



DEVELOPMENT OF PICKLE FROM MARINE MOLLUSKS AND ITS SHELF-LIFE ASSESSMENT DURING STORAGE

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Abstract: In the southwestern part of Bangladesh, mollusk such as oysters, blood clam, mussels and horn snails are used only for lime production, whereas they also have other potential purposes of use. The flesh of edible blood clam (*Anadara granosa*) and oyster (*Crassostrea madrasensis*) are not utilized by the people because of lack of awareness about the high nutritive value of the seafood products. Keeping this in mind, an attempt was made to utilize mollusk flesh as pickle. The shelf life of the pickled product developed from blood clam and oyster were assessed for a period of 6 months at ambient temperature (28-32 °C). Three types of oils, such as mustard oil, sesame oil and soybean oil were used for pickle preparation, where mustard oil was selected as the best pickle on the basis of biochemical, microbiological and sensory evaluation. Trimethylamine nitrogen, total volatile base nitrogen, total plate count and sensory scores of pickles with mustard oil were significantly increased ($P < 0.001$) at the end of storage period though all scores were at acceptable limit. The cost of preparing pickle was found very low. It may also contribute to our blue economy by marketing and exporting pickles to foreign countries. In future, the pickle prepared from unutilized mollusk meat may be used as food for tribal people in Bangladesh and also have economic value in the tourist area for foreign tourists.

Keywords: Pickle, blood clam (*Anadara granosa*), oyster (*Crassostrea madrasensis*), mustard oil, sesame oil and soybean oil.

Introduction

The Sundarbans, the southwestern coastal region of Bangladesh, has a unique brackish water ecosystem, which is suitable for a wider range of mollusk species. Mollusks are the second largest phylum of invertebrate animals with varieties of form, structure, habit and habitat (Kotpal, 2007). There are at least 200,000 known living species of mollusk throughout the world (Solaiman, 2006). Mollusks are capable of adapting to a wider range of habitats. Most mollusks are marine, some are found in terrestrial and freshwater habitats (Wye, 1989). About 36 mollusk species including 7 species of bivalves are available in estuarine areas and the mangrove floor of the Sundarbans Reserved Forests (Zaman *et al.*, 2011). Among them, blood clam, oyster, horn snail, small sea snail, murex snails are reported along the *Chuna* River in the southwestern part of Bangladesh (Biswas, 2017 and Rahman, 2018).

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With the variation from country to country, people consume approximately 14% of animal protein from marine fisheries. As marine mollusks are the excellent sources of protein, carbohydrate, lipid, vitamins and minerals, these are accepted as human food throughout the world (Marget *et al.*, 2013). The worldwide consumption of mollusk is increasing due to its health-promoting potentiality with a focus on their antiviral, anti-inflammatory and antimicrobial properties (Ahmed *et al.*, 2018). The meat of some edible mollusk is delicious and nutritious (Ramesh and Ayyakkanu, 1992). Consumption of mollusk may be greatly increased by making better use of the existing catch through developing different value-added products.

Various value-added products, such as pickle, sauce, soup, curry, boiled meat with sauces, etc. produced from mollusk meat have high demand in Australia, Japan, South Korea, India and United States of America (Hackney, 1990 and, Renitta and Patterson, 2012). Value-added fishery products mean mince or mince-based products, battered and breaded or coated products and surimi-based products. So, mollusk is popular not only in raw form, but also in processed form. All over the world, food technologists are focusing much attention on the development of products based on consumer acceptance (Marget *et al.*, 2013). However, in our country there is a very limited use of mollusks. Only a small number of people from Rakhain community and some other tribes of the coast traditionally have long been eating green mussel, clam and oyster meat (Solaiman *et al.*, 2006). In the southwestern part of Bangladesh, the local people use only the shell of mollusk for lime production, although the unused flesh has high nutritional value. Edible mollusks such as blood clam (*Anadara granosa*) and oyster (*Crassostrea madrasensis*) collected from the *Chuna* River along the Sundarbans contain 8-16% protein, 1-3.5% lipid, 1.22-4.8% ash and 72-82% moisture (Nayan, 2017).

