in a liquid medium and peanut butter

Determination of tyramine, cadaverine, tryptamine, putrescine, and histamine either in a liquid medium or in peanut butter was carried out by high-performance liquid chromatography (HPLC) according to Hwang et al. (2011) with some modifications as following :

Mix 5g of the homogenized sample with 20ml of perchloric acid (0.4M) using a vortex mixer then centrifuge at 3000xg at 4°C for 10min, the residue was extracted again with an equal volume of perchloric acid.

The collected supernatants were combined and adjusted to 50ml with perchloric acid (0.4M), then filtered and stored at 4°C±1 for high-performance liquid chromatography (Agilent 1100 series; Agilent, Santa Clara, CA) analysis within a week.

Sensory evaluation

All samples were subjected to sensory evaluation at 0, 6, and 12 weeks of storage at 4, 25 and 37° C for flavor (50), color (25) and texture (25) (Gaballa, 2005).

Statistical analysis

Standard deviation (SD) and significant differences between the mean values of the estimated tests were performed using the Software Package Statistical 9.1 for Windows, Stat Soft, Tulsa, Oklahoma, USA, 2009. Differences were considered significant at $P<0.05$.

Results and Discussion

Effect of storage temperature on survival probiotics

Storage temperature affected the survivability of *B. infants* and *L. plantarum*. The average populations of mentioned strains reduced with increasing storage time (Table 1). The reduction was higher at 37° C compared to the storage of 4° C and 25° C. *L. plantarum* and *B. infants* were initially incorporated at about 10^8 CFU g^{-1}.

After six weeks of storage at 4, 25 or 37° C, *L. plantarum* survival reduced in sample storage at 37° C while sample storage at 4° C or 25° C did not show a reduction. *L. plantarum* reduced on all treatments during storage at 4, 25, and 37° after 12 weeks.

After storing treatments for one, two, and four weeks, treatments B and C did not show a high reduction in probiotics at all temperature storage. However, probiotics showed a negligible decrease in CFU after 12 weeks of storage at 4, 25, and 37°C for all treatments. Klu et al. (2014) reported that Bifidobacteria had the highest survivability in all probiotic products during storage.

Studying of probiotic bacteria suitability at different storage temperatures is essential to evaluate the storage condition and quality of non-refrigerated probiotic food. The counts of bacteria recovered from samples were high at 4 and 25° C compared with counts at 37°C. Bruno and Shah (2003) reported that high storage temperature accelerates the metabolic and activities of probiotics, leading to depletion of nutrients and loss of cell viability. Peanut butter is the best matrix and has been shown to protect probiotics (Klu et al., 2014 and Min et al., 2017). Also, Granato et al. (2010) mentioned that the choice of food matrix is vital for the viability of probiotics during storage.

Degradation of biogenic amines by probiotic strains

Study the risk assessment of BA, such as tyramine and histamine in fermented food is essential for the toxicity evaluation of these compounds (Eom et al., 2015). The tabulated data in Table 2 showed that biogenic amines' values were decreased continuously in the liquid

medium during the incubation period. The results indicated that the reduction of biogenic amines content was relevant to the activity of probiotic bacteria cells. From the data it can be noticed that biogenic amines content of treatment inoculated with *B. infantis* decreased from an initial value (25 ppm) to 8.90 ±0.009, 7.63 ±0.010, 5.62 ±0.023, 5.91 ±0.081 and 6.14 ±0.011 ppm for tryptamine, putrescine, histamine, tyramine, and cadaverine, respectively with decreasing ratio 64.40, 69.48, 77.52, 76.36 and 75.44 % at pH 6.8 followed by treatment at pH 7.8 and 5.8 respectively while it was 9.46 ±0.008, 8.54 ±0.111, 5.97 ±0.013, 6.39 ±0.009 and 7.08 ±0.132 ppm at pH 6.8 for sample inoculated with *L. plantarum*. The data also showed that samples had high histamine decreasing ratio at all pH values compared with other amines. Tapingkae et al. (2010) reported that the optimum pH for biogenic amines degradation is between 6.5 and 8.3. *Staphylococcus xylosus* No. 0538 degraded tyramine by 4% and histamine by 38% and total BA by 16% by applying starter cultures in fermented Anchovy.

