Development of pickle from marine mollusks and its shelf-life assessment during storage

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Fisheries and Marine Resource Technology Discipline

Khulna University

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A Thesis

By

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DECLARATION

Development of pickle from marine mollusks and its shelf-life assessment during storage

The results presented in the thesis with the above mentioned title are entirely of the candidate's own investigations and no part of the results has been accepted for any degree nor is it being concurrently submitted for any degree.

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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. 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 rich nutritive value of the seafood products. Keeping this in mind, an attempt was made to utilize mollusk flesh as pickle and the shelf life of the pickled product developed from blood clam (*A. granosa*) and oyster (*C. madrasensis*) were assessed for a period of 3 months at ambient temperature (35-37°C). Different oils such as mustard oil, sesame oil and soybean oil were used for pickle preparation. Pickles prepared using mustard oil were 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. These are ready-to-eat products, and were stored for a period of 6 months for *A. granosa* and *C. madrasensis*. The cost of preparing pickle was lower than India. The pickle may be a great income source for the people of southwestern part of our country. It may also contribute to our blue economy by marketing and exporting pickles to foreign countries.

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DEDICATED TO

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Chapter-1

Introduction

The mollusk composes the second largest phylum Mollusca of invertebrate animals with varieties of form, structure, habit and habitat (Oliver, 1989). Mollusks have adapted to a wider range of habitats. Most mollusks are marine, some are found in terrestrial and freshwater habitats (Wye, 1989 and Khan, 2011). There are at least 200,000 known living species of mollusk throughout the world which includes snails, clams, oysters, mussels, squids, scallops, octopuses, and chitons (Solaiman, 2006 and Hossain *et al.*, 2004).

The southwestern coastal region of Bangladesh has a unique brackish water ecosystem, Sundarbans. This humid and tropical climate is suitable for a wider range of mollusk species. About 36 mollusk species including seven species of bivalves are available in estuarine areas and the mangrove floor of the Sundarbans Reserved Forests (Zaman *et al.*, 2011). Of them, blood clam, oyster, horn snail, small sea snail, murex snail are reported along Chuna river in the southwestern part of Bangladesh (Biswas, 2017 and Rahman, 2018).

In recent years, seafood and seafood-based products have become very popular worldwide, as they are rich in protein, vitamins, minerals and polyunsaturated fatty acids (Venugopal, 2003). People consume approximately 14% of animal protein from marine fisheries, but it varies from country to country. Marine mollusks are the excellent sources of protein, carbohydrate, lipid, vitamins and minerals. As a result, they are now used 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 (Khan and Liu, 2019). The marine mollusk too forms protein rich and low fat food. The meat of some edible mollusk meat is delicious and nutritious (Ramesh and Ayyakkanu, 1992). Various value added products such as pickle, sauce, soup, curry, boiled meat with sauces and so on produced from mollusk meat have high demand in Australia, Japan, South Korea and United States of America (Hackney, 1990). In India, the neighboring country of Bangladesh, pickle, soup, condiments are

prepared from mollusks (Renitta & Patterson, 2012). In India, about two-third of the total landed fish are consumed in fresh condition, and the remaining is preserved by various methods. 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 uses of mollusks. Only a small portion of people from Rakhaing 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, while the unused flesh has high nutritional value. Edible mollusks such as blood clam (*Anadara granosa) and* oyster *(Crassostrea madrasensis)* collected from Chuna river along Sundarbans contain 8-16% protein, 1-3.5% lipid, 1.22-4.8% ash and 72-82% moisture (Nayan, 2017).

Nutritional value of marine mollusks such as blood clam, oyster, turban snail, horn snail were carried out by Lah *et al.* (2006), Padidela & Thummala (2015) and Marget *et al.* (2013). In Bangladesh nutritional analysis of some freshwater mollusks (*Pila globosa, Bellamya bengalensis, Melania tuberculata, Lamellidens marginalis*, and *Helix sp*) was carried out by Baby et al. (2010). A very few studies are available about marine mollusks in Bangladesh. Biswas (2017) and Rahman (2018) reported on the seasonal variation in molluscan abundance and diversity in 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, nutritional value of mollusks meat various methods are evolved for the processing and preservation of mollusks meat to prepare value added products such as pickle, sauce, condiment, soup throughout the world and quality of these products within the storage time were evaluated (Gupta & Basu, 1985 and Jayachandran *et al.*, 1987). Renitta & 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). However, in Bangladesh there is no work on the value addition of edible mollusks meat though limited work was done to prepare

various value added products from fish such as mola fish pickle (Pervin *et al.*, 2010), thai pangus pickle (Shikha *et al.*, 2018). Therefore, the present study is designed to prepare pickle by unused edible mollusks flesh which could be an initiative for the proper utilization of mollusks in Bangladesh. Pickling is a simple and effective method for preserving the easily perishable food materials for a long period and for a long journey (Chandrasekar *et al.*, 1978). It is also comparatively cheaper method of preservation and yielding an acceptable product with a reasonable storage life at ambient temperatures. Hence, pickled products from blood clam and oyster will be developed and their shelf-life will be assessed for a period of 3 months. The pickle prepared from unutilized mollusk meat may have used as food for tribal people in Bangladesh and also have economic value in the tourist area for foreign tourists.

