Journal of Nutrition and Food Security
Shahid Sadoughi University of Medical Sciences
School of Public Health
Department of Nutrition
Shahid Sadoughi University of Medical Sciences
School of Public Health

eISSN: 2476-7425 pISSN: 2476-7417 JNFS 2025; 10(1): 143-154 Website: jnfs.ssu.ac.ir

Potential of Lactiplantibacillus Plantarum 20174 for Vitamin B12 Production in Pineapple: An Enhanced Nutritional Quality and Storage Stability of Processed Jelly

Mayuri Rastogi; MSc[1], Bushra Shaida; PhD[2], Vandana Singh; PhD[1], Zoobiya Islam; MSc[1] & Saleem Siddiqui; PhD[*3]

[1] School of Allied Health Sciences, Sharda University, Greater Noida, U.P., India; [2] Department of Food Technology, Jamia Hamdard University, New Delhi, India; [3] Sharda School of Allied Health Science, Sharda University, Greater Noida, U.P., India.

ARTICLE INFO

ORIGINAL ARTICLE

Article history:
Received: 17 Jul 2023
Revised:17 Apr 2024
Accepted: 28 Apr 2024

Corresponding author:
saleem.siddiqui@sharda.ac.in
Sharda School of Allied Health Science, Sharda University, Greater Noida, U.P., India.

Postal code: 201310
Tel: +91 941 63397847

Keywords:
Vitamin B12;
Cyanocobalamin;
Lactiplantibacillus plantarum;
Microbial fermentation;
Pineapple fruit jelly.

ABSTRACT

Background: Vitamin B12 is the micronutrient, present naturally in animal products. The present study was conducted with the aim of developing a naturally fortified vitamin B12 rich fruit product, which can easily be consumed by vegan population. **Methods:** Pineapple (*Ananas comosus*) fruit pulp was fermented with *Lactiplantibacillus plantarum* 20174 to produce vitamin B12 rich jelly. The selected microbial strain grew well in pineapple (*Ananas comosus*) fruit pulp matrix. The physiochemical properties and nutritional content were analysed and compared for the jellies prepared from fermented (fermented pineapple jelly or FPJ) and spontaneous fermented (control pineapple jelly or CPJ) pineapple pulp. Both CPJ and FPJ were packed and stored for 30 days at low (4 °C) and ambient (30 °C) temperature conditions, and were subjected to analysis at 10 days intervals. **Results:** The Total soluble solids (TSS), pH, and reducing sugars were higher in CPJ than FPJ. The FPJ contained 8.17 µg vitamin B12/100 g, while it was not detected in CPJ. There was no microbial growth detected in FPJ throughout the storage period when stored at 4 ºC, rendering it safe for consumption up to 30 days at both the storage temperatures. The decrease in scores of various sensory attributes during storage was little and the developed FPJ was still in the "liked very much" category throughout the storage period. The vitamin B12 content of FPJ was stable during storage and sufficient enough to fulfil an individual's daily requirement. **Conclusion:** The developed fermented pineapple jelly can be recommended as an excellent vegan source of vitamin B12.

Introduction

Vitamin B12 is a water soluble vitamin and is an important nutrient. Its deficiency may lead to megaloblastic anaemia and some other neurological disorders (Yahn *et al.*, 2021). Vitamin B12 is present largely in animal food sources and dairy products (Azad *et al.*, 2020, Sobczyńska-Malefora *et al.*, 2021). However, these days due to increased popularity of vegan and vegetarian diets, there is a necessity to develop more plant-based food products providing the requirement of vitamin

This paper should be cited as: Rastogi M, Shaida B, Singh V, Islam Z, Siddiqui S. ***Potential of Lactiplantibacillus Plantarum 20174 for Vitamin B12 Production in Pineapple: An Enhanced Nutritional Quality and Storage Stability of Processed Jelly.*** *Journal of Nutrition and Food Security (JNFS)*, 2025; 10 (1): 143-154.

[DOI: 10.18502/jnfs.v10i1.17766]

B12 for vegans.

Fruits are excellent sources of vitamins and other nutrients but lack in vitamin B12. One of the most consumable fruit from tropical and subtropical regions is pineapple, which is widely known for its great aroma and flavour (Ali *et al.*, 2020). The fruit pulp is rich in antioxidant, polyphenols, phytochemicals, vitamins and minerals (Chaudhary *et al.*, 2019, Shamsudin *et al.*, 2020). The fruit is consumed as raw for its pulp or juices, or processed into various food products, jelly being one of the most popular ones.

