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Evaluation of sensory, biochemical, and microbial quality of fermented shrimp paste product during long-term cold storage

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Abstract

Shrimp paste (Ngapi) is the oldest method of preservation and is used as a condiment in culinary in almost all Asian countries. It has specific taste and flavor properties developed due to aging or fermentation. Therefore, the primary goal of the current study was to investigate the shelf-life of shrimp paste produced in the laboratory. The sensory characteristics, biochemical, and microbiological aspects of low salt fermented shrimp paste products were conducted at various storage periods (0, 10, 20, 30, 50, 70, and 90 days). Ngapi samples were kept at chilled storage of -2.2±0.5°C during the experimental period. Sensory attributes were determined by expert panel members, and nutritional, biochemical, and microbial quality were determined by the standard validated methods. Sensory investigation revealed that the quality was adequate during the storage period. The protein, lipid, and moisture decreased significantly ($p<0.05$), although ash and total volatile base-nitrogen increased during the experimental period. Throughout the investigation, the microbial quality remained within permissible limits. Finally, the findings of the study demonstrated that the nutritional, biochemical, and microbiological quality of shrimp paste (Ngapi) products could be preserved for a longer storage duration, providing a safer food item for consumers especially ethnic people in Bangladesh.

1. Introduction

Fermentation is a well-known and conventional food preservation or processing technique that increases the shelf life and enhances the nutritional properties of foods (Visessanguan *et al.*, 2004; Kim *et al*., 2014; Lv *et al.*, 2020). Shrimp paste is one of the most frequent preservation methods in South and Southeast Asian countries due to effective, simple technology and the availability of low-cost raw materials and equipment that are generally used in the shrimp fermentation process (Cai *et al.*, 2017). Nonetheless, shrimp paste is widely used in cuisine because of its umami flavor, delicious nutritional value, and appetite-stimulating aroma (Pongsetkul *et al.*, 2015). Shrimp paste is also known as "Xiajiang" in China, "Terasi" in Indonesia, "Saeu-jeot" in Korea, "Mamruoc" in Vietnam, "Kapi" in Thailand, "belacan" in Brunei and Malaysia, and "Ngapi" in Bangladesh and Myanmar (Kim *et al.*, 2014). Ngapi is a popular fermented fishery product for some ethnic groups of people of the Hill Tract region in Bangladesh (Chakma *et al.*, 2015) and is produced by converting shrimp into high-value-added products (Irianto and Giyatmi, 1997). Under ambient conditions, typical shrimp pastes are naturally fermented with 25-30% salt. Traditional fermentation of shrimp paste products is not fully emphasized in some essential points such as proper salt percentage, quality of raw shrimp, storage periods, and hygienic conditions. Owing to the addition of sand,

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soil, and polluted raw materials, as well as unhygienic conventional manufacturing, the taste and smell are often inaccurate. Furthermore, high salt content in food processed for longer periods can improve taste and flavor sensitivity while also raising blood pressure and increasing the risk of cardiovascular disease (Riis *et al.*, 2021). Fermented foods are not only nutritionally and dietary valuable, but they also can play a role in well-being (Peralta *et al.*, 2008). After fermentation, it develops a high proportion of nutritional composition and essential micro-macro elements (Chuon *et al.*, 2014; Faithong and Benjakul, 2014). Because of the prolonged fermentation, fermented shrimp pastes are high in free amino acids and peptides, as well as antioxidant activity (Peralta *et al.*, 2008). Ngapi, on the other hand, might be contaminated by rough handling, unhealthy and ineffective operation practices, bad travel, and a lack of enough storage time (Chakma *et al.*, 2015). Due to a lack of proper understanding, microorganisms are the most serious factor that contributes to product degradation (Chakma *et al.*, 2020). Lack of appropriate preservation encourages microbial attack as well as environmental oppression such as lipid oxidation and moisture gain, both of which have negative effects on nutritional and sensory quality (Howgate *et al.*, 1992).