Objectives of this study

- To prepare pickle from mollusk meat.
- To assess shelf-life of the pickles.

Chapter-2

Literature review

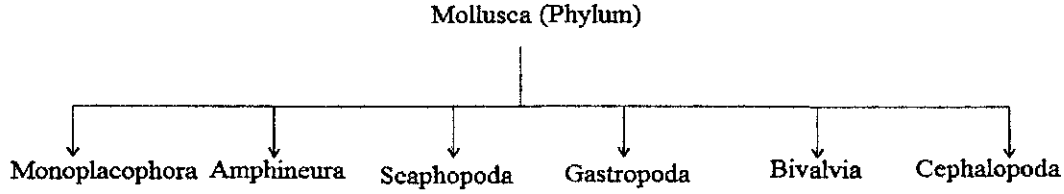
Chapter-2

Literature review

2.1 Taxonomy of mollusk

The history of molluscan taxonomy started from Aristotle (384-322 B.C), who divided the phylum mollusks into two groups, Malachia (present day cephalopods) and Ostracodermal (all shelled mollusks). Later, Linneaus (1758), made a great contribution in molluskan taxonomy and he used the term mollusks to describe large number of soft bodied animals, including slug, tunicates, octopus, jelly fish, star fish etc. He also used the term 'Testacea' to describe gastropods, bivalves, chitons, cephalopods and barnacles. Since then, the molluscan fauna are under various systematic revisions, especially in the recent works (Griffith & Pidgeon, 1834; Bouvier, 1887; Knight, 1952). Globally, about 120,000 species of mollusks have been described so far (Chase, 2002). While the diverse, large shelled groups are relatively well known because of familiarity of shells among people, many smaller species are poorly known.

Mollusks are divided into six classes according to their symmetry and the characters of food, shell, mantle, gills, nervous system and radula.



Source: (Kotpal, 2007)

Anadara granosa comes under the class bivalvia, order arcoida family arcidae, subfamily anadarinae (Narasimham, 1988).

Crassostrea madrasensis (Indian oyster) is belonging to the class bivalvia, subclass pteriomorpha, order ostreida, family ostreidae, subfamily crassostreinae (Hylleberg & Kilburn, 2002).

2.2 Morphological features of mollusk

A great variety of morphological differences is shown in term of mollusks body plan. They are front-to-back bilaterally symmetrical with well-defined nerve gangils, respiratory, blood circulatory, digestive, reproductive and excretory organs (Bambaradeniya, 2006). Mollusks have two characters 'radula and mantle' which are not found elsewhere. They are triploblastic, coelomate, unsegmented and bilaterally symmetrical. Body is divided into head, mantle, foot and visceral mass. Shell is calcareous and when present usually univalve or bivalve. Circulatory system is mainly of closed type. Respiration is direct or by gills or lungs or both. Nervous system of paired ganglia, connectives and nerves. Ganglia usually form a circumetric ring (Kotpal, 1996).

Monoplacophora is a class of phylum mollusca. Their body is bilaterally symmetrical with a dome-shaped mantle. Shell is flattened with a limpet-shape. They have broad and flat foot, five pairs of gills. The second class of mollusca is amphineura which have elongated body with reduced head, flat and ventral foot and radula. Scaphopoda is the third class and which have tusk-shells, conical foot, mouth with tentacles. They have no head and eyes. The largest and fourth class of phylum molluska is gastropoda. They have well developed head with eyes, tentacles, radula, large and flat foot. Shell is present or absent and usually coiled. Snails and slugs are gastropod aniamals. Bivalves have laterally compressed bodies enclosed by a shell consisting of two hinged parts. Bivalves as a group have no head and they lack some usual molluscan organs like the radula and the odontophore. Cephalopods have bilateral symmetrical body, a prominent head, and a set of arms or tentacles (muscular hydrostats) modified from the primitive molluscan foot (Kotpal, 1996).