From the data, it can be noticed that reduction of biogenic amines was obvious in probiotic *B. infantis* compared with *L. plantarum,* and biogenic amine degradation activity was observed in *B. infantis*, so suggesting that using this strain as probiotic can mainly prevent BAs accumulation (Table 2). Also, Bifidobacteria had several good effects on human health, including the reduction of cholesterol (Modler, 1994) and reduced diarrhea in children (Klu et al., 2014).

Incorporation of probiotic bacteria in peanut butter affected the reduction of biogenic amine during storage at different temperatures. Data presented in Tables 3, 4 & 5 show that control samples (A) contained higher amounts of biogenic amines than those contained probiotic bacteria at all storage conditions, specially tyramine and cadaverine.

Moreover, degradation of biogenic amines was less at 37°C comparing with 4° and 25°C. According to Latorre Moratalla et al. (2012), high processing and fermentation temperatures of dry sausage increase decarboxylase activity of *L. curvatus* and, hence, favored amine accumulation. From the data, it can be noticed that tyramine was the dominant amine in amounts, followed by the cadaverine and putrescine. Histamine and tryptamine are other biogenic amines that may appear of peanut butter during storage. The data also showed that a drastic reduction of biogenic amine production was at 25°C in the sample (D) followed by samples (B) and (C). Numerous bacteria have been found to control enzymes that oxidize biogenic amines or to have negative decarboxylase activity (Naila et al., 2010). *Bacillus* species could be used to control biogenic amine production in food when used as a starter culture (Eom et al., 2015). starter culture selection is fundamental to guarantee the quality of the products (Martin-Alvarez et al., 2006). *L. paraplantarum* incorporation with starter during industrial cheese for decreasing the concentration of histamine and tyramine (Guarcello et al., 2016).

Dapkevicius et al. (2000) reported that the degradation of histamine by lactic acid bacteria was observed in the ensiled fish slurry. According to Capozzi et al. (2012) who reported that we could apply the selected *L. plantarum* strains in wine during fermentation to degrade biogenic amines. A mixture of probiotic bacteria, including *Bifidobacterium infants* and *Bifidobacterium longum*, is sufficient for histamine allergic (Dev et al., 2008). Diamine oxidase can degrade histamine to an undetectable level in the food system and food for human consumption (Naila et al., 2010).

According to Mokhtar et al. (2012) who mentioned that *L. plantarum* could fracture biogenic amines such as histamine rather than producing them.

Biogenic amines are found in food of animal origins such as eggs, fish, and meat in high concentrations (above 50 µg/g), which can induce chemical poisoning, while in fresh food, it was below 10µg/g.

The concentration of amines in food depends on microorganisms and conditions for the activity of enzymes produced by microorganisms (Martin et al., 2016). The inclusion of probiotic bacteria into the peanut butter resulted in a drastic reduction of biogenic amine production. This phenomenon may be due to some probiotic bacteria such as *Bifidobacteria* can produce antimicrobial compounds called bacteriocin (Cheikhyoussef et al., 2009; Casaburi et al., 2016). pH values of treatments containing probiotic bacteria had a slight decrease at 4°C for 12 weeks compared with control. Mean values at zero time were 6.80 while they were 6.86, 6.75, 6.76 and 6.74 for control, B, C, and D treatments after 12 weeks of storage, respectively. However, at the other storage temperatures (25 and 37°C), the data revealed that a significant decrease in pH values was observed.

TABLE 1. Survival of probiotic bacteria (log CFU/g) at pH 6.8 during the storage of peanut butter at different temperatures (Mean ±Standard Deviation).