Mysids and *Acetes* sp. are a by-catch fishery with a broad catch composition in Bangladesh's coastal area (Hossain *et al.*, 2020). Due to a lack of consideration for the finished product's quality and storage terms, some Ngapi manufacturers develop a very restricted amount of shrimp paste product without utilizing an appropriate amount of salt. However, adding salt in amounts greater than 20% of the total weight of food products can prevent pathogenic and putrefactive bacteria from growing without the use of other preservatives (Lee and Lee, 2014). In Bangladesh, however, preserving Ngapi or shrimp paste items with a little salt (2.5-5%) is a typical procedure (Hossain *et al.,* 2020). Keeping this in mind, the present study was performed to keep shrimp paste product (Ngapi) prepared using 5% salt in chilled storage conditions (-2.2±0.5°C) for a longer period of up to 90 days and to investigate if there are any variations in sensory, biochemical, and microbiological properties of Ngapi product over the 90 days storage period.

2. Materials and methods

2.1 Collection and sorting of shrimp samples

A total of 5 kg of wild marine species *Mysids* and *Acetes* sp. (mean length of 4.4±0.2 cm and mean weight 0.47±0.3 g) were obtained from Alipur Fish Landing Center, Patuakhali, Bangladesh from August to October 2019. The collected samples were packed in an icebox with the required amount of ice (ice: shrimp = 2:1) and transported to the Seafood Processing, Quality, and Safety Laboratory at the Patuakhali Science and Technology University, Bangladesh. The shrimp samples were kept in the refrigerator (SJ-VX79E-SL, Sharp, Japan) at -2.2°C for two days before starting Ngapi product was made.

2.2 Preparation of fermented shrimp paste (Ngapi)

Mysid and *Acetes* species were properly cleaned with tap water. The samples were dried by using a drying tray made of the iron-meshed wooden frame for three days at 25-31°C. After that, the partially dried (25-35% moisture) sample was transferred to a hot air oven (105° C) and kept for up to 6 hrs in laboratory conditions. Then shrimp powder was made by grinder machine (HR-12, Meat Grinder, WHA, co. Ltd., China) and 5% salt, following Alam (2007) and the required amount of water and then adequately mixed to prepare the Ngapi product (Figure 1). Afterwards, the initial Ngapi was packed into an airtight container and wrapped in polythene sheet for 10 days aging at ambient conditions. Furthermore, Ngapi was packed in airtight zip polythene bags and kept for up to the experimental periods of 90 days at chilled storage conditions (-2.2±0.5°C).

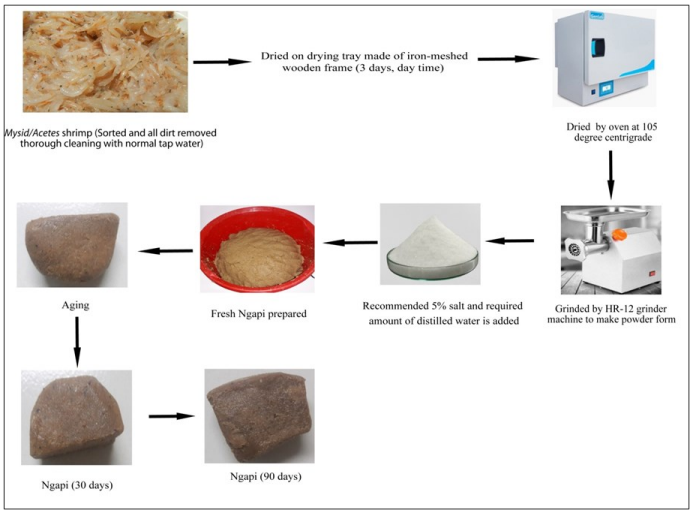

Figure 1. The schematic diagram for the preparation and storage of Ngapi fermented product.

2.3 Sensory properties of Ngapi

Ngapi samples were reviewed by a trained panel of 20 male and female panelists (1:1) who had ingested this paste regularly. The sensory quality evaluation method was used based on the combination of Fish Inspection and Quality Control (FIQC), Department of Fisheries (DoF), Ministry of Fisheries and Livestock, Government of Bangladesh, and Howgate *et al.* (1992) with slight modifications. Sensory attributes of fresh (0 days), 10 days (Aging), 30, 50, 70, and 90 days storage of fermented shrimp paste were assessed based on defect characteristics (Table 1). The defect scores of the Ngapi products were calculated by grading as presented in

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Table 2.

Table 1. Sensory assessment of Ngapi based on defect characteristics.