Fermentation enhances organoleptic properties and nutritional value of several fruit products. *Lactobacillus* is generally recognized as a safe (GRAS) microorganism, widely used for fermentation and production of nutrient-rich food products (Ayivi *et al.*, 2020). *Lactobacillus* strains enhances the antioxidant and antimutagenic activities (Heydari *et al.*, 2023). Several species of lactobacillus have also been used for vitamin production in food matrixes (Prazdnova *et al.*, 2022, Song *et al.*, 2021). However, the production of vitamin B12-rich commercial fruit products is still the subject of on-going study. Thus, the present study was designed to formulate a vitamin B12 rich fermented pineapple pulp jelly using *Lactiplantibacillus plantarum* 20174. The product was further evaluated for the changes in its nutritional and sensory properties during storage.

Materials and methods

Preparation of pineapple fruit substrate

Fresh pineapple fruit was procured from local market, New Delhi, India**.** The fruit was washed under tap water (2 min. at 8 ^{o}C) and peeled with sharp knife. The pulp was removed and cut into small cubes. The fruit cubes were minced into thick paste using electric blender (Philips Mixer Grinder, model, HL7777/00) and strained through muslin cloth. The juice was removed and thick fruit pulp was stored in glass bottle at -18 ^{o}C for further use.

Two pineapple pulp recipes, control pineapple pulp (CPP) without inoculation for spontaneous fermentation, and fermented pineapple pulp (FPP) inoculated with *Lactiplantibacillus plantarum* 20174 were prepared as follows. 400 g pineapple pulp was added with 40% sucrose and 300 ml distilled water, which were kept in air-sealed glass container separately for both recipes (CPP and FPP). Both glass bottles were sterilized in water bath at 90 ^{o}C for 15 minutes with continuous stirring followed by rapid cooling at room temperature. This procedure was required to destroy microbes and prevent contamination until further use. Before inoculation, the pH was adjusted to 6.0 by adding 0.1 M NaOH solution in both recipes.

Microbial strains and culture preparation

The bacterium strain, *Lactiplantibacillus plantarum* strain 20174 was procured from MTCC, India. It was in lyophilized form and was reactivated in 1000 ml DeMan, Rogosa and Sharpe (MRS) broth. The MRS broth contained following components in 1000 ml distilled water: Proteose peptone 10 g; yeast extract 5 g; beef extract 10 g; dextrose 20 g; tween-80 1 ml; ammonium citrate 2g; sodium acetate 5g; magnesium sulphate 0.1 g; manganese sulphate 0.05 g; and dipotassium phosphate 2 g. The pH was adjusted to 6.0 before autoclaving at 121 ^{o}C, and the reactivated cells were again transferred to MRS agar plate for 24 hours to check the potency and viable cell count. A loopful of cells was inoculated in 100 ml MRS broth and incubated for 48 hours at 37 ^{o}C until it reached 9.0 log CFU/ml. The inoculum optical density (OD_{600}) was measured at 1.0 at wavelength 600 nm.

Preparation of fermented pineapple jelly

*Fermentation***:** The pasteurized pineapple pulp (FPP) was inoculated with 1.2 to 1.4 $\times 10^{9}$ cfu/g bacterial suspension in aseptic conditions and kept for fermentation in anaerobic conditions for 72 hours at 37 ^{o}C. The CPP was kept in anaerobic condition for spontaneous fermentation, for 72 hours at 37 ^{o}C. After 72 hours of fermentation, the anaerobic fermentation jars were opened and samples were kept at -18 ^{o}C for further treatment.

*Jelly preparation***:** Both pineapple pulp (CPP and FPP) were used for jelly preparation. The pineapple pulp was homogenised and 400 g pulp

[DOI: 10.18502/jnfs.v10i1.17766]

was poured into heavy base steel vessel and brought to a boil with continuous stirring. Food grade gelatine (10 g), citric acid (2 g), pineapple flavour, 12% glucose solution, and 180 g cane sugar were added in both recipes with continuous homogenization. The hot pulp was poured in silicon moulds and labelled separately as FPJ and Control Pineapple Jelly (CPJ). The moulds were kept at 4 ^{o}C for 2-3 hours for solidification. The solid jellies were removed from moulds and coated with starch powder to prevent sticking together. The jellies were individually wrapped in plastic air-proof coating and stored in air tight container separately at low (4 ^{o}C) and ambient (30 ^{o}C) temperature conditions for 30 days. The analysis of various parameters was carried out regularly at 10-day intervals.

Quality analysis

Determination of physiochemical properties: Physiochemical properties were estimated by used AOAC 2005 methods (Bogha *et al.*, 2020). The titrable acidity (942.15 method) is the measurement of total available hydrogen ions in solution. A 10 g of sample was mixed with 100 ml distilled water. 25 ml of diluted jelly fruit pulp was taken in volumetric flask, and 2-3 drops of phenolphthalein indicator was added. The Solution was titrated against 0.1 M NaOH till the constant pink colour appearance. The titrable acidity was expressed in percentage, and calculated with following formula (Pawar *et al.*, 2023).

$$Titrable\ acidity = (Titre\ value \times Normality\ of\ NaOH \times volume\ made \times equivalent\ weight \times 1000)/(Volume\ of\ extract \times weight\ of\ sample \times 100)$$

The pH of the cooked pulp was estimated using pH meter (Digital pH Meter HTLP-081). The pH meter was calibrated with acid and base buffers (Bogha *et al.*, 2020). Total soluble solids (%) were measured at room temperature using digital refractometer (Hanna Instruments Sucrose Refractometer, India) (Bogha *et al.*, 2020, Pawar *et al.*, 2023).