As for example, *Anadara granosa*, which is known as blood clam or blood cockle under the class of bivalvia. Their body is enclosed in a bivalve shell and laterally compressed. They have no head, eyes, tentacles and radual. Foot is often hatched-shaped and extending between mantle lobes. They have medium sized shell. The shell is fairly thick, ovate, convex, inflated, equivalve and inequilateral; dorsal margin straight, anterior end rounded, sloping ventrally, posterior end obliquely rounded and ventral margin concave. Inner shell white in color, ventral, anterior and

posterior margins of valves with crenulations corresponding to the ribs outside, impressions of ribs are faintly visible. Mantle covering the bulk of visceral mass which is thin, but at the edges it is thick and muscular and bears the impressions of crenulated shell margin. Foot is large, laterally compressed with a ventral groove. Soft body is blood red in color (Narasimham, 1988).

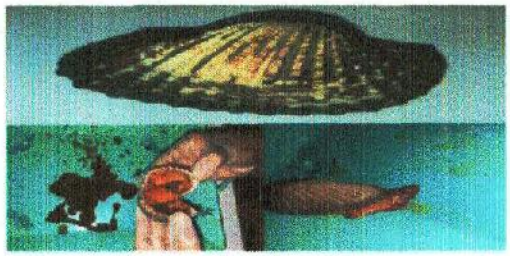


Figure 2.1: Blood clam (*Anadara granosa).*

Crassostrea madrasensis is another example of bivalvia. The shell valves of *Crassostrea madrasensis* are very irregular in shape and usually elongated. Outer surface of shell valves has numerous foliaceous laminae with sharp edges. Width of shell is 0.38 to 0.64mm and thickness is 0.14 to 0.36mm in length. The left valve is deep and the right one slightly concave. Hinge is narrow and elongated; it is sometimes elevated and has a medial depression in some oysters. Adductor muscle is situated subcentrally, reuniform and dark purple in color. The outer surface is grey, green or light purple depending on the area in which the oysters occur due to the presence of detritus, algae etc. The inner surface of valves is smooth, glossy and white in color with purplish black coloration along the margins of the valves (Rao, 1987).

Figure 2.2: Oyster (*Crassostrea madrasensis).*

2.3 Habitat and distribution of mollusk

Mollusks are noted to different modes of life because of their adaptive alternation. They are distributed in most of the parts of the earth and exhibit variety of forms. Most of the mollusks are aquatic and a few are adapted to terrestrial environment. They penetrate the abysses of the sea, coral reefs, mudflats, deserts and forests, rivers, lakes and underground. Gastropoda are mostly marine, some are freshwater or terrestrial. Cephalopoda are marine and free-swimming. Bivalvia are mostly marine and a few are freshwater (Kotpal, 1996).

As for example, *Anadara granosa* is a bivalve mollusk. They are shallow burrower, filter feeder, intertidal species lives in silty bottom area. They are found widespread in the Indo-West Pacific, from East Africa to Polynesia, North to Japan and south to northern and eastern Australia (FAO, 2017). The distribution of mollusks is depended heavily on the salinity of the water, and they were found most commonly under brackish sand and fine, soft, mud bottoms (Pathansali & Soong, 1958). They can be adaptable to salinity range from 14 to 300 and optimum temperature ranging from 20 °C to 30 °C (FAO, 2017).

Crassostrea madrasensis is a typical euryhaline species and found especially well in turbid brackish waters like estuaries, creeks, bays to a depth of 15-16 meters growing to a large size with heavy meat. It is also found in sheltered areas like ports and harbours where it occurs in large numbers attached to pillars, walls of wharves and buoys and along open coasts where hard substrata like rocks or stones are present for settlement. The Species is widely distributed in India and also in Pakistan coast (Rao, 1987).

2.4 Food and feeding habit of mollusk

Some mollusks are herbivorous while others are carnivorous. They feed on every possible food and vary in size from giant squids and clams to little snails, a millimeter long. Bivalves mainly live on micro-organisms, but the larval forms for some time lead ectoparasitic life in the gills of fishes. All cephalopods live on small fishes and crustaceans but gastropods show a different food habits. They are mostly vegetable feeders and some are predaceous (Kotpal, 1996).

As a shallow burrower and benthic feeder, the feeding habit of *Anadara granosa* is related to the bottom feed where they live. They mostly depend on the organic detritus (98% were found in cockle's intestine) and also on phytoplankton and unicellular algae (FAO, 2017).

Crassostrea madrasensis are sessile and filter feeders. They feed on the detritus, diatoms, dinoflagellates and zooplankton (Thangavelu, 1988).

2.5 Importance of mollusks

2.5.1 Ecological importance

The second largest phylum, mollusks play a great importance in the ecology and economy of the world. They are important ecosystem engineers which help to structure aquatic bottom environments and provide habitat, protection and food to a wide array of other taxa. They are used as bio-indicators to monitor the health of both freshwater and the marine environments. As major calcareous organisms with an extensive fossil record, they can provide important information on past climate events and oceanic changes, thus, increasing our understanding of predicted future changes (Fortunato, 2015).