Characteristics of Ngapi	Defect Characters	Defect Points
Color	Natural color and shiny	1
	Brownish color	2
	Slightly to brownish shining	3
	Moderately brownish shining	4
	Blackish inner and dark brownish	5
Odor	Natural odor	1
	Slight off sour	2
	Slightly to moderate fishy	3
	Faint sour odor	4
	Extremely sour and become	5
Texture	Firm and flexible	1
	Slightly moderate soft	2
	Soft and slightly juicy	3
	Very soft or rigid surface	5
Flavor	Natural and slightly salty	1
	Moderate aroma flavor	3
	Very strong and spoiled	5
General Appearance	Excellent	1
	Good	2
	Slightly to moderate good	3
	Bad	4
	Very bad	5
Overall Acceptability	Highly acceptable	1
	Slight to moderate acceptable	2
	Slightly unacceptable	3
	Very unacceptable	4
	Rejected	5
Total Defect Point Gain		
Grand Average Defect Point		

Table 2. Grading of Ngapi fermented products.

Grade	Defect score	Level of acceptance
A	<2	Excellent and highly acceptable
B	2 -<3	Good and acceptable
C	>3 - <5	Poor/bad and slightly unacceptable
D	>5	Rejected

Defect points were estimated by the following equation:

$$\text{Grand defect mean value/Grade} = \frac{\text{Total Point Gain}}{\text{No. of characteristics}}$$

2.4 Determination of proximate compositions

After the preparation of Ngapi, the following procedures were analyzed following the standard methodology of AOAC (2005). According to the method, moisture content was determined using a hot air oven (HAS/50/TDIG/SS, Genlab, UK) at 105°C until a constant weight (g) was obtained. Ash content was determined by muffle furnace (HM-9MP, Raypa, Spain) at 550°C for 6 hrs. Crude protein content was analyzed by using the Kjeldahl apparatus (Bloc Digest 12, JP Selecta, Spain) where a 6.25 conversion factor was used to convert total nitrogen to crude protein. The fat content was measured by using the Soxhlet apparatus (J-SH3, JISICO, Korea). For all the parameters triplicate (n=3) samples were used. The energy value of all samples was calculated by Crisan and Sands (1978):

Energy value (kcal/100 g) = (2.62 × % Protein) + (8.37 × % fat) + (4.2 × % Carbohydrate)

2.5 Analysis of total volatile base-nitrogen and pH value

The values of TVB-N (mg N/100 g) of the Ngapi samples were estimated by the methodology of (Antonacopoulos and Vyncke, 1989). In this method, the digested sample was distilled under a semi-automatic distilling unit and then titrated with 0.01 N H_2SO_4 solution mixed indicator for ammo-nia titrations. About 10 mL of distilled water was added to 10 g of Ngapi samples and homogenized with a homogenizer (Sonic Ruptor 400, OMIN, UK) for about 3 min at the speed of 8000 rpm. Samples were prepared in triplicate, and pH values were measured by inserting the pH meter electrode (HI5522-01 Benchtop, Romania).

2.6 Microbiological analysis of Ngapi

In the case of microbial load analysis, 5 g of Ngapi samples were taken aseptically into a polyethylene bag and homogenized in 45 mL with 0.9% NaCl solution. The test tubes of homogenate samples were made up to 6 dilutions with triplicates and total plate count (TPC), total *Salmonella* count (TSC), total *Escherichia coli* count (TEC), and total *Vibrio* count (TViC) by using the pour plate technique where different selective media were used as the medium and Anambra 100 mm as a model plate (Sousa *et al.*, 2020). The samples containing serial plates were kept in an incubator (ThermaCell-CO2 80, QLS, UK) at 32°C for 24-72 hrs based on Nutrient Agar media for APC, EMB Agar media for TEC, XLD Agar media for TSC and TCBS Agar media for TViC. The bacterial colonies were developed on the plates and were counted by using a colony counter and counted as 30 to 300 colonies were selected for TPC, TSC, TEC, TViC and recorded as CFU/g were converted as log CFU/g. The total plate counts were evaluated below the following equation:

$$\text{TPC} = \frac{\text{Plate count} \times \text{dilution factor} \times 10 \times \text{volume of sample (mL)}}{\text{Weight of used sample (g)}}$$

2.7 Statistical analysis

Statistical Package for Social Science (SPSS) software version 23.0 (SPSS Inc., Chicago, Illinois, USA) was used to conduct all analyses. The differences

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between means were examined using the Least Significant Difference method (LSD, ANOVA), and all data were reported as mean ± standard deviation (SD). A Games-Howell nonparametric post hoc analytic approach was also used for performing multiple comparisons. Values with a significance level of $p<0.05$ were considered substantially different.