Determination of proximate and nutritional composition: Proximate composition including moisture, ash, crude fibre, protein, and total carbohydrates of the developed product were estimated as per AOAC 2005 Official Methods (Bogha *et al.*, 2020). "Fats were estimated by organic solvent extraction method (Sarungallo *et al.*, 2019). A 2.0 g sample was taken in an extraction thimble, and 200 ml petroleum ether was added while the mantle was heated. After 8 hours of continuous heating, the petroleum ether was evaporated. Then, the fat free sample was cooled and weighed. Finally, the total fats (%) were calculated using the formula:

$$\%\ Fat = \frac{Weight\ of\ dried\ sample}{Weight\ of\ the\ sample} \times 100$$

Total crude fibre was also determined by AOAC 2005 official method (Bogha *et al.*, 2020). Briefly, 3.0 g fat free sample was digested with 2.0 N sulphuric acid, followed by 2.0 N sodium hydroxide. The sample was washed thrice and dried at 105 ^{0}C for 7 hours followed by ashing in muffle furnace. The presence of crude fibre was estimated by using the following formula(Kuria *et al.*, 2021) :

$$Crude\ Fiber(\%) = (Initial\ weight\ of\ dried\ sample - final\ weight\ (ash) \times 100)/(Weight\ of\ the\ sample)$$

Determination of Vitamin B12: Vitamin B12 in prepared sample was estimated in cyanocobalamin form after extraction and purification as described by Xie *et al.* with few modifications (Xie *et al.*, 2019). 5 g crushed and pureed sample was mixed with 25 ml sodium hydroxide and acetic acid extraction buffer with 1%sodium cyanide solution. About 0.05 g α-Amylase was added and incubated for 15-20 minutes at 40 ^{o}C. Add 20 ml sodium acetate solution and sample was again incubated at water bath at 37 ^{o}C for 30 minutes. The sample was allowed to cool down at room temperature and centrifuged to 6.800 rpm for 10 min. Supernatant was collected and residue was again centrifuged with extraction buffer and centrifuged again.

[DOI: 10.18502/jnfs.v10i1.17766]

Total supernatant was collected at 25 ml, followed by filtration. After filtration, the extract was purified by passing through 0.22 µm syringe filter and sample was collected in amber sample vials. The filtrate was stored in dark for further HPLC analysis with C-18 column.

Microbiological analysis

The microbiological analysis was carried out on standard plate count and yeast and mold count as suggested in previous researches(Choo *et al.*, 2018, Lim *et al.*, 2022). The samples were serially diluted in sterile 0.1% peptone water before being plated onto microbiological media using spread plate technique. Triplications of each sample were made. Further, Plate Count Agar (PCA) technique was used to quantify the number of aerobic mesophilic bacteria in each sample, whereas Potato Dextrose Agar (PDA) supplemented was used to quantify the number of yeast and mold. The plates were incubated at 37 ^{0}C for 48 hours aerobically for bacterial growth, while at 25 ^{0}C they were incubated for 120 hours to grow yeast and mold.

Sensory evaluation

Both FPJ and CPJ were subjected to sensory evaluation by 30 semi trained panellists on 9-point hedonic scale (Nagar and Rastogi, 2022). The panellist included 18 females and 12 males between the ages of 20-58. The evaluation of jellies was based on colour, taste, texture, aroma, and overall acceptability. The 9-point hedonic scales, where 9=extremely liked, 8=liked very much, 6=liked slightly, 5='neither liked not disliked' and 1='extremely disliked' were used. Every 10 day during storage, the process was repeated with same panellists.

Data analysis

All the experiments were performed in triplicates and presented in mean±standard deviation. The results were statistically analysed by one-way ANOVA and Tukey's test using SPSS software (IBM version 22). P-value of 0.05 was taken as significant difference.

Results

Proximate and nutritional composition of jelly:

The proximate and nutritional composition of CPJ and FPJ, the two variants of jellies, was presented in **Table 1**. The moisture content of the jellies ranged from 23.45% to 23.74% and was not significantly different in the two variants. The fibre content in the jellies ranged from 2.92-3.14%, and it was slightly but significantly lower in FPJ than CPJ. The ash content ranged from 1.94% and 2.08% and it was significantly higher in FPJ than CPJ.

FPJ exhibited a lower concentration of crude fats (0.17%) as compared to CPJ (0.22%). The protein content in FPJ was significantly higher (6.78%) in comparison to CPJ (0.94%). The total carbohydrates were significantly lower in FPJ (67.23%) as compared to CPJ (73.45%).