Pickling of fish, shellfish and mollusk to enhance their keeping quality is an ancient practice and there are historical evidences to suggest that is followed by the ancient Indians, Egyptians and Chinese (Subhendu, 2013). Pickling is one of the safest means of easy preservation of fish/ shellfish. Pickle prepared from finfish and crustaceans in various styles are gaining acceptance in recent days. The technology is simple and can be adopted by the rural people/ fisher folk after short training. Nutritional value of marine mollusks was discussed by Lev *et al.* (2007), Padidela and Thummala (2015), Baby *et al.* (2010) and Marget *et al.* (2013). Biswas (2017) and Rahman (2018) reported on the seasonal variation in mollusk abundance and diversity in the Sundarbans Mangrove Forest. Mollusks marketing system in Bangladesh was investigated by Solaiman *et al.* (2006). However, there is little work on the utilization of mollusks meat as human food in Bangladesh. Considering the importance of mollusks health-promoting potentiality and nutritional value, various methods are evolved for the processing and preservation of mollusks meat to prepare value added products throughout the world and quality of these products during storage was evaluated (Gupta and Basu, 1985 and Jayachandran *et al.*, 1987). Renitta and Patterson (2012) reported on the quality and shelf-life assessment of underutilized marine gastropod pickle. Preparation of horse conch (*Pleuroploca trapezium*) meat pickle and its shelf-life studies were done by Chellaram (2015). Until now, limited work was done to prepare various value-added products from fish such as mola fish pickle (Shahabuddin *et al.*, 2010) and Thai pangus pickle (Santhiya *et al.*, 2013). Therefore, the present study is designed to prepare pickle from unused edible mollusks flesh.

Materials and methods

Collection and processing of raw material: Edible mollusk such as blood clam (*A. granosa*) and oyster (*C. madrasensis*) were collected from the intertidal zone of the *Chuna* River along the Sundarbans Mangrove Forest situated in Munshiganj, Shyamnagar and Satkhira.

Raw materials were washed in the laboratory with potable water and shucked. Intestine was removed and the meat was thoroughly washed with potable water to remove all the dirt, mucus and pigments on the foot muscle. The edible parts were cut into small pieces and washed repeatedly.

Preparation of pickle by blood clam and oyster using different oils: Different oils used for preparing of pickle are given in Table 1. Small pieces of meat were cooked in a pressure cooker for 30 minutes and blanched. The cooked meat was marinated with salt, chili powder and turmeric for 30 minutes at room temperature. Marinated meats were fried in oil (mustard oil/ sesame oil/soybean oil) till golden brown color appeared. Chopped onion, garlic and ginger paste were also fried in the remaining oil and then *masala* paste was made by mixing other ingredients except vinegar. Fried meat was added to the mixture and boiled for 5 minutes. Then, vinegar was added and wait until its absorption (Renitta and Patterson, 2012; Shikha *et al.*, 2018). Finally, the pickles were stored at 28-32 °C in jars for six months to evaluate their shelf-life. Each of the jars contained 300 g pickle. Out of 18 jars, 9 contained blood clam pickles and another 9 jars contained oyster pickles. Among 9 jars of blood clam pickles, 3 individual treatments were deviated equally as mustard oil, soybean oil and sesame oil, where each treatment had three replications. In the same way, other 9 jars contained oyster pickles. Then, the prepared pickles were brought under biochemical, microbiological and organoleptic evaluation to find out the best recipe of blood clam and oyster pickles. Initially the samples were evaluated at 15-day intervals for first three months and then 30-day intervals up to six months to minimize cost and time.