3. Results and discussion

3.1 Sensory properties of shrimp paste (Ngapi)

The sensory properties of fermented shrimp paste (Ngapi) are presented in Table 3. The color of the fermented shrimp paste showed fresh brownish at "day 0" of storage but the color turned slightly blackish in storage time of 90 days. The odor of the Ngapi changed from a natural odor to a moderate natural odor during the study period. The texture of the samples was soft and flexible in the initial time but became moderately rigid in the surface area in 90 days of storage time. The flavor of the shrimp paste product also changed from natural and slightly salty to a natural moderate aroma flavor. The general appearance of Ngapi samples was good and accepted for human consumption up to the experimental period of 90 days. The overall sensory quality of fresh Ngapi (0 days) was highly satisfactory with a total grade point of 1.82 and considered as "A" grade quality. The panelists remarked fresh Ngapi was excellent and highly acceptable for the consumers. On the other hand, the overall sensory quality of Ngapi an aging condition (10 days) was good and acceptable with a total grade of point 2, which is considered "B" grade quality. Finally, the sensory quality of 90 days of storage Ngapi was also good and acceptable, which is also considered as "B" grade quality by the expert panelists. The findings of the study coincide with Pongsetkul *et al.* (2015), who conducted on the sensory quality of Kapi production in Thailand. The finding of Peralta *et al.* (2008) also found that the brown coloring shrimp paste is a sign of MRP (Maillard reaction products) structure and the value of MRP highly increased during the increase of fermentation time. The differences in sensory attributes among samples can be regulated by the raw materials properties, compositions, processing techniques, and conditions (Beriain *et al.*, 2000).

Like Ngapi, belacan is a similar paste in Malaysia which is a thick paste with a greyish pink to greyish purple color. Belacan has a strong, pungent shrimp taste with a pleasant flavor, and umami is produced after several weeks of fermentation (Adnan, 1984). Like that, the present study also takes approximately the same storage periods for Ngapi fermentation to make its entire sensory attribute more perfect and acceptable. Yet belacan has a strong umami taste which is probably also the same cause of giving pleasant umami flavor in this present study like belacan products. The color of the low salt fermented shrimp paste changed from red and yellow to a dull red color, which could be due to the changes in the content of Maillard reaction products (MRP) and astaxanthin (Cai *et al.*, 2015). The grade point was slightly reduced due to the denaturation of protein which softens the product and oxidation may result in yellowish color but overall, it was good to appear and showed good natural flavor. The variation in sensory attributes might be caused by different pigment contents in shrimp spp., processing technique, as well as ingredients, added. The carbonyl groups of aldehydes and ketone can react with amino groups of free amino acids by peptides enzyme during hydrolysis which results in yellow or brown color (Yarnpakdee *et al.*, 2014). That should be the possible cause of color change in experimental storage Ngapi products. Though it reduced some of its quality attributes but still usable and acceptable while stored for a long

Table 3. Organoleptic characteristics of fresh, aging, and storage of Ngapi.

Sensory attributes	Periods (Days)					
	0 day	10 days	30 days	50 days	70 days	90 days
Color	Fresh brownish color	Brownish color	Brownish color	Moderate brownish	Brownish to blackish	Slightly blackish
Odor	Natural odor	Natural odor	Natural odor	Moderate natural odor	Moderate natural odor	Moderate natural odor
Texture	Soft and flexible	Soft	Soft	Moderate soft	Moderate soft	Moderate rigid
Flavor	Natural and slightly salty	Natural flavor	Natural flavor	Natural flavor	Natural moderate flavor	Natural moderate flavor
General appearance	Excellent	Excellent	Excellent	Good	Good	Good
Overall acceptability	Highly acceptable	Slightly acceptable	Moderate acceptable	Acceptable	Acceptable	Acceptable
Overall grade point	1.82	2.20	2.00	2.30	2.50	2.50
Overall grade	A	B	B	B	B	B
Grade characteristics	Excellent acceptable	Good and acceptable	Good and acceptable	Good and acceptable	Good and acceptable	Good and acceptable

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time in storage condition.