Vitamin B12: Fruits and vegetables are poor source of vitamin B12. The fermented pineapple jelly contains high amount (8.17 µg/100 g) of vitamin B12 while the unfermented pineapple jelly did not show any detectable amount of vitamin B12 **(Table 1).** The changes in vitamin B12 content of FPJ stored at 4ºC and 30°C are presented in **Figure 1.** There was no significant change observed in vitamin B12 content during storage at 4 ^{o}C; however, at ambient temperature conditions of 30 ^{o}C, no change was observed up to 10 days and a slight decrease was observed at later storage periods.

Table 1. Nutritional composition of jellies.

Composition	Fermented pineapple jelly	Control pineapple jelly
Moisture (%)	23.74±0.24^a	23.45±0.54^a
Fibre (%)	2.92±0.18^a	3.14±0.21^b
Ash (%)	2.08±0.89^a	1.94±0.54^b
Crude fats (%)	0.17±0.45^a	0.22±0.91^b
Protein (%)	6.78±0.87^a	0.94±0.18^b
Total carbohydrates (%)	67.23±1.23^a	73.45±1.76^b
Vitamin B12 (µg/100 g)	8.17±0.45	Not detected

Values are presented in mean± standard deviation (n=3).Values with the same superscript within a row indicate a non-significant difference (P>0.05).

[DOI: 10.18502/jnfs.v10i1.17766]

Total soluble solids (TSS): TSS was slightly higher in CPJ than FPJ. The initial TSS of FPJ and CPJ were 45.25% and 46.20 %, respectively. The TSS in FPJ was not significantly affected throughout the storage period at both the storage temperatures; however, there was a slight but significant decrease observed in TSS after 20 days of storage at 30°C (**Table 2**). In CPJ, it was not significantly affected up to 20 and 10 days of storage at 4°C and 30°C, respectively. It then decreased significantly at later storage periods.

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Titratable acidity: The initial acidity of FPJ and CPJ were 0.72% and 0.69%, respectively (**Table 2).** The acidity in FPJ was not significantly affected up to 20 days of storage at 4 °C and 10 days at 30 °C, but then increased at both the storage temperatures at later storage periods. In CPJ, it was not significantly affected up to 20 days of storage at both the storage temperatures but then increased significantly at later storage periods. During 30 days of storage at 4 °C, the acidity of FPJ and CPJ increased from 0.72% to 0.78% and 0.69% to 0.72%, respectively. However, when stored at 30 °C, the acidity increased to 0.80% and 0.79% in FPJ and CPJ, respectively during this period.

*pH***:** FPJ and CPJ were found to have initial pH values of 2.89 and 3.19 (**Table 2**). During storage at low temperatures, the pH was reduced to 2.81 and 2.98 for FPJ and CPJ, respectively. Furthermore, when stored at 30 °C, final pH was observed to be 2.79 and 2.95 for FPJ and CPJ.

*Reducing sugars***:** As shown in **Table 2**, the initial content of reduced sugars was lower in FPJ (28.23%) than CPJ (33.34%). During storage period of 30 days at 4 °C, the reducing sugar content was increased to 29.76% and 33.89% for FPJ and CPJ. On the other hand, higher reduction in sugar was observed when stored at ambient temperature of 30 °C. It was 32.34% in FPJ and 35.12% in CPJ at 30 days of storage.

*Total plate and total yeast and mold count***:** The total plate count (TPC) and total yeast and mold count (TYMC) are presented in **Table 3.** The microbiological analysis of FPJ indicated no microbial count throughout the storage period when stored at 4 °C. On the other hand, CPJ showed the presence of bacteria, mold and yeasts by 30 days of storage at 4 °C.

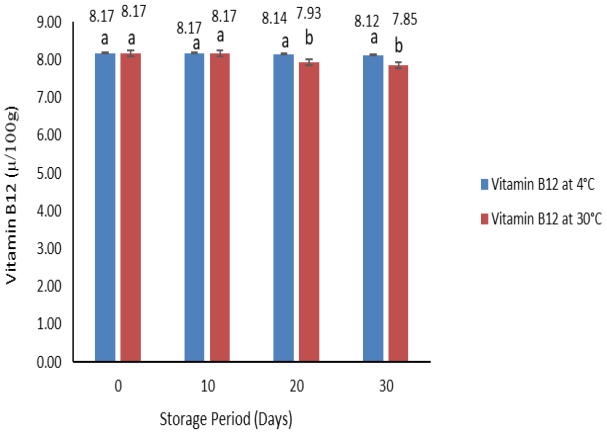

Figure 1. Changes in Vitamin B12 during storage; Data are presented in mean±standard deviation (n=3). Mean values with different superscripts on each bar indicate a significant difference (P<0.05).