Table 1. Standard recipe for blood clam and oyster pickle

Ingredients name	Quantity	Ingredients name	Quantity
Cooked mollusk meat	500 g	Mustard seeds	20 g
Garlic	125 g	Pachforon	5 g
Ginger	100 g	Salt	30 g
Onion	300 g	Oil (Mustard oil/ sesame oil/soybean oil)	300 g
Chili powder	30 g	Vinegar	60 mL
Turmeric powder	5 g	Tamarind (Pulp)	30 mL
Coriander powder	50 g	Sugar	2 TS

Biochemical and microbiological analysis: The protein and lipid content of pickle, prepared from both the species were estimated according to the method of Association of Official Analytical Chemists (AOAC, 1990). Trimethylamine nitrogen (TMA-N) and total volatile base nitrogen (TVB-N) were determined according to Beatty and Gibbons (1937). Total bacterial count (TBC) was determined by the standard pour plate method using nutrient agar and incubating the plates at 27°C (Gupta and Basu, 1985; Jayachandran *et al.*, 1987 and, Renitta and Patterson, 2013). pH was measured using a digital pH meter after blending 10 g of sample with 100 mL of distilled water for 30s.

Sensory evaluation: The sensory evaluation (appearance, color, flavor, odor, taste, texture) of the pickles was carried out by serving pickles to a panel consisting of nine members. The organoleptic scores for the product were with the rating of 9 for extremely good, 8 for very good, 7 for good, 6 for very fair to good, 5 for very fair, 4 for fair, 3 for poor, 2 for very poor and 1 for extremely poor.

Economic analysis: The cost of blood clam and oyster pickle production was divided into fixed costs and operational costs. Equipment and labor cost were marked as fixed costs, where ingredient cost and utility charge are known as operational costs. The total cost of pickle preparation for 500 g of each pickle was calculated.

Statistical analysis: The statistical analysis was performed using R Studio version 3.6.0. At first the proximate data (percentage data) were transformed by ARCSIN formula and count data (organoleptic scores) were transformed by square root. Shapiro-Wilk test was performed for normality test. Then One-Way ANOVA and Tukey test were done to evaluate differences among the treatments.

Results

Biochemical and microbiological analyses: The proximate composition of pickle prepared from blood clam and oyster are presented in Table 2 and 3. The protein and lipid content of blood clam pickles using three different oils (mustard oil, sesame oil and soybean oil) were $29.91 \pm 0.01\%$, $22.23 \pm 0.04\%$, $26.43 \pm 0.06\%$ and $17.32 \pm 0.03\%$, $15.26 \pm 0.03\%$, $15.89 \pm 0.07\%$, respectively. Pickles prepared from oyster using mustard oil, sesame oil and soybean oil contained $11.52 \pm 0.04\%$, $10.23 \pm 0.03\%$, $11.12 \pm 0.02\%$ protein and $30.49 \pm 0.01\%$, $27.41 \pm 0.06\%$, $29.23 \pm 0.03\%$ lipid, respectively. Pickles prepared from blood clam showed higher protein content than oyster pickles ($P < 0.01$). Whereas, oyster pickle using different oils contained higher lipid content than blood clam pickles ($P < 0.01$). However, the lipid contents of both pickles using mustard oil were higher due to the presence of oils over the solid particles ($P < 0.01$).

Table 2. Proximate composition of raw meat of blood clam and oyster

Parameters	Raw meat	
	Blood clam	Oyster
Protein (%)	12.92 ± 0.22	14.86 ± 0.19
Lipid (%)	1.63 ± 0.37	1.64 ± 0.09

Table 3 indicates the differences of biochemical and microbiological quality among the prepared pickles. The one-way ANOVA showed significant differences ($P < 0.001$ and $P < 0.01$) among these three pickles. The biochemical and microbiological quality of oyster pickles prepared using three different oils were also significantly different ($P < 0.001$ and $P < 0.01$) at the initial day of storage (Table 4). Pickles prepared from mustard oil for both species were selected as the best.