2.5.2 Economical importance

Historically, mollusks have been important to humans in many ways, and today they are an economically important group worldwide. Commercially importance mollusks are all essentially marine and broadly come under two major categories, (i) edible and (ii) ornamental. Squids, mussels, oysters, clams etc., which are used for human consumption come under the edible molluscan fisheries. The marine mollusks are very important since many of them are used as food, they are also used as crafts making, to dye cotton, yarn and clothes, etc., so it is that they have received special attention and become a natural resource of economic importance (Santhiya *et al.*, 2013).

2.5.2.1 Nutritional value of mollusks

Marine mollusks are the source of protein, carbohydrate, lipid and minerals. Four gastropods such as *B. zeylanica, M. virgeineus, B. spirata* and *T. radiates* along the Kanyakumari coast of India contain 43.2 %, 39.8%, 36%, 28.55% protein , 19.6%, 19.5%, 13.7%, 6.7% carbohydrate and 6.2%, 4.7%,1.45%,1.1% lipid respectively

(Margret, 2013). An edible bivalve from India, *Parreysia cylindrica* which contains protein varied between 55.67% and 40.21%, carbohydrate ranged from 5.21 to 16.67% and lipid ranged from 6.63 to 2.11% (Padidela & Thummala, 2015). The protein content of *Mactra violacea* is 11.9 %, fat 1 %, ash 3.2 % and moisture 80 %. It is a good source of protein and minerals but lower fat content (Laxmilatha, 2009). The bivalves contain both 4 macro minerals and 2 trace minerals. Among the macro minerals, calcium, sodium, magnesium and potassium are found in bivalves. The trace minerals such as iron and zinc are also found in mollusks (Padidela & Thummala, 2015).

(*Anadara grsnosa* contain 13.04% protein, 2.00% lipid, 0.88% ash and 87.30% moisture (Rahman *et al*, 2012). Blood clam, *Anadara granosa* has high rate of nutrition. It has 20.10% of protein content; 1.18% of lipid; 2.64% of carbohydrate; 75.80% of water; and 0.20% of ash (Gunawan & Heldinto).

Crassostrea madrasensis is considered as a great delicacy throughout the world. It contains 82.64%, 9.41%, 3.25% 3.2% and 1.01% of moisture, protein, fat, carbohydrate and ash respectively (Asha *et al*, 2014).

2.5.2.2 Health-promoting efficacy of mollusks

As a source of colorants, shells, foodstuff, and customary medication, mollusks have traditionally been an integral part of human life traversing diverse ethos from an ancient time (Benkendorff, 2010). Being a source of healthy food and because of the ability of certain species to be value added for the production of functional or health-promoting foodstuff, shelled mollusks even in modern times provide vital role in a number of customary medications (Benkendorff *et al.*, 2015; Lev & Amar, 2007; Zhong, 1999). Mollusks have been used as remedy for many ailments like inflammatory disorders by the Medieval Eastern-Mediterranean, primeval Greco-Romans, and European societies historically, while they are still in use for the same purpose in India, Zimbabwe, and Latin America. Clam and oyster species were used as medication for skin ailments, arthritis, and stomach ulcers in China (Ahmad *et al.*, 2018).

2.5.2.3 Mollusks consumption and associated health benefits

Mollusks consumption as a part of daily diet has proven health benefits because they are rich in vital nutrients and active secondary metabolites, and they have the ability to enhance immune response (Benkendorff, 2010). In fact, a number of Asian communities consume many mollusks species for the associated health-promoting effects (Kim & Pallela, 2012).

Normal cooking practices generally increases digestibility of proteins by denaturation (Venugopal, 2006), which results in the production of bioactive peptides from the corresponding proteins (Suleria *et al.*, 2015). Production of such bioactive peptides associates a number of biological activities, including immunomodulation, antimicrobial, antihypertensive, and antithrombotic activities (Murray & FitzGerald, 2007). To add further, mussel and squid, when pan fried in olive oil enhances the levels of squalene (skin protectant) and monounsaturated fatty acids (Kalogeropoulos *et al.*, 2004), a substantial decrease is occurred in the levels of sitosterol and cholesterol (Ozogul *et al.*, 2015) while, riboflavin (bioactive vitamin B2) remains unaffected during cooking (Venugopal, 2006).

Molluscan consumption has been reportedly associated with a number of health benefits such as, a significant reduction in hypertension is associated with consumption of blue mussel (fermented) and oyster sauces due to the presence of biologically active peptides (Wijesekara & Kim, 2010).

2.5.2.4 Value added products from mollusks

Value added food products are raw or pre-processed commodities whose value has been increased through the addition of ingredients or processes that make them more nutritive and attractive to the buyer and or more readily usable by the consumer (Hennessy *et al.*, 2004). Therefore, +the dual advantages of value addition, namely, finding ways for better utilization of some fish species and providing protein and mineral rich convenience foods are note worthy (Venugopal *et al.*, 1995).