Sensory attributes: The scores for various sensory attributes are presented in **Table 4**. It was observed that colour, taste, texture, aroma and overall acceptability scores of the freshly prepared jellies (0-day of storage) were above 8.0, thereby they were in the category of liked very much. The sensory scores were found slightly higher in FPJ than CPJ.

[DOI: 10.18502/jnfs.v10i1.17766]

Table 2. Changes in Total soluble solids, acidity, pH and total reducing sugars of pineapple jelly during storage at different temperatures.

Days of storage	Fermented pineapple jelly		Control pineapple jelly	
	Storage temperature			
	4 °C	30 °C	4 °C	30 °C
Total soluble solids (%)				
0	45.25±0.76^{a}	45.25±0.75^{a}	46.20±0.82^{a}	46.20±0.81^{a}
10	45.24±1.23^{a}	45.25±1.20^{a}	46.20±0.91^{a}	46.19±0.93^{a}
20	45.24±0.28^{a}	45.20±0.32^{a}	46.19±0.45^{a}	45.41±0.34^{b}
30	45.25±0.62^{a}	44.85±0.70^{b}	46.14±0.65^{b}	45.32±0.71^{b}
Acidity (%)				
0	0.72±0.16^{a}	0.72±0.15^{a}	0.69±0.34^{a}	0.69±0.28^{a}
10	0.72±0.05^{a}	0.74±0.23^{a}	0.69±0.14^{a}	0.70±0.18^{a}
20	0.74±0.18^{b}	0.78±0.19^{b}	0.70±0.35^{a}	0.71± 0.12^{a}
30	0.74±0.20^{b}	0.78±0.18^{b}	0.72±0.19^{b}	0.79±0.23^{b}
pH				
0	2.89±0.11^{a}	2.89±0.21^{a}	3.19±0.07^{a}	3.19±0.08^{a}
10	2.87±0.08^{a}	2.85±0.09^{a}	3.15±0.13^{a}	3.14±0.17^{a}
20	2.84±0.12^{b}	2.81±0.15^{ab}	3.03±0.33^{b}	2.99± 0.22^{b}
30	2.81±0.19^{b}	2.79±0.17^{b}	2.98±0.18^{b}	2.95±0.20^{b}
Reducing sugars (%)				
0	28.23±0.11^{a}	28.23±0.21^{a}	33.34±0.07^{a}	33.34±0.08^{a}
10	28.50±0.08^{a}	29.20±0.09^{b}	33.45±0.13^{a}	34.21±0.17^{b}
20	28.92±0.12^{a}	30.94±0.15^{c}	33.80±0.33^{b}	34.95± 0.22^{c}
30	29.76±0.19^{b}	32.34±0.17^{d}	33.89±0.18^{b}	35.12±0.20^{d}

Data are presented in mean ± standard deviation (n=3). The mean values with different superscripts in each column of a parameter differ significantly (P<0.05).

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Table 3. Microbial load (CFU/ml) of pineapple jelly during storage at different temperatures.

Days of storage	Microbial counts							
	Fermented pineapple jelly				Control pineapple jelly			
	Storage temperature							
	4°C		30 °C		4 °C		30 °C	
	TPC	TYMC	TPC	TYMC	TPC	TYMC	TPC	TYMC
0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
10	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	1.2×10^{2}	2.1×10^{2}
20	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	1.7×10^{2}	2.5×10^{2}
30	N.D.	N.D.	2.5×10^{2}	3.1×10^{2}	1.4×10^{2}	2.1×10^{2}	2.9×10^{2}	4.1×10^{2}

***CFU**: Colony Forming Units; **TPC**: Total Plate Count; **TYMC** : Total yeast and mold count; **N.D**: Not detected*

Since only the developed product FPJ contained vitamin B12, the sensory evaluation during storage was conducted only for FPJ. The changes in various sensory attributes of FPJ stored at 4 ºC and 30 °C are presented in **Figure 2**. The colour and appearance were reduced from 8.59 to 8.51 during 30 days storage at 4 °C and from 8.59 to 8.48 during storage at 30 °C. The taste of FPJ was decreased from 8.77 to 8.75 at 4°C, and 8.77 to 8.73 when stored at 30 °C for 30 days. The consistency score decreased from 8.9 to 8.85 at 4 °C while from 8.9 to 8.81 at 30 °C. Gradual decrease was observed for overall acceptability scores during storage at both the temperatures. Marginal changes have been observed in aroma during storage at both the temperatures. The average overall acceptability score after 30 days storage was 8.86 and 8.82 when stored at 4 ºC and 30 °C, respectively. In the present study, the decrease in scores of various

[DOI: 10.18502/jnfs.v10i1.17766]

sensory attributes was little and the developed FPJ was still in "liked very much category" after storage of 30 days at both low and ambient temperature conditions.