Table 3. Proximate composition of pickle prepared from blood clam and oyster with three different oils

Parameters	Pickle					
	Clam pickle			Oyster pickle		
	Mustard oil	Sesame oil	Soybean oil	Mustard oil	Sesame oil	Soybean oil
Protein (%)	29.91 ± 0.01	22.23 ± 0.04	26.43 ± 0.06	11.52 ± 0.04	10.23 ± 0.03	11.12 ± 0.02
Lipid (%)	17.32 ± 0.03	15.26 ± 0.03	15.89 ± 0.07	30.49 ± 0.01	27.41 ± 0.06	29.23 ± 0.03

Mean \pm Standard deviation

Sensory evaluation and shelf-life assessment of pickles: The results of sensory evaluation of blood clam and oyster pickles using three different oils at initial day were showed in Table 4 and 5. The sensory evaluation was done by the panelists. The sensory scores of pickles using different oils were significantly different ($P < 0.001$ and $P < 0.01$). In case of both species, pickles prepared with mustard oil showed much better result than sesame oil and soybean oil. Considering proximate

composition, biochemical, microbiological and sensory evaluation, pickle prepared from blood clam and oyster using mustard oil was selected as the best and their shelf-life were analyzed for six months.

Table 4. Quality assessment of *A. granosa* pickle and *C. madrasensis* pickle with different oils at initial day

Parameters	Pickle							
	Clam pickle				Oyster pickle			
	Mustard oil	Sesame oil	Soybean oil	F	Mustard oil	Sesame oil	Soybean oil	F
TMA-N (mg/100 g)	2.49±0.02	2.74±0.02	2.91±0.04	152.6***	2.89±0.04	3.02±0.06	3.59±0.03	210.94***
TVB-N (mg/100 g)	4.97±0.04	5.11±0.06	4.93±0.03	13.697**	4.81±0.02	5.09±0.06	5.45±0.03	197.08***
pH	5.25±0.05	5.45±0.05	5.35±0.05	12**	5.45±0.05	5.65±0.05	5.6±0	16**
TPC (log cfu/g)	2.25±0.04	2.41±0.02	2.53±0.02	59.917***	2.38±0.04	2.49±0.04	2.55±0.02	16.147**

Mean ± standard deviation. *** $P < 0.001$ - Significant. ** $P < 0.01$ - Significant
 TMA-N = Triethylamine nitrogen; TVB-N = total volatile base nitrogen; TPC = total plate count; CFU = colony forming units.

Table 5. Sensory evaluation of *A. granosa* pickle and *C. madrasensis* pickle with different oils at initial day

Organoleptic characteristics	Pickle							
	Clam pickle				Oyster pickle			
	Mustard oil	Sesame oil	Soybean oil	F	Mustard oil	Sesame oil	Soybean oil	F
Appearance	9±0	8.1±0.8	8±0.3	233.01***	8.9±0.3	7.5±0.5	7.5±0.5	211.94***
Color	8.9±0.3	8±0.3	8±0.3	167.39***	9±0	8.2±0.4	8±0.3	187.9***
Odor	8.8±0.3	6.5±0.1	7.5±0.5	78.129***	7.8±0.1	7±0.3	7±0.3	74.01***
Taste	8.8±0.3	6.2±0.3	7.2±0.2	286.7***	7.5±0.5	6.7±0.2	6.5±0.1	149.7***
Texture	8.2±0.4	6±0.2	6.5±0.1	89.3***	7.9±0.7	6±0	6±0	238.7***
Flavor	8.8±0.3	7±0	7.2±0.2	58.917***	8.5±0.1	6.5±0.1	6.5±0.1	69.19***
Overall acceptability	8.8±0.3	6.9±0.9	7.4±0.6	153.67***	8.3±0.6	6.9±0.8	6.9±0.7	345.89***

Mean ± standard deviation. *** $P < 0.001$ - Significant.

Quality assessment: The changes in the pH during storage of both pickles are given in Table 6. The pH in the case of *A. granosa* and *C. madrasensis* meat pickles prepared using mustard oil were initially 5.25±0.07 and 5.45±0.05, respectively, and stabilized to 3.8±0.07 and 3.9±0.1 at the end of 180 days of storage period, respectively. The one-way ANOVA showed that there was a significant decreasing ($P < 0.001$) in pH with the increasing storage days of both the pickles.

TMA-N contents of both pickles were initially low such as 2.49±0.03 and 2.89±0.04 mg/100 g (Table7) and then gradually increased to 11.29±0.03 and 13.53±0.08 mg/100 g during the storage period. The statistical analyses showed that there was a significant difference ($P < 0.001$) among the days of storage in TMA-N contents of both pickles.