Value addition and diversification of processed seafood is an important need in fish processing. Value addition means "any additional activity that changes the nature and presentation of the product thus adding value to it for sale." Present market trends are indicative of extensive growth in demand for ready-to-cook or ready-to

serve convenience products processed out of a variety of fin and shell fishes. The technology for ready-to-eat food products preparation is now rapidly advancing in India (Saritha & Patterson, 2012).

Value added fish based products will help to promote improved public health, social welfare and better quality of life. Development of value added products for exporting to foreign countries could be an effective measure towards poverty alleviation (Prajapati, 2007).

Value added foods also serve as convenience foods. A food may be classified as convenience food if it meets the following criteria:

- The food must have undergone considerable amount of food preparation by the manufacturer before it reaches the retailer.
- It must require minimal cooking or processing before consumption by the consumers.
- The preparation time before consumption should be minimal (Manohar *et al.*, 2005).

The growing appetite for value added sea food is being mainly fueled by changing life styles. The fish processing industry is faced with new consumer demands and trends such as convenience without quality or nutrition loss coupled with cost reduction and food safety (Etheridge, 2010).

Ready-to-cook (RTC) foods are convenience foods which need some preparations like cooking, frying, reconstitution, dilution etc. before consumption. The demand for such foods is increasing at a fast pace due to social and economic patterns, urbanization, increase in buying power, awareness about health foods, changes in meal pattern and existing food habit and a desire to taste new products (Manohar *et al.*, 2005). Subsequently, convenience foods are processed foods which are designed to save consumer's time in the kitchen and reduce costs due to spoilage. They are developed specifically to preserve the oversupply of products available at the time of harvest in order to stabilize the food markets in developed countries. By the turn of the century, every household uses convenience foods in one form or another (Shiby *et al.*, 2007).

There are several value added convenience foods prepared from different edible mollusks such as clam pickle, oyster pickle, curry, mussel pickle, oyster soup, oyster sauce that are available in the Indian market (Gupta & Basu, 1985 and Jayachandran *et al.*, 1987).

2.6 Availability of mollusks in Bangladesh

Bangladesh is enriched with unique aquatic resources for aquaculture development. The fisheries resources of Bangladesh are highly diversified in terms of types, resources and types of the commodities produced. The coastal area of Bangladesh is one of the highly productive areas of the world for its geographical position and climatic condition (Islam, 2003). The influence of the mangrove forests is one of the unique features of the coastal areas of Bangladesh, which support a high number of fishes and other commercially important aquatic organisms (Ahmed, 1990). About 301 marine and 26 freshwater mollusk species are available in Bangladesh.

In our coastal area we have sandy, muddy and rocky grounds, mangrove areas and coral reef which are suitable for molluscan habitats. It has been found that there are 327 species (marine 301 and freshwater 26) of mollusks available in Bangladesh (Solaiman *et al.*, 2006). Bangladesh has an abundant resource of marine bivalves. Clams, edible oysters, windowpane oysters and mussels are the major bivalve groups in order of abundance and mainly distributed in the South Eastern coastal region of Bangladesh, more specifically in the Cox's Bazar coastal area. There are three species of mollusks that occur naturally in the coastal waters of Bangladesh, namely, green mussel, clam and oyster. Four species of edible oysters such as *Crassostrea gryphoides*, *C. madrasensis*, *C. belcheri* and *Saccostrea cucullata* have been identified from various natural beds. They are found on rocks, bricks, sluice gates, bridge posts, boulders and mangrove roots in the coastal areas. Only one species (Placuna placenta) of windowpane oyster has been identified in the waters around Cox's Bazar, Gotibhanga (Moheshkhali) and Teknaf. Two species of cockles, *Anadara granosa* and *A. rhombea* have been have been found along the Banckkhali, Gotibanga, Chakaria and Teknaf estuaries and *Mytilus edulis* (green mussel) has been identified in the estuary of the Naf river and in St. Martins (Solaiman *et al.*, 2006). Relative abundance of oyster and clam are found in coastal area of greater

Khulna region, especially in the Sundarbans Mangrove Forests. The availability of mollusks is higher in the lower region compared to the upper northern areas. Green mussel is not found in the greater Khulna region (Shahabuddin *et al.*, 2010). SUFER funded project by Wahab *et al.* (2004) and Salam *et al.* (2004) found that about 700 tons of oyster (with meat and shells) are annually collected from the Sundarbans in Satkhira region.