Discussion

Proximate composition: The results acquired from the experiment accomplished on pineapple fruit pulp showed the moisture content of the jellies ranged from 23.45% to 23.74%. There was insignificant variation in moisture content on vitamin B12 rich FPJ and CPJ. These findings are, however, inconsistent with the findings of (Ogodo *et al.*, 2017), where higher mositure in food matrix was observed after fermentation in maize flour. The moisture content of the jellies in present investigation was lower than the reported values by other researchers. The moisture content of jams was reported to be rang from 26.78% to 28.52% and in fruit marmalades it was 31.23% to 33.37% (Naeem *et al.*, 2017, Rana *et al.*, 2021).

Table 4. Sensory evaluation (9-point hedonic scale) of pineapple jellies at 0-day of storage.

Sensory attributes	Fermented pineapple jelly	Control pineapple jelly
Colour	8.59±0.32[a]	8.71±0.12[a]
Taste	8.77±0.27[a]	8.33±0.29[b]
Texture	8.90±0.17[a]	8.60±0.29[b]
Aroma	8.30±0.43[a]	8.00±0.12[b]
Overall acceptability	8.91±0.53[a]	8.28±0.31[b]

Values are presented in mean± standard deviation (n=3). Values with the same superscript within a row indicate a non-significant difference (P>0.05).

The amount of ash present in fruit matrixes indicated the presence of minerals. In the present study, the fermented jelly showed a significant increase (14%) in ash content compared to non-fermented jelly. The higher ash content in FPJ compared to CPJ could be due to higher reduction of certain chemical components such as carbohydrates, fibres, and fats during fermentation. (Septembre-Malaterre *et al.*, 2018) also reported similar increase in ash content of mango pulp during fermentation, and attributed it to decreased carbohydrate content.

Crude fibres are required in human body for healthy digestion, maintaining gut microbiota, and lowering the risk of heart disease and colon cancer. According to the Food Standard and Safety Authority of India (FSSAI), a product must have at least 3 g of fibre per 100 g product in order to be labelled a "good source of fibre." In the present study, it ranged from 2.92% to 3.14% and was thus conforming to the prescribed standards. (Tuolienuo and Galyuoni, 2022) also reported 3.00 % crude fibres in jam prepared from pineapple and pumpkin pulp blends. In the present investigation, the reduced amount of fibre in FPJ could be the result of metabolization, enzymatic breakdown, and utilisation of fibres by the microorganisms responsible for fermentation. Similar decrease in fibre content during fermentation was reported by other workers as well (Kuria *et al.*, 2021, Ogodo *et al.*, 2017, Ozabor *et al.*, 2020).

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[DOI: 10.18502/jnfs.v10i1.17766]