The TVB-N values were found to be significantly increased with storage period in both the pickles ($P < 0.001$). The TVB-N content ranged from 4.97 ± 0.05 to 22.44 ± 0.07 mg/100 g in *A. granosa* pickle and 4.81 ± 0.02 to 27.93 ± 0.03 mg/100 g in *C. madrasensis* pickle (Table 6) during storage period.

Table 6. Biochemical and microbiological quality of *A. granosa* pickle and *C. madrasensis* pickle using mustard oil

Parameters	TMA-N (mg/100 g)		TVB-N (mg/100 g)		pH		TPC (log cfu/g)	
	Clam pickle	Oyster pickle	Clam pickle	Oyster pickle	Clam pickle	Oyster pickle	Clam pickle	Oyster pickle
Initial	2.49±0.03	2.89± 0.04	4.97±0.05	4.81±0.02	5.25±0.07	5.45±0.05	2.25±0.04	2.38±0.04
15 th day	2.88±0.05	4.14± 0.06	5.19±0.01	6.16±.06	4.9±0.14	5.25±0.05	2.37±0.02	2.62±0.01
30 th day	3.49±0.09	4.46±0.09	5.84±0.04	7.39±0.06	4.65±0.07	4.95±0.05	2.59±0.04	2.72±0.08
45 th day	3.96±0.07	5.88± 0.04	6.63±0.07	8.19±0.05	4.45±0.07	4.7±0.1	2.83±0.03	2.91±0.03
60 th day	4.81±0.02	6.09±0.05	8.48±0.08	11.32±0.06	4.4±0.14	4.5±0.1	2.9±0.03	3.18±0.05
75 th day	5.08±0.04	7.34±0.06	10.52±0.06	11.5±0.01	4.15±0.07	4.35±0.05	3.19±0.06	3.33±0.02
90 th day	6.7±0.12	8.98±0.08	12.32±0.01	13.94±0.12	4.05±0.07	4.15±0.07	3.69±0.05	4.02±0.05
120 th day	8.21±0.04	10.85±0.04	15.27±0.05	16.87±0.03	4±0	4.05±0.07	3.9±0.08	4.22±0.07
150 th day	10.12±0.06	11.14±0.07	18.39±0.05	19.18±0.03	4±0	4±0	3.97±0.03	4.32±0.01
180 th day	11.29±0.03	13.53±0.08	22.44±0.07	27.93±0.03	3.8±0.07	3.9±0.1	4.2±0.05	4.4±0.04
F	2877.8***	5278.3***	9180.3***	12649***	114.69***	260.11***	416.88***	469.49***

Mean ± standard deviation. *** $P < 0.001$ - Significant; TMA-N = Trimethylamine nitrogen; TVB-N = total volatile base nitrogen; TPC = total plate count; CFU = colony forming units.

The microbial qualities of both pickles are shown in Table 6. The initial bacterial load (TPC) in *A. granosa* and *C. madrasensis* pickles was 2.25 ± 0.04 and 2.38 ± 0.04 log cfu/g, respectively. During storage period, the TPC increased steadily to 4.2 ± 0.05 and 4.4 ± 0.04 log cfu/g in *A. granosa* and *C. madrasensis* pickles, respectively. The bacterial load in *A. granosa* pickle was lower than *C. madrasensis* pickle.

Organoleptic characteristics evaluation: The organoleptic characteristics of both the pickles are given in Table 7. Blood clam pickle remained in acceptable condition (7.28 ± 0.5) while oyster pickle showed lower acceptability (6.08 ± 0.4) throughout the storage period of 180 days. The difference in appearance, color, odor, taste, texture, flavor and overall acceptability of *A. granosa* and *C. madrasensis* pickle were significantly changed during storage ($P < 0.01$). In appearance and color, the blood clam pickle was better than oyster pickle throughout the storage period. Moreover, the odor of blood clam pickle was remained well while the oyster pickle showed very fishy odor. However, taste of the oyster pickle was very pleasant at the end of storage. The texture of both pickled products became spongier at the end of storage. No rancid flavor was found during the storage period of both pickles.