2.7 Uses of mollusks in Bangladesh

The mollusks play a significant role in the world fishery today. Large quantities of edible oyster are exploited and harvested from natural beds in some developed and developing countries like Japan, USA, Canada, United Kingdom, China, Korea, the Philippines, India and In some countries these are also cultured. The edible oyster contain glycogen, lipids, protein, vitamins specially A, B and D and several essential minerals. Oyster is used as food and fish bait by some coastal dwellers and the shells are also used to make lime. Mussels and clams are important food source in many parts of the world, including Europe, South America, and the Far East, and they have considerable potential as a valuable protein source for many developing countries (Solaiman *et al.*, 2006 and Shahabuddin *et al.*, 2010). However, in Bangladesh there is very limited use of mollusks species and the uses are as follows:

2.7.1 Human consumption of mollusk meat

People from Rakhaing and some other tribes of the coastal area use green mussel, clam and oyster meat as their food. The main dishes they prepare from mollusks meat are chatni, curry, bhuna, light fry and chop. No hotel/restaurants are found selling mollusk dish in the coastal cities (Khulna, Chittagong and Cox's Bazar) however, in Dhaka some hotel and restaurants are selling mollusk dishes (Solaiman *et al.*, 2006). Name of hotels and restaurants of Dhaka city which use mollusk meat are given in table 1.

Table 2.1: Mollusk meat consumption in different restaurants of Dhaka

Name of hotels	Meat needed (kg /year)	Name of hotels	Meat needed (kg /year)
Purbani	10-15	La Vinchi, Kawran bazer	5
Sheraton	15-20	Crystal garden, Gulshan-2	5
Sonargaon	10-15	Abakash, Mohakhali	5

Source: (Solaiman *et al.*, 2006)

2.7.2 Mollusk meat used in shrimp hatcheries

In different coastal shrimp hatcheries mollusk meat is fed to mother shrimp. Most of the hatchery owners prefer fresh mollusc meat to feed the mother shrimp. They collect mollusk mest from the mollusk meat wholesalers (Solaiman *et al.*, 2006).

2.7.3 Use of shell in poultry and fish feed industries

In the poultry and fish feed industry the mollusk shell has huge demand as an essential source of calcium in the feed. The present supply of mollusk shell can not meet the high demand of this sector. In general, 1-2% crushed mollusk shell is used in the feed preparation in the poultry and fish feed industry. Mollusk shell powder is collected from crushing mils located in the coastal areas mainly from Patia, Chittagong and Khulna region. There are about 8 crushing mills are found in Chittagong Cox's Bazar area and 9 mills are in Khulna region. Around 10 mt dry shell powder is produced monthly in each mill. 36 kg small particle and 2 kg powder are obtained after crushing 40 kg mollusk shell. Small particles are used in fish and poultry feed industries and the powder is used in shrimp gher (Solaiman *et al.*, 2006).

2.7.4 Lime making

In Bangladesh, mollusk shell is mainly used for making lime. There are 20 lime producing factories are found in Cox's Bazar region and about 30 lime factories are found in Khulna region (Solaiman *et al.*, 2006).

2.7.5 Ornamental and decorative uses

There is a high demand of mollusk shell for the use in ornamental and decorative purposes especially in the Cox's Bazar, Teknaf, St. Martins area. Different decorative and ornamental items made from mollusk shell are sold in these areas. A large number of people are engaged in collecting, cleaning, processing of shell and preparation of various types of ornaments, showpieces and home decorating items from the shells. Usually the tourists from all over the country and overseas buy these items. These items are also supplied to the different parts of the country (Solaiman *et al.*, 2006).

2.7.6 Paint making/shining

A number of households are involved in making paint and shining materials (special kinds of lime) from mollusk shell in Cox's Bazar and Bagerhat areas. The paints are used in painting walls, floor, flower vase and other items and the shining materials are used after painting the walls (Solaiman *et al.*, 2006).

2.8 Pickling as a method of preservation

Pickling is the process of preserving or extending the lifespan of food by either anaerobic fermentation in brine or immersion in vinegar. It is an ancient gastronomic craft of preserving food. Historically, India, Egyptians, Chinese used pickling for food preservation. In East Asia, vinaigrette (vegetable oil and vinegar) is also used as a pickling medium. The pickling procedure typically affects the food's texture, taste and flavor. The resulting food is called a pickle, or, to prevent ambiguity, prefaced with pickled. Foods that are pickled include vegetables, fruits, meats, fish and eggs. A distinguishing characteristic is a pH of 4.6 or lower, which is sufficient to kill most bacteria. Pickling can preserve perishable foods for months. It is one of the simplest and most effective ways to preserve perishable foods for months for out of season use and for long journeys.

Now-a-days, pickles are used by a number of people as an important side dish of delicacy. Pickles prepared from fish and shellfish are good appetizer and highly nutritious. Fish pickles are very popular taste wise and a variety of methods are available in India. Today, India exports large quantities of fish and shellfish pickles to Middle East and to other parts of the world to meet the demands. The awareness of people about the consumption of fishery products is increasing day-by-day throughout the world. The food technologists are focusing much attention on the development of fishery products (Waghmare *et al.*, 2015).