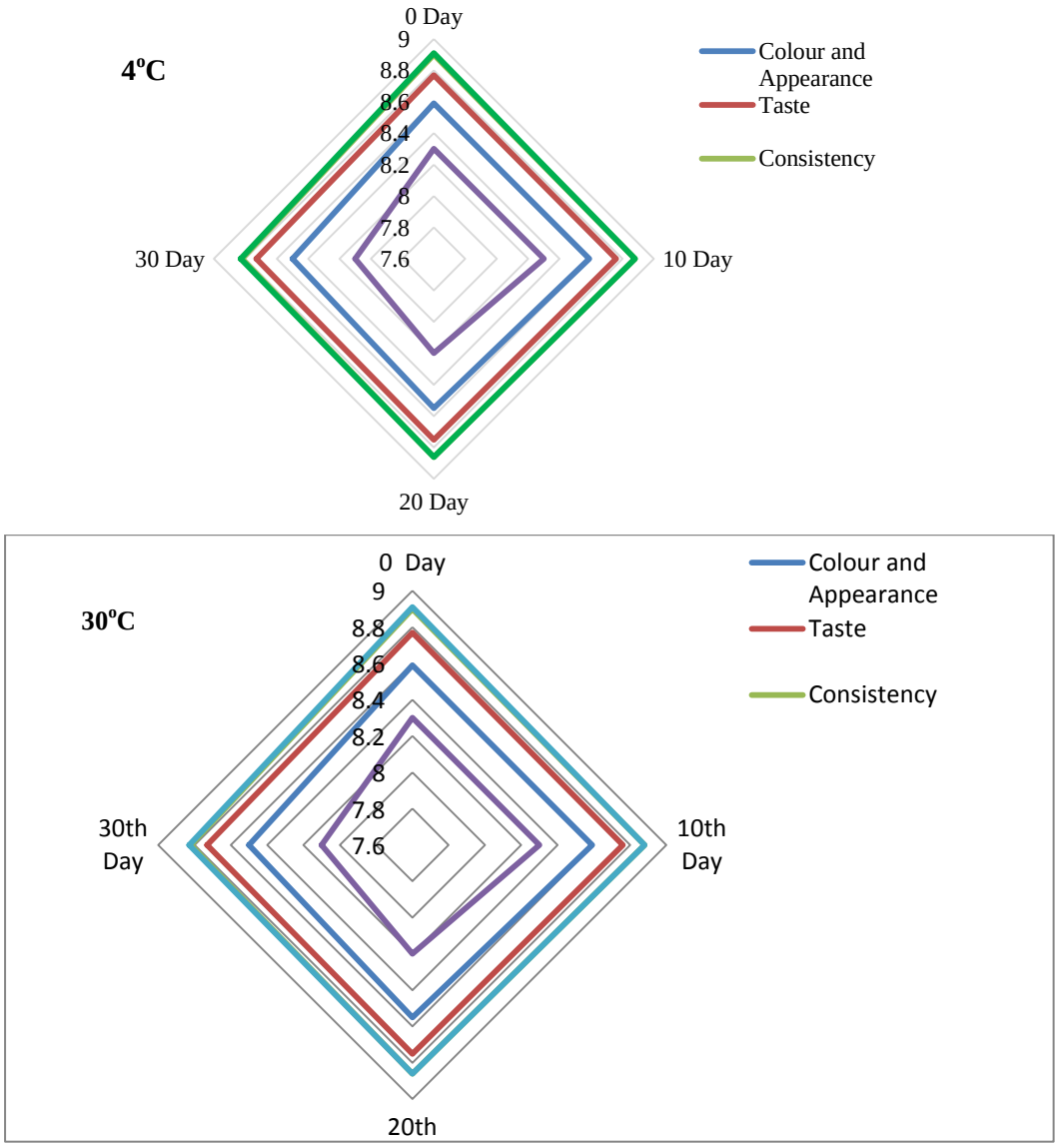

Figure 2. Sensory attributes of FPJ during storage at low (4°C) and ambient temperature (30°C) conditions.

In the present study, the crude fat percentage was observed to be lower in FPJ than CPJ. The reduction in fats during fermentation was also reported in soya milk and was attributed to its utilization for energy production by the microbes (Obadina *et al.*, 2013). Ogodo *et al.* also observed similar decrease in fat content due to fermentation in maize flour (Ogodo *et al.*, 2017).

The total carbohydrates in the present investigation were significantly lower in FPJ as compared to CPJ. However, the amount of carbohydrates, which was largely because of sugars were still sufficient enough to provide palatability and sweetness in FPJ. The decrease in carbohydrate content in fermented jellies could be due to its utilisation by microbes responsible for fermentation (Rehman *et al.*, 2020, Zhang *et al.*, 2023).

Fruits and vegetables are poor sources of proteins. Fermentation is considered one of the promising methods to enhance protein content in fruit products. Similarly, in the present study the protein content in FPJ was significantly higher than CPJ. (Afoakwah *et al.*, 2023) also reported that protein content was increased by 6.5 fold in potato-pineapple jam prepared from fermented

[DOI: 10.18502/jnfs.v10i1.17766]

pulp. Singhal *et al*. also observed that fermented bamboo shoots contained higher protein content (Singhal *et al.*, 2021). Kantachote *et al.* earlier reported an increase in amino acids content of coconut water when fermented with *Lactiplantibacillus plantarum (Kantachote et al., 2017).*

Vitamin B12 is heat stable and required for proper brain functioning and prevention of anaemia. Prokaryotes synthesizes vitamin B12 in food matrixes by two alternative pathways: aerobic and anaerobic (Azad *et al.*, 2020, Yahn *et al.*, 2021). In the present experiment, fermentation of pineapple pulp with *Lactiplantibacillus plantarum* 20174 led to synthesis of vitamin B12, and hence, FPJ contained its sufficient amount while it was lacking in CPJ. Accordingly, fermentation of coconut water with *Lactobacillus brevis*, *in-situ* fermentation of wheat, and bran mix was reported to enrich vitamin B12 content of these food products (Kantachote *et al.*, 2017, Xie *et al.*, 2019).

Changes in FPJ and CPJ during storage: During storage, total soluble solids in FPJ remained stable when stored at low temperature, while marginal reduction was observed when stored at ambient temperature of 30 ^{o}C. The marginal decrease in total soluble solids could be due to utilisation of soluble polysaccharides and metabolites by the microorganisms for their growth (Rehman *et al.*, 2020). The TSS of fermented dragon fruit juice was found to decreased from 28.70 to 25.1 when stored at ambient temperature (Choo *et al.*, 2018).

The titrable acidity was higher in FPJ than CPJ, which increased during storage. The increased acidity and thus reduced pH in FPJ pulp could be attributed to lactic acid fermentation of the pulp. Rana *et al.* also observed increased acidity and reduced pH of mixed fruit jam during storage (Rana *et al.*, 2021). Similar changes in pH and acidity were also reported in fermented blueberry jam (Chaudhary *et al.*, 2020) and in dragon fruit jelly (Panchal *et al.*, 2018).