Treatment	B			C			D					
Storage Period (wk)	4° C	25° C	37° C	4° C	25° C	37° C	4° C		25° C		37° C	
							X	Y	X	Y	X	Y
0	8.96 ±0.105	8.96 ±0.009	8.96 ±0.110	8.96 ±0.011	8.96 ±0.012	8.96 ±0.009	3.961 ±.005	3.724 ±0.741	3.811 ±0.003	3.156 ±0.117	3.562 ±0.132	3.316 ±0.512
1	8.96 ±0.019	8.924 ±0.080	8.857 ±0.121	8.945 ±0.014	8.914 ±0.011	8.919 ±0.112	4.541 ±0.121	4.423 ±0.118	4.541 ±0.315	4.332 ±0.111	4.511 ±0.331	4.462 ±0.101
2	8.935 ±0.081	8.909 ±0.003	8.845 ±0.121	8.398 ±0.010	8.447 ±0.026	8.342 ±0.103	4.620 ±0.230	4.411 ±0.042	4.711 ±0.114	4..358 ±0.132	4.321 ±0.118	4.195 ±0.131
4	8.748 ±0.022	8.833 ±0.110	8.623 ±0.118	8.267 ±0.024	8.29 ±0.031	8.26 ±0.210	4.551 ±0.111	4.263 ±0.302	4.382 ±0.016	4.253 ±0.352	4.115 ±0.215	4.012 ±0.228
6	8.279 ±0.301	8.623 ±0.118	8.122 ±0.122	8.174 ±0.005	8.248 ±0.025	8.158 ±0.032	4.350 ±0.118	4.211 ±0.261	4.493 ±0.001	4.210 ±0.113	3.973 ±0.001	3.421 ±0.006
8	7.903 ±0.210	7.952 ±0.109	7.602 ±0.132	6.903 ±0.001	7.716 ±0.015	6.654 ±0.016	4.151 ±0.001	3.852 ±0.006	3.951 ±0.131	3.818 ±0.116	3.825 ±0.001	3.782 ±0.111
10	7.532 ±0.301	8.322 ±0.311	7.099 ±0.151	6.699 ±0.015	7.699 ±0.106	6.301 ±0.101	4.320 ±0.081	3.516 ±0.161	3.721 ±0.008	3.822 ±0.004	3.839 ±0.052	3.418 ±0.192
12	6.954 ± 0.002	6.954 ± 0.110	6.602 ± 0.140	6.301 ± 0.016	6.491 ± 0.115	5.954 ±0.115	4.123 ±0.001	3.911 ±0.019	3.950 ±0.101	3.321 ±0.139	3.711 ±0.192	3.452 ±0.008

A= Control without probiotic
B= Treatment inoculated with *B. infants*
C= Treatment inoculated with *L. plantarum*
D= Treatment inoculated with *L. plantarum* + *B. infants*
X= *B. infants*, Y= *L. plantarum*

TABLE 2. Degradation of biogenic amines (25 mg/100 ml) by probiotics in a liquid medium at different pH after 24 hr of incubation (Mean ±S.D).

Biogenic amine	By	pH 5.8		pH 6.8		pH 7.8	
		Concentration (mg/ 100 ml)	% reduction	Concentration (mg/ 100 ml)	% reduction	Concentration (mg/ 100 ml)	%reduction
Tryptamine	*B. infants*	10.81 ±0.008	56.76	8.90 ±0.009	64.40	10.30 ±0.022	58.80
	L. plantarum	11.50 ±0.001	54.0	9.46 ±0.008	62.16	10.96 ±0.131	56.16
Putrescine	*B. infants*	10.19 ±0.131	59.24	7.63 ±0.010	69.48	9.45 ±0.003	62.22
	L. plantarum	10.98 ±0.113	56.08	8.54 ±0.111	65.84	9.88 ±0.210	60.48
Histamine	*B. infants*	7.50 ±0.051	70.00	5.62 ±0.023	77.52	6.72 ±0.007	73.42
	L. plantarum	8.56 ±0.003	65.76	5.97 ±0.013	76.12	7.08 ±0.005	71.68
Tyramine	*B. infants*	7.92 ±0.001	68.32	5.91 ±0.081	76.36	8.22 ±0.111	67.12
	L. plantarum	8.72 ±0.210	65.12	6.39 ±0.009	74.44	8.91 ±0.132	64.36
Cadaverine	*B. infants*	9.84 ±0.011	60.68	6.14 ±0.011	75.44	9.83 ±0.123	60.68
	L. plantarum	9.98 ±0.235	60.08	7.08 ±0.132	71.68	10.01 ±0.115	59.96

The values decreased from 6.80 at zero time to 6.63, 6.65 and 6.62 for treatments B, C, and D during storage at 25° C while decreased to 6.57, 6.79 and 6.55 during storage at 37° C (The data not tabulated). Similar results were observed by Gab-Alla and Gad (2001), who mentioned that incorporating *Bifidobacterium lactis* Bb-12 in peanut butter caused a slight decrease in pH during storage at 25 and 37° C for 20 weeks. Also, Khalil and Mansour (1998) reported that mayonnaise containing encapsulated Bifidobacteria had a pH value lower than the control sample.