2.9 Evaluation of pickled fisheries products

Shell-fishes are highly perishable. Spoilage of shell-fish is due to autolytic and microbial reactions. Natural enzymes present in their tissues retain much of their activity after the death and continue to operate although changes are mostly catabolic. The rates at which these reactions are produced depend on temperature, type of fish and time period.

The shelf life of processed fisheries products mainly depends on the freshness of the raw materials at the time of processing. Hence, it is necessary to ensure that only fresh shell-fish is used for food production purposes. It is very vital to study these changes in order to understand the quality of fish products during storage. The freshness quality of fish and fisheries products can therefore be understood by periodically assessing the biochemical, microbial and sensory qualities (Pancharaja *et al.*, 2008).

2.9.1 Biochemical evaluation

Protein and fat in fish and shell-fish undergo changes during processing like drying, freeze drying, pickling, canning etc. and during storage (Pancharaja *et al.*, 2008).

i. Changes in protein during storage

a) Total Volatile Base Nitrogen (TVB-N)

b) Trimethyl amine (TMA)

ii. Changes in lipids during storage

a) Free fatty acids (FFA)

b) Thiobarbituric acid value (TBA)

iii. Changes in pH

i. Changes in protein during storage

a) Total Volatile Base Nitrogen (TVB-N)

TVB-N is one of the most widely used measurements of sea food quality. Microbiological spoilage of sea food causes the formation of volatile bases such as ammonia, dimethyl amine and trimethyl amine. The measurement of these compounds is an indirect method of assessment of freshness quality of sea food. This method is not capable of identifying the early stages of deterioration because TVBN values are not linearly related to the length of time (days) if the species being evaluated was stored in ice and it cannot be used to predict the storage life of that species. However, these values are useful for assessing quality during later stages of storage and therefore can be routinely used as a standard method for assessing the quality changes in chilled, frozen, dried and canned seafood (Pancharaja *et al.*, 2008). The maximum admissible limit of TVBN for fish and fishery products are presented in Table 2.

Table 2.2: Levels of TVB-N in fish and fishery products

Item	Maximum admissible limit
Fresh fish and shell-fish	35 – 40 mg/ 100 g
Frozen fish	<30 mg / 100g
Salted and sundried fish	100 – 200 mg / 100 g
Canned sea food	<20 mg / 100 g
Pickled products	30-40 mg / 100 g

Source: (Connell, 1995)

b) Trimethyl amine (TMA)

Trimethyl amine is a pungent volatile amine often associated with the typical fishy odour of spoiling sea food. Its presence in spoiling shell-fish and fish is due to the bacterial reduction of trimethyl amine oxide (TMAO) which is naturally present in the living tissue of many marine shell-fish species. The chief advantages of TMA analysis over enumeration of bacterial numbers are that TMA determinations can be performed far more quickly and often reflect more accurately the degree of spoilage than do bacterial counts. Values of TMA depend upon species, time of year, location of catching, stage of spoilage, types of processing and or storage and method of analysis (Pancharaja *et al.*, 2008). The acceptability limit of the volatile basic compound is 10–15 mg/100 g for human consumption (Renitta & Patterson 2013).

ii. Changes in fish lipids during storage

Two types of changes take place in the lipids during processing and preservation of fish and shell-fish. They are hydrolytic changes and oxidative changes. Both these changes reduce the organoleptic quality and shelf life of fish products. These changes result in rancidity (Charley, 1982).

iii. Changes in pH during storage

Factors that affect texture of food include moisture content, composition and pH (Charley, 1982). Huss (1988) reported that the pH of fish immediately after being caught was between 6.0 and 6.5. The fish were acceptable up to a pH of 6.8, but were considered to be spoiled above a pH of 7.0.

2.9.2 Microbiological evaluation

Food spoilage denotes the disruption of the original nutritional value, texture and flavor of the food. The food becomes unsuitable for human consumption (Gram & Dalgaard, 2002). It is a metabolic process that causes foods to be undesirable or unacceptable for human consumption due to changes in sensory characteristics (Burkepile *et al.*, 2006; Sherratt *et al.*, 2006). As soon as a fish dies, spoilage begins. Bacteria enter through the gills and into the blood vessels, through the lining of the belly cavity and eventually through the skin. In the flesh they grow and multiply rapidly, producing disagreeable odors and flavors (Johnston *et al.*, 1994).