The reducing sugar content increased in both FPJ and CPJ during storage, the increase being more at 30 ^{o}C than 4 ^{o}C. The increase in reducing sugar was caused by degradation of insoluble polysaccharides into soluble ones during storage and conversion of non-reducing sugars into reducing sugars under low pH conditions, the conversion being faster at higher temperature. Similar increase in reducing sugar was also reported in other fruits products (Aimi Azira *et al.*, 2021, Bekele *et al.*, 2020, Ikegaya *et al.*, 2020).

During storage, no significant changes were observed in vitamin B12 content in FPJ stored at 4 °C; however, the jellies stored at ambient temperature showed slight but significant decrease in vitamin B12 content during storage. Therefore, on the basis of the findings of the present investigation, it can be recommended to store the prepared FPJ at low temperature of 4 ^{o}C. However, storage at ambient temperature of 30 ^{o}C also retained sufficient amount of vitamin B12 in FPJ that could fulfil individual adult's daily requirement of 2.4 µg/day, as prescribed by (National Institute of Health, 2021). The present study thus indicated that vitamin B12 is a stable vitamin with respect to temperature of storage. Similar observations were made by other workers too (Czarnowska-Kujawska and Paszczyk, 2021, Sobczyńska-Malefora *et al.*, 2021, Zhang *et al.*, 2023). In the present study, the marginal loss of vitamin B12 during storage of FPJ at ambient temperature of 30 ^{o}C could be due to the influence of relative humidity and packaging material, as suggested by other workers (Czarnowska-Kujawska and Paszczyk, 2021, Hemery *et al.*, 2020, Zhang *et al.*, 2023).

The microbiological analysis of the FPJ revealed that there was no growth of bacteria, yeast, or molds till 30 days of storage at 4 °C, while the marginal microbial infestation was observed during later storage periods at 30 ^{o}C. The low microbial count in FPJ can be attributed to organic acids produced during fermentation and thereby its low pH, and proper pasteurization of pulp. Since the microbial counts in FPJ were lesser than the permitted limit of 1×10^{3} CFU/ml (Chaudhary *et al.*, 2020), the developed FPJ was microbiologically safe for consumption at both the storage

[DOI: 10.18502/jnfs.v10i1.17766]

temperatures and up to the study period of 30 days.

In the present study, the sensory scores were slightly higher in FPJ than CPJ**,** indicating that fermentation improved the overall acceptability of the product. The acceptability of FPJ was studied for 30 days of storage. A gradual decrease in all the sensory attributes was observed during storage at both 4 ^{0}C and 30 ^{0}C. The decrease in the overall acceptability of FPJ during storage could be attributed to increased acidity and various associated biochemical changes. Since these changes leading to decreased acceptability were slower at low temperature storage of 4 ^{o}C, the overall acceptability scores were higher for FPJ stored at low temperature compared to ambient temperature conditions. Similar results had earlier been reported for the sensory scores during storage at different temperatures for coconut water (Kantachote *et al.*, 2017), strawberry jam (Ikegaya *et al.*, 2020), pineapple coconut jam (Chaudhary *et al.*, 2019) and fermented sweet lemon juice s (Hashemi *et al.*, 2017).

Fermentation in fruit pulp, therefore, has a great potential for producing value-added fruit products with enhanced vitamin B12. Fermentation can also be one of the most effective tools for sustainable use of these otherwise perishable fruits. Other than *Lactobacillus,* microbes like *Propionibacterium or Pseudomonas sp.* can also be employed in the future for fermentation, the different strains of which may show better efficiency in other fruits to produce vitamin B12-enriched fruit products.

Conclusion

The experiment's findings showed that *Lactiplantibacillus plantarum* 20174 fermented pineapple fruit pulp could be a good source of vitamin B12. Furthermore FPJ, when individually wrapped in a plastic air-proof coating and stored in an airtight container, showed excellent (liked very much in the category of hedonic scale) taste, aroma, overall acceptability, and nutritive value up to 30 days of storage, both at low (4 ^{o}C) and ambient (30 ^{o}C) temperature conditions. The FPJ served as an excellent source of vitamin B12 that could fulfill an individual adult's daily requirement of 2.4 µg/day. Additionally, the microbiological investigation for bacteria, fungi, or yeast showed that the developed FPJ was microbiologically safe for consumption throughout the storage period. The developed fermented pineapple jelly, therefore, can be recommended as an excellent vegan source of vitamin B12. Further investigations need to be carried out with respect to its diversification and packaging.

Funding

Financial support was provided by Sharda University under the Seed Fund scheme (SU/SF/2022/14).

Conflict of interest

The authors declared no conflict of interests.

Authors' contributions

Rastogi M and Singh V contributed to design and implementation of research, Islam Z, and Shaida B and Siddiqui S contributed to planning, result analysis, and manuscript writing.

Acknowledgement

The authors would like to thank the staff at Sharda University for support and facilities they provided.

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[DOI: 10.18502/jnfs.v10i1.17766]