Economic analysis: The economic analysis of pickle prepared from *A. granosa* and *C. madrasensis* shown in Table 8. The cost of ingredients needed for pickle preparation, labor cost, equipment cost (pan, spoon, pressure cooker) and utility charge were included in the economic analysis. Total Tk 205.7 was needed for 500 g pickle preparation.

Table 7. Changes in organoleptic quality of *A. granosa* pickle and *C. madrasensis* pickle

F	Organoleptic Characteristics										
	180 th day	150 th day	120 th day	90 th day	75 th day	60 th day	45 th day	30 th day	15 th day	Initial	Overall acceptability
25.05556**	8.2±0.1	8.5±0.2	8.5±0.2	8.9±0.3	8.9±0.3	9±0	9±0	9±0	9±0	9±0	Clam pickle
25.0778***	7.2±0.2	7.4±0.2	7.7±0.3	8.2±0.4	8.5±0.1	8.5±0.1	8.7±0.4	8.7±0.4	8.8±0.3	8.9±0.3	Oyster pickle
9.91667ND	8.4±0.1	7.6±0.1	8.7±0.2	9±0	9±0	9±0	9±0	9±0	9±0	8.9±0.3	Clam pickle
55.1164***	7.4±0.1	7.5±0.1	7.8±0.2	8.2±0.4	8.4±0.2	8.5±0.1	8.8±0.3	8.8±0.3	9±0	9±0	Oyster pickle
27.61905***	7.4±0.2	7.1±0.3	7.8±0.1	8±0.3	8.2±0.4	8.3±0.3	8.5±0.1	8.7±0.5	8.8±0.3	8.8±0.3	Clam pickle
61.4321***	4.9±0.2	5±0.1	5.2±0.1	5.5±0.6	5.8±0.3	6.1±0.3	6.5±0.1	6.8±0.3	7.2±0.3	7.8±0.1	Oyster pickle
25.3871**	7±0.2	8.3±0.1	7.3±0.1	7.5±0.5	7.9±0.7	8.4±0.6	8.7±0.4	8.8±0.3	8.9±0.2	8.8±0.3	Clam pickle
21.2344***	5±0.3	5.3±0.1	5.4±0.3	5.7±0.2	5.9±0.3	6.3±0.1	6.4±0.2	6.9±0.1	7.2±0.3	7.5±0.5	Oyster pickle
93.08***	6.1±0.3	6.3±0.2	6.6±0.2	6.9±0.6	7.2±0.3	7.5±0.2	7.8±0.1	8.3±0.3	8.5±0.1	8.2±0.4	Clam pickle
35.001**	5.8±0.3	6±0.2	6.2±0.3	6.6±0.3	7.2±0.3	7.5±0.5	7.8±0.1	8±0.3	8.0±0.3	7.9±0.7	Oyster pickle
39.5**	6.6±0.3	6.8±0.2	7±0.1	7.2±0.8	7.5±0.5	8.2±0.4	8.4±0.2	8.8±0.3	8.9±0.2	8.8±0.3	Clam pickle
19.005**	6.2±0.3	6.6±0.2	6.9±0.2	7.2±0.4	7.5±0.5	7.8±0.1	7.9±0.9	8.2±0.4	8.5±0.1	8.5±0.1	Oyster pickle
8.066667**	7.28±0.5	7.43±0.9	7.65±0.6	7.9±0.9	8.1±0.7	8.4±0.6	8.6±0.5	8.8±0.3	8.9±0.2	8.8±0.3	Clam pickle
33.1023***	6.08±0.4	6.3±1.1	6.5±0.3	6.9±0.8	7.2±0.7	7.5±0.4	7.6±0.9	7.9±0.9	8.1±0.8	8.3±0.6	Oyster pickle

Mean ± standard deviation. *** $P < 0.001$ - Significant. ** $P < 0.01$ - Significant.

Discussion

The pickled products prepared from blood clam and oyster: The present study revealed that pickles prepared from blood clam and oyster using mustard oil were the better pickle than pickles with sesame oil and soybean oil. Santhiya *et al.* (2013) reported that *Thaipangus* pickle prepared with mustard oil had higher acceptability than *Thaipangus* pickle with soybean oil.