3.2 Nutritional value of Ngapi

The nutritional values of Ngapi are shown in Table 4. At the beginning of storage time (0 days) the protein content was 42.80% and decreased gradually up to 90 days of the experimental period (35.17%). There was a significant difference ($p<0.05$) in nutritional composition and energy value during the storage periods. The lipid content (2.21% to 1.03%) of Ngapi was found to be decreased significantly ($p<0.05$) throughout the 90 days of storage periods. However, ash content encountered to be increased gradually from 19.45% to 25.73% followed by moisture content from 35.17% to 38.07%. The energy value of Ngapi was the highest (132.19 Kcal/100 g) in 0 days but decreased to 103.46 Kcal/100 g in the storage period of 90 days.

In the present study, protein content varied significantly ($p<0.05$) from fresh to 90 days of storage condition, and it ranged from 42.80 to 35.17% whereas Clucas and Ward, (1996) stated that protein content was found to be ranged from 30 to 40% in Ngapi, fermented shrimp product. Further, in the storage time, its protein content lowered to 35.17%, this reduction is mainly due to the denaturation character of protein and peptides. Daroonpunt *et al.*, (2016) showed that the protein content of traditional Thai shrimp paste (kapi) is ranged from 17.9-42.8%, which is in agreement with the present study. Similar protein values were revealed by Majumdar *et al.* (2015) but were lower than the present study conducted by Chuon *et al.* (2014) and Faithong and Benjakul (2014). In the case of lipid content, it was decreased from the fresh condition to aging and storage condition and is mainly by lipid oxidation or its degradation. Chuon *et al.* (2014) also displayed that the lipid molecule also decreased which was similar to the present study. The ash content increased significantly ($p<0.05$) from 19.45% to 21% during 90 days of the storage period. This probably seems due to the moisture variation occurrence in the product. The energy value which was calculated from protein, lipid, and carbohydrate composition was found approximately a good amount initially but gradually decreased a little further in aging and chilled conditions. The main causes are denaturation of protein, further lipid reduction, and very low carbohydrate arising with storage time. Apart from this, the fresh condition of Ngapi showed very good energy value that would be helpful for consumers. The moisture content increased from 35.17 to 38.33% due to the absorption of moisture from the surrounding environment and declined to 36.67% after proper vacuum storage in ambient conditions.

3.3 Total volatile base-nitrogen value of Ngapi

The TVB-N value of Ngapi followed a substantially ($p<0.05$) upward trend throughout the storage periods from 16.73 at 0 days to 39.43 mg N/100 g at 90 days (Figure 2). The TVB-N value in fish and fishery products is an important indicator of spoilage even longer storage in low temperatures might increase the value of TVB-N (Egan *et al.*, 1981). The present finding is acceptable for human consumption which is fairly consistent with the findings of Chakma *et al.* (2020). The changes in TVB-N and its residue compounds reflect the prolonged deterioration of proteins and other protein compounds. This can be achieved by the process of protease enzyme either in the presence of microbial action or enzymes produced by microorganisms during fermentation (Riebroy *et al.*, 2007). However, slight differences in TVB-N compound in fishes varied intra-inter species, fishing time, location age, and sex (Sadok *et al.*, 1996).

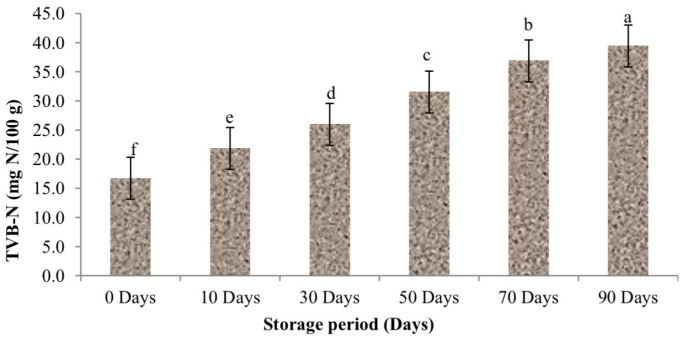

Figure 2. TVB-N changes in Ngapi have been preserved for up to 90 days. Bars with different notations are statistically significantly different ($p<0.05$). in different storage periods performed through Games-Howell nonparametric post hoc analysis approach.