Table 6 shows that flavor, color and texture are not significantly different in control and samples containing probiotic bacteria at zero time. On the other hand, the scores of all organoleptic properties were significantly higher when compared to the control sample after 12 weeks of storage at 4, 25, and 37°C. Sensory panelists rated peanut butter treated with probiotic bacteria as being more acceptable than control in all sensory characteristics understudy after 12 weeks of storage at 4, 25, and 37°C. That could be attributed to the production of antimicrobial substances by Bifidobacteria that suppress many bacterial strains (Casaburi et al., 2016). Khalil and Mansour (1998) observed that the addition of encapsulated Bifidobacteria to mayonnaise resulted in improved sensory properties.

TABLE 3. Biogenic amine contents (mg/kg) of peanut butter containing probiotic bacteria during storage at 37° C (mean ±Standard Deviation).

Amine	Treatments at pH 6.8				
	Storage time (wk)	A	B	C	D
Tryptamine					
	3	1.92 ±0.005	0.89 ±0.014	0.92 ±0.118	0.53 ±0.201
	6	2.56 ±0.031	0.62 ±0.025	0.73 ±0.110	0.41 ±0.309
	9	4.09 ±0.052	0.71 ±0.007	0.79 ±0.105	0.38 ±0.053
	12	4.15 ±0.017	0.53 ±0.120	0.65 ±0.024	0.31 ±0.007
Putrescine					
	3	3.15 ±0.009	2.08 ±0.108	2.17 ±0.038	1.86 ±0.102
	6	5.1 ±0.135	1.8 ±0.117	1.99 ±0.152	1.43 ±0.105
	9	8.03 ±0.008	1.46 ±0.113	1.58 ±0.239	1.09 ±0.118
	12	10.51 ±0.008	1.29 ±0.051	1.32 ±0.310	0.65 ±0.191
Histamine					
	3	1.97 ±0.017	1.52 ±0.103	1.59 ±0.095	1.2 ±0.199
	6	3.79 ±0.026	1.08 ±0.108	1.19 ±0.035	0.75 ±0.231
	9	5.85 ±0.009	0.68 ±0.129	0.99 ±0.160	0.52 ±0.191
	12	8.1 ±0.152	0.57 ±0.116	0.72 ±0.137	0.32 ±0.182
Tyramine					
	3	52.36 ±0.172	40.36 ±0.114	40.91 ±0.171	29.13 ±0.019
	6	76.24 ±0.019	27.48 ±0.126	28.01 ±0.230	18.25 ±0.225
	9	96.67 ±0.035	23.13 ±0.135	23.83 ±0.158	12.73 ±0.102
	12	133.14 ±0.007	12.2 ±0.113	12.95 ±0.220	5.26 ±0.018
Cadaverine					
	3	13.1 ±0.008	10.35 ±0.157	10.92 ±0.105	5.17 ±0.515
	6	15.46 ±0.051	8.31 ±0.129	8.82 ±0.006	3.98 ±0.420
	9	19.31 ±0.093	6.92 ±0.138	7.08 ±0.058	2.96 ±0.316
	12	20.56 ±0.070	4.78 ±0.116	5.02 ±0.211	2.3 ±0.008

A= control without probiotic, B= Treatment containing *B. infantis*
C= Treatment containing *Lactobacillus plantarum*.
D= Treatment containing *B. infantis* + *L. plantarum*.
Biogenic amine content in control at zero time; Tryptamine= 0.89, Putrescine= 2.31, Histamine= 1.31, Tyramine= 43.11, Cadaverine= 11.31

TABLE 4. Biogenic amine contents (mg/kg) of peanut butter containing probiotic bacteria during storage at 25° C (Mean ± Standard Deviation).