In the case of marine shell-fishes, the bacteria found are 90 to 100 percent gram negative rods and are concentrated on the skin, gills and intestines in increasing order. According to their sensitivity to salt, they can be further divided into three

(1) Halophobic or salt sensitive eg., *Achromobacter* and *Pseudomonas* which cannot tolerate salt concentrations higher than 6 %;

(2) Halotolerant consisting of spore formers, Micrococci and some anaerobes are growing even up to saturation point of salt, the rate of growth being inversely proportional to salt concentration;

(3) Halophilic or salt loving which grow best in the presence of salt. It is the last group of bacteria which cause spoilage in salt cured or dried fish.

Some of halotolerant fungi also grow on salted and dried fish eg. *Sporendema epizooum* (Govindan, 1985).

2.9.3 Sensory evaluation

Sensory evaluation is the identification, scientific measurement, analysis and interpretation of the properties (attributes) of a product as they are perceived through the five senses of sight, smell, taste, touch and hearing. Sensory analysis is used to answer questions about product quality, questions relating to discrimination, description and preference. Discrimination is of particular relevance in the context of product quality control, in shelf life studies and in investigation of possible taints.

Descriptive tests are more appropriate in the product development context, where there is a desire to develop a product that matches a known target quality or to reformulate an existing product using different ingredients or processes or to

investigate the differences among a range of experimental and or commercial products. Preference and acceptability tests are aimed at establishing whether product differences are recognized by the consumer and are seen to be improving liking or acceptability. These methods lie on the fringe between sensory analysis and consumer research and have different panel recruitment criteria from those for discrimination or descriptive tests. These assessors need to be representative of the target consumer population and preferably to have little or no sensory training (Carpenter *et al.*, 2000).

Appearance is very important in food perception. If a product does not have an appearance that is visually acceptable (as compared with previous images stored in the memory), a person may decide not to eat the food. Visual appraisal can have a significant effect on the other senses (Carpenter *et al.*, 2000). The appearance of food contributes immeasurably to one's esthetic appreciation of them (Charley, 1982).

Flavor is the combination of taste and odor. It may be influenced by sensations of pain, heat, cold and by tactile sensations. Flavor has a major role in determining the acceptability of foods and as with texture, it is a dynamic process as changes in intensity occurs over time. Our sense of smell can detect many different odors when sniffed through the nose, but is also important for detecting volatiles given off by food items in the mouth as part of the flavor perception. The sense of smell is a key input to sensory analysis.

Texture plays an important role in the overall acceptance of a product. Consumers expect certain products to have a particular texture. Texture is therefore one of the major criteria used by consumers to assess the quality and freshness of foods (Carpenter *et al.*, 2000). Texture, from the sensory stand point refers to the sense of feel of food in the mouth. Words of texture are crisp, hard, soft and chewy (West *et al.*, 1988). Crispiness of food contributes to the pleasure derived from eating them. The texture of food is important not only per say but also because of the effect of texture on taste. The appearance, odor, mouth feel and taste influence the overall acceptability of food. Even though appearance, odor and taste are acceptable, a food may be rejected on the basis of mouth feel (Charley, 1982).

Chapter-3

Materials and methods

Chapter-3

Materials and Methods

3.1 Collection and Processing of raw material

Edible mollusk such as blood clam (*Anadara granosa*) and oyster (*Crassostrea madrasensis*) were collected from the intertidal zone of Chuna river along the Sundarbans Mangrove Forest which is situated in Munshiganj union, Shyamnagar upazila of Satkhira. The mollusk was transported to the Biology Laboratory, Khulna University for producing pickle.

Raw materials were washed 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 for further pickle preparation.

3.2 Experiment-1: Preparation of pickle by blood clam and oyster using different oils

3.2.1 Ingredients used for pickle preparation

The ingredients were used for the preparation of pickle from blood clam (*Anadara granosa*) and oyster (*Crassostrea madrasensis*) using different oils are given in the Table 3.

Table 3.1: Standard recipe for blood clam (*A. granosa*) and oyster (*C. madrasensis*) pickle

Ingredients name	Quantity	Ingredients name	Quantity
Meat (Cooked)	500 g	Mustard seeds	20 g
Garlic	125 g	Pach foron	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

3.2.2 Preparation of pickle from blood clam (*A. granosa*) and oyster (*C. madrasensis*) using different oils

At first small pieces of meat were cooked in a pressure cooker for 30 min and blanched. Then the cooked meat were marinated with some salt, chili powder and turmeric for 30 min at room temperature. Marinated meat were fried in oil (Mustard oil/sesame oil/soybean oil) till golden brown color appeared. Chopped onion, garlic and ginger paste were fried in the remaining oil till brown color and then others ingredients except vinegar were mixed and made a masala paste.

Fried meat were added to that mixture and boiled for 5 min. Vinegar was added and waiting till its absorption. Cooled down the pickle at room temperature and stored in glass bottles at room temperature.