Table 4. Proximate composition of fresh, aging, and stored Ngapi during 90 days storage condition.

Parameters	Percentage (%)					
	0 day	10 days	30 days	50 days	70 days	90 days
Protein (%)	42.80±0.54^{a}	38±0.13^{b}	37.47±0.29^{b}	37.19±0.09^{c}	37.09±0.03^{c}	35.17±0.09^{d}
Lipid (%)	2.21±0.04^{a}	2.09±0.07^{a}	1.54±0.11^{b}	1.29±0.04^{c}	1.17±0.04^{d}	1.03±0.05^{e}
Ash (%)	19.45±0.10^{e}	21±0.11^{d}	24.27±0.03^{c}	24.86±0.06^{b}	24.99±0.03^{b}	25.73±0.22^{a}
Moisture (%)	35.17±0.13^{c}	38.33±0.10^{a}	36.67±0.15^{b}	36.18±0.11^{b}	36.11±0.04^{b}	38.07±0.13^{a}
Energy Value (kcal/100 g)	130.66±0.68^{a}	117.11±0.27^{b}	111.06±0.36^{c}	108.24±0.18^{d}	106.87±0.21^{e}	100.75±0.21^{f}

Values are mean±SE of triplicate (n = 3) samples. Values with different superscripts are statistically significantly different ($p<0.05$).

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3.4 pH value of Ngapi

The pH value of the Ngapi product increased significantly ($p<0.05$) throughout the storage periods (Figure 3). The pH value increased from 7.42 to 8.17 during the entire experimental period, which is a good indicator of the freshness quality of the Ngapi product. Similar findings were observed by Clucas and Ward, (1996), where the pH value of Ngapi ranged from 7.6 to 7.8. Figure 3 shows that increasing pH value during chilled conditions may be correlated with the progress of volatile compounds and the activity of enzyme reaction can bestow to the changes of pH in low temperatures (Berg, 1964; Chakma *et al.*, 2020).

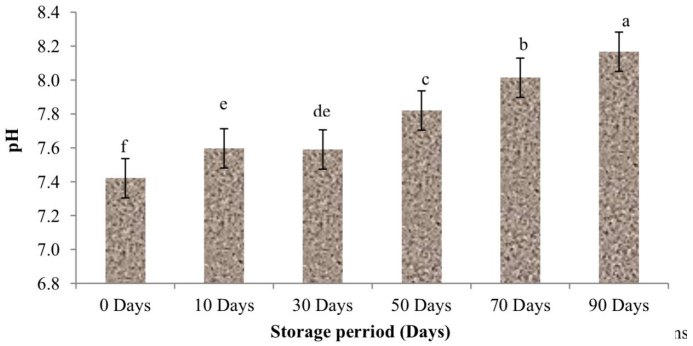

Figure 3. pH changes in Ngapi have been preserved for up to 90 days. Bars with different notations are statistically significantly different ($p<0.05$). in different storage periods performed through Games-Howell nonparametric post hoc analysis approach.

3.5 Microbiological quality of Ngapi

At the initial stage of Ngapi, the bacterial count was 1.23±0.03 log CFU/g for total plate counts (TPC) whereas at the end of the storage period, the TPC was 4.70±0.00 log CFU/g. The TSC ranged from 1.34±0.00 log CFU/g at 90 days to 3.68±0.00 log CFU/g at 10 days followed by TEC ranging from 1.71±0.00 log CFU/g at 70 days to 3.36±0.00 log CFU/g at 10 days during the experimental period and TViC from 2.28±0.01 log CFU/g at 30 days to 3.71±0.00 log CFU/g at 0 days of storage time (Figure 4).