Amine	Treatments at pH 6.8				
	Storage time (wk)	A	B	C	D
Tryptamine					
	3	2.23 ±0.042	0.83 ±0.118	0.91 ±0.011	0.31 ±0.081
	6	2.86 ±0.051	0.69 ±0.210	0.7 ±0.201	0.29 ±0.132
	9	3.13 ±0.009	0.65 ±0.018	0.68 ±0.330	0.2 ±0.109
	12	5.32 ±0.116	0.42 ±0.117	0.45 ±0.152	0.13 ±0.116
Putrescine					
	3	2.96 ±0.061	1.92 ±0.114	2.05 ±0.119	0.92 ±0.231
	6	4.91 ±0.115	1.5 ±0.160	1.77 ±0.351	0.64 ±0.150
	9	8.07 ±0.321	1.01 ±0.331	1.35 ±0.240	0.58 ±0.119
	12	10.1 ±0.251	0.89 ±0.280	1.06 ±0.192	0.37 ±0.107
Histamine					
	3	1.63 ±0.037	0.83 ±0.092	1.06 ±0.117	0.76 ±0.009
	6	3.72 ±0.009	0.69 ±0.092	0.85 ±0.109	0.7 ±0.103
	9	6.02 ±0.118	0.48 ±0.035	0.55 ±0.008	0.28 ±0.008
	12	7.89 ±0.312	0.32 ±0.106	0.38 ±0.107	0.16 ±0.005
Tyramine					
	3	53.1 ±0.116	28.11 ±0.008	31.51 ±0.025	18.11 ±0.115
	6	81.09 ±0.093	20.13 ±0.109	24.72 ±0.132	12.3 ±0.123
	9	101.22 ±0.135	15.81 ±0.311	21.09 ±0.081	8.46 ±0.137
	12	141.5 ±0.256	8.07 ±0.076	10.22 ±0.331	2.09 ±0.141
Cadaverine					
	3	13.19 ±0.056	7.82 ±0.039	8.19 ±0.251	4.09 ±0.081
	6	18.09 ±0.108	5.13 ±0.27	5.64 ±0.371	3.21 ±0.035
	9	20.22 ±0.085	4.65 ±0.281	4.99 ±0.095	1.96 ±0.056
	12	21.5 ±0.139	2.39 ±0.381	2.92 ±0.083	0.82 ±0.046

A= control without probiotic, B= Treatment containing *B. infantis* C= Treatment containing *Lactobacillus plantarum*.
D= Treatment containing *B. infantis* + *L. plantarum*.
Biogenic amine content in control at zero time; Tryptamine= 0.89, Putrescine= 2.31, Histamine= 1.31, Tyramine= 43.11, Cadaverine= 11.31

TABLE 5. Biogenic amine contents (mg/kg) of peanut butter containing probiotic bacteria during storage at 4° C (Mean ±Standard Deviation).

Amine	Treatments at pH 6.8				
	Storage time (wk)	A	B	C	D
Tryptamine					
	3	1.21 ±0.1012	0.81 ±0.211	0.89 ±0.302	0.42 ±0.110
	6	1.82 ±0.2131	0.72 ±0.008	0.80 ±0.135	0.39 ±0.225
	9	2.08 ±0.332	0.56 ±0.019	0.62 ±0.023	0.29 ±0.138
	12	2.79 ±0.123	0.42 ±0.035	0.52 ±0.039	0.29 ±0.180
Putrescine					
	3	2.95 ±0.315	2.01 ±0.022	2.52 ±0.163	1.72 ±0.025
	6	4.53 ±0.377	1.53 ±0.181	1.98 ±0.117	1.23 ±0.135
	9	7.92 ±0.193	1.21 ±0.009	1.51 ±0.025	0.96 ±0.425
	12	10.08 ±0.421	1.08 ±0.119	1.30 ±0.100	0.42 ±0.196
Histamine					
	3	1.35 ±0.118	1.07 ±0.111	1.19 ±0.215	0.91 ±0.005
	6	3.02 ±0.315	0.86±0.213	0.97 ±0.098	0.71 ±0.010
	9	5.06 ±0.002	0.56 ±0.019	0.69 ±0.013	0.32 ±0.009
	12	7.10 ±0.110	0.38 ±0.012	0.49 ±0.172	0.18 ±0.004
Tyramine					
	3	50.19 ±0.1002	39.51 ±0.220	40.01 ±0.005	27.32 ±0.009
	6	75.02 ±0.1120	25.62 ±0.350	31.12 ±0.216	16.59 ±0.132
	9	96.11 ±0.1031	21.35 ±0.002	22.72 ±0.065	10.39 ±0.182
	12	132.16 ±0.0221	10.29 ±0.110	11.36 ±0.029	3.26 ±0.191
Cadaverine					
	3	13.52 ±0.008	9.63 ±0.421	9.82 ±0.119	4.19 ±0.009
	6	15.28 ±0.116	7.56 ±0.620	8.09 ±0.129	3.81 ±0.019
	9	19.15 ±0.350	6.12 ±0.006	6.92 ±0.038	2.54 ±0.250
	12	20.01 ±0.215	3.62 ±0.132	4.03 ±0.129	1.98 ±0.112