Shelf-life assessment of pickles from blood clam and oyster with mustard oil *Biochemical and microbiological analysis:* The pH is one of the main spoilage indicators in pickled products. The acid pH mostly reduces the microbial load but acidophiles can grow in this pH too. The low pH during storage may be due to the activity of certain types of the bacteria, which is producing acid (Venugopal *et al.*, 1995). In the present study, a decrease in pH from 5.25 ± 0.07 to 3.8 ± 0.07 and 5.45 ± 0.05 to 3.9 ± 0.1 was observed in clam and oyster pickles, respectively. Similar decreasing trend of pH in blood clam pickle by Gupta and Basu (1985) was 4.50 to 4.36. Carpenter *et al.* (1995) reported decrease in pH from 5.1 to 4.9 for pickle from *Nemipterus japonicus*. The decreasing trend in pH of fish and mussel pickles have also been reported by Venugopal *et al.* (1995) and Vijayan *et al.* (1995).

Table 8. Total cost of pickle prepared from *A. granosa* and *C. madrasensis*

Cost	Name	Quantity	Price (Tk)
Ingredient cost	Cooked meat	500 g	Underutilized
	Garlic	125 g	10
	Ginger	100 g	10
	Onion	300 g	12
	Chili powder	30 g	11
	Turmeric powder	5 g	2
	Coriander powder	50 g	9
	Mustard seeds	20 g	2
	Pachforon	5 g	2
	Salt	30 g	1.2
	Mustard oil	300 g	32
	Vinegar	60 mL	6
	Tamarind (Pulp)	30 mL	2
	Sugar	2 TS	1.5
		Total ingredients cost	
Labor cost			40
Equipment cost			64
Utility charge			3
Total cost			205.7

Trimethylamine (TMA) is one of the major components contributing to the odor of decomposing fish product (Huss, 1988). This volatile amine is produced by bacterial degradation of trimethylamine Oxide. The present study showed a gradual increase in TMA-N levels from 2.49 ± 0.03 to 11.29 ± 0.03 and 3.59 ± 0.03 to 13.53 ± 0.08 for *A. granosa* and *C. madrasensis*, respectively, which are within the limit of acceptability throughout the storage period of 180 days. The acceptability limit of

the volatile basic compound is 10–15 mg/100 g for human consumption (Connell, 1995). 2.4 to 12.84 mg/100 g and 3.6 to 14.86 mg/100 g TMA-N are reported in *C. ramosus* and *H. pugilinus* respectively for 240 days of storage by Renitta and Patterson (2012). Hennessy *et al.* (2004) reported 2.38 to 6.72 mg/100g TMA-N for *Nemipterus japonicus* pickle. Kalogeropoulos *et al.* (2004) have reported similar increase in TMA-N concentration during the storage period of 180 days in clam pickle.

TVB-N comprises mainly TMA and ammonia (NH₃) that are produced by both bacterial and endogenous enzyme (Wahab and Amin, 2004). The increase in TVB-N in the later stages of storage may attribute to the bacterial spoilage (Shiby *et al.*, 2007). The production of TVB-N resulted in the reduction of organoleptic scores too. The release of TMA and NH₃ produces some off odors in food products. The present study showed 22.44±0.07 and 27.93±0.03 mg/100 g TVB-N, respectively, for *A. granosa* and *C. madrasensis* at 180 days which are in the acceptable limit (30-40 mg/100 g) by Connell (1995). Gradual increase in TVBN values from 9.80 to 32.20 mg/100g in fish pickle of *Nemipterus japonicus* is reported by Wijesekara and Kim (2010). Renitta and Patterson (2012) have reported 5.2 to 24.62 mg/100 g and 7.24 to 28.45 mg/100g in *C. ramosus* and *H. pugilinus* pickles, respectively, throughout the 240 days of storage. The production of off flavor correlates well with the reduction of organoleptic scores, and similar results have been noted in preparation of clam pickle by Thangavelu *et al.* (1988).