The analysis of Ngapi products showed acceptable results in terms of microbial load. Daroonpunt *et al.* (2016) found that the total bacterial count in shrimp paste products ranged from 3.11 to 5.46 log CFU/g, which coincided with the experimental findings. The frozen temperature has a direct effect on microbial load and decreases from the initial storage to the longer storage period (Emire and Gebremariam, 2010; Majumdar *et al.*, 2015). In contrast, the bacterial count increased drastically with increasing storage periods (Obemeata, 2011; Gandotra, 2012). The quantity of the total *Salmonella* spp. count and coliform spp. declined gradually with the increase in storage period but the total *Vibrio* spp. counts fluctuated over the storage periods (Figure 4). However, the present bacterial counts did not exceed (<5 log CFU/g) the permissible limit (Roberts, 1998) except for TPC values. Most of the studies claimed that the lack of proper nutrition and storage conditions hinders microbial growth. In terms of Ngapi production, using salt in an appropriate amount might help to control microbial activity. Proper salting and reducing water activity by proper drying and high heat were applied before making Ngapi which helped in having a very lower bacterial load in fresh conditions, which was neglected.

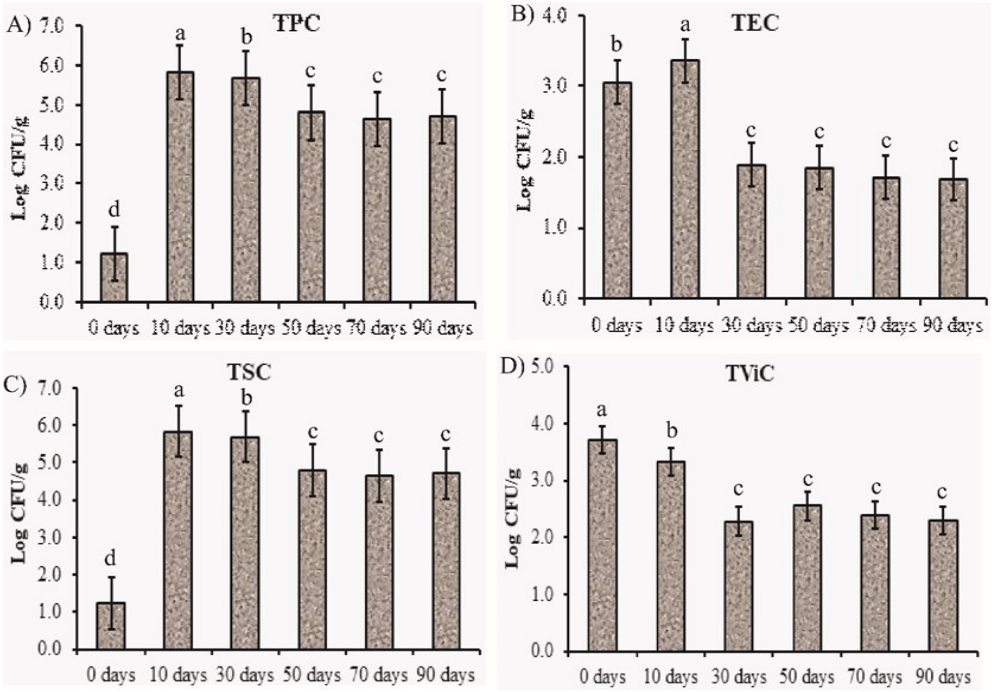

Figure 4. Microbial changes of Ngapi fermented paste products stored at -2.2±0.5°C. Bars with different notations are statistically significantly different ($p<0.05$).

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The bacterial load throughout the study was an acceptable range that can be applied for the reduction of protein and lipid content, which is to provide growth nutrients for proteolytic and lipolytic bacteria. At the same time, the lowering of moisture is responsible for the maintenance of water activity, which is hindering microbial growth. Furthermore, Ngapi contained a negligible quantity of carbohydrates which is another possible reason for the reduction of microbial growth during storage conditions, because insufficient substrates hinder bacterial growth (Faithong *et al.*, 2010; Pongsetkul *et al.*, 2014).

4. Conclusion

The present study shows that Ngapi remains within the acceptable limit throughout the long-term storage periods of 90 days in terms of sensory, nutritional, biochemical, and microbial quality. Since the substance contains a comparatively lower amount of salt it can be beneficial to human health by decreasing the high risk of cardiovascular disease. To summarize, the current study may serve as a subtle reminder to local processors, processing industries, fish traders, and policymakers to produce and familiarize Ngapi shrimp paste at the national and international levels.

Conflict of interest

The authors declare no conflict of interest.

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