A= control without probiotic, B= Treatment containing *B. infantis* C= Treatment containing *Lactobacillus plantarum*.
D= Treatment containing *B. infantis* + *L. plantarum*.
Biogenic amine content in control at zero time; Tryptamine= 0.89, Putrescine= 2.31, Histamine= 1.31, Tyramine= 43.11, Cadaverine= 11.31

TABLE 6. Sensory properties of peanut butter containing probiotic bacteria during storage at different temperatures.

Sensory properties	Flavor			Color			Texture		
Treatments									
				Storage at 4° C					
	0 wk	**6 wk**	**12 wk**	**0 wk**	**6 wk**	**12 wk**	**0 wk**	**6 wk**	**12 wk**
A	45.8 a	38.4 b	35.4 d	22.2 a	19.8 c	14.8 b	22.4 a	19.8 c	14.6 c
B	45.4 a	45.0 a	39.2 b	22.4 a	21.4 a	17.8 a	22.2 ab	21.2 ab	16.8 b
C	45.2 a	43.6 a	37.0 c	22.2 a	20.4 b	15.6 b	21.2 b	20.6 bc	16.6 bc
D	45.6 a	45.6 a	41.4 a	22.2 a	21.8 a	19.2 a	23.0 a	22.2 a	19.4 a
				Storage at 25° C					
A	45.8 a	38.00 c	35.25 c	22.2 a	18.25 b	15.25 c	23.4 a	18.5 c	15.0 c
B	45.4 a	42.25 a	37.50 b	22.4 a	20.75 a	19.5 a	23.2 ab	21.5 ab	19.0 a
C	45.2 a	40.75 b	36.0 c	22.2 a	19.0 b	16.50 b	22.2 b	21.0 b	17.25 b
D	45.6 a	43.25 a	39.5 a	22.2 a	20.50 a	20.6 a	24.0 a	22.25 a	19.25 a
				Storage at 37° C					
A	45.8 a	35.25 c	34.75 b	22.2 a	16.0 b	14.75 b	23.4 a	17.25 c	14.50 c
B	45.4 a	38.75 a	36.75 a	22.4 a	19.0 a	18.25 a	23.2 ab	20.0 a	17.25 a
C	45.2 a	37.25 b	35.2 b	22.2 a	16.75 b	15.50 b	22.2 b	18.75 b	15.75 b
D	45.6 a	39.50 a	37.5 a	22.2 a	19.75 a	18.5 a	24.0 a	20.0 a	18.25 a

A= Control
B= Treatment containing *B. infants*
C= Treatment containing *L. plantrum*
D= Treatment containing *B. infantis* + *L. plantrum*
Mean with different letter in the same column indicate significant differences between treatments at the same temperature (p 0.05)

Conclusion

In conclusion, this study revealed that good quality peanut butter could be produced by incorporating probiotic bacteria. *B. infantis* had the most excellent validity, followed by *Lactobacillus plantarum*. Peanut butter is a suitable food matrix to give probiotics. Furthermore, probiotic bacteria could also reduce the formation of biogenic amine during storage in different conditions.

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