The present study showed increasing trend in TPC for both pickles. The TPC for both pickles were found 2.25±0.04 to 4.2±0.05 and 2.38±0.04 to 4.4±0.04 log cfu/g, respectively. The microbial count was reported to remain satisfactory till 90 days of storage at ambient temperature as the count remained in the range of 4 log cycles (Sahu *et al.*, 2012). Renitta and Patterson (2012) have reported similar increasing trend for *C. ramosus* and *H. pugilinus* pickles. However, Wijesekara and Kim (2010) have reported that the total counts were in the range of 10³ to 10⁴ cfu/g in the clam pickle. Similar increase in TPC was encountered in the range of 10³ to 10⁴ cfu/g in prawn pickle (Kumar and Basu, 2001).

Organoleptic characteristics evaluation: The present study revealed that *A. granosa* pickle had good appearance, dark brown color and no rancid flavor until the end of the storage period while *C. madrasensis* pickle showed lower acceptance than blood clam pickle. The texture of both pickles became soft due to absorption of oil while at the end meat became spongy. Vijayan *et al.* (1989) have reported that the development of dark color and rancid flavor were noticed in catfish pickles during the storage period of 6 months. The production of rancid flavor may be due to the rancidity of lipid took place by oxidation as well as lipolytic enzymes. This correlates well with the mean panel scores and became unacceptable. Renitta and Patterson (2012) reported that *H. pugilinus* meat pickle developed a dark color and rancid flavor from the sixth month onwards and *C. ramosus* meat pickle had good appearance, slightly dark color and no rancid flavor until the end of 3 Months storage. Organoleptic evaluation of rohu pickle stored at ambient temperature showed gradual increase in overall acceptability from 0 to 72 days (Waghmare *et al.*, 2015). Kumar and Basu (2001) noticed increasing trend of overall acceptability of prawn pickle up to 90 days of shelf life study.

Economic analysis : Pickles are good appetizer and add to the palatability to starch based bland tasting dishes besides being highly nutritious. As no costly equipment is involved, an industry can be started with very low investment. However, strict hygiene and sanitary conditions are to be maintained. This product has got good export potential, particularly in those countries where large number of Asians lives. At present there exist an expanding export and domestic market for fish and prawn pickle. In the present study, the total cost of ingredients for 500 g pickle preparation

was 98.7 Tk. The cost of mollusks flesh was not included in the economic analysis as the flesh is underutilized in the southwestern part of Bangladesh. In India, 400 g clam pickle is sold at Rupee 259 (Source: Amazon. in). The local people of southwestern part of Bangladesh use only the shell of mollusk for lime production while the flesh of blood clam and oyster are unused. Blood clam and oyster contain 14 % flesh and around 70 % shell of their body weight. The lime farmers from southwestern part of Bangladesh collect mollusk at 240 Tk/40 kg. From 40 kg raw mollusk they get 28 kg shell and the 5.6 kg of flesh which are unused during the lime production. Farmers produce 28 kg limes from 28 kg mollusk shell and they sell 28 kg lime at Tk. 420 (Nayan, 2017). Whether they prepare pickle from these unused clam and oyster flesh they can earn at least Tk. 2400/5.6 kg more. Preparing pickle from blood clam and oyster can be a great earning source for the local lime farmer of southwestern part of Bangladesh.

Conclusion

It is evident from the present study that the pickle products of good quality can be prepared by utilizing these underutilized marine mollusks and preserved in acceptable condition for a reasonable period at ambient temperature. In case of pickle prepared from blood clam and oyster with mustard oil had higher acceptability than pickles with sesame oil and soybean oil because of the known smell. Both of the pickles had a longer shelf-life and were safe for human consumption up to six months. The length of shelf life depends on spice ingredients, amount of oil and quality of sealing cap of the container. The costs of preparing these pickles were also lower than pickle from India. The local people from southwestern part of our country can earn more by preparing these pickles. This study has proved that the pickling is one of the best methods for the better utilization of these underutilized marine mollusks in Bangladesh. These pickles prepared from edible mollusks may be a part of our blue economy by marketing and exporting of these products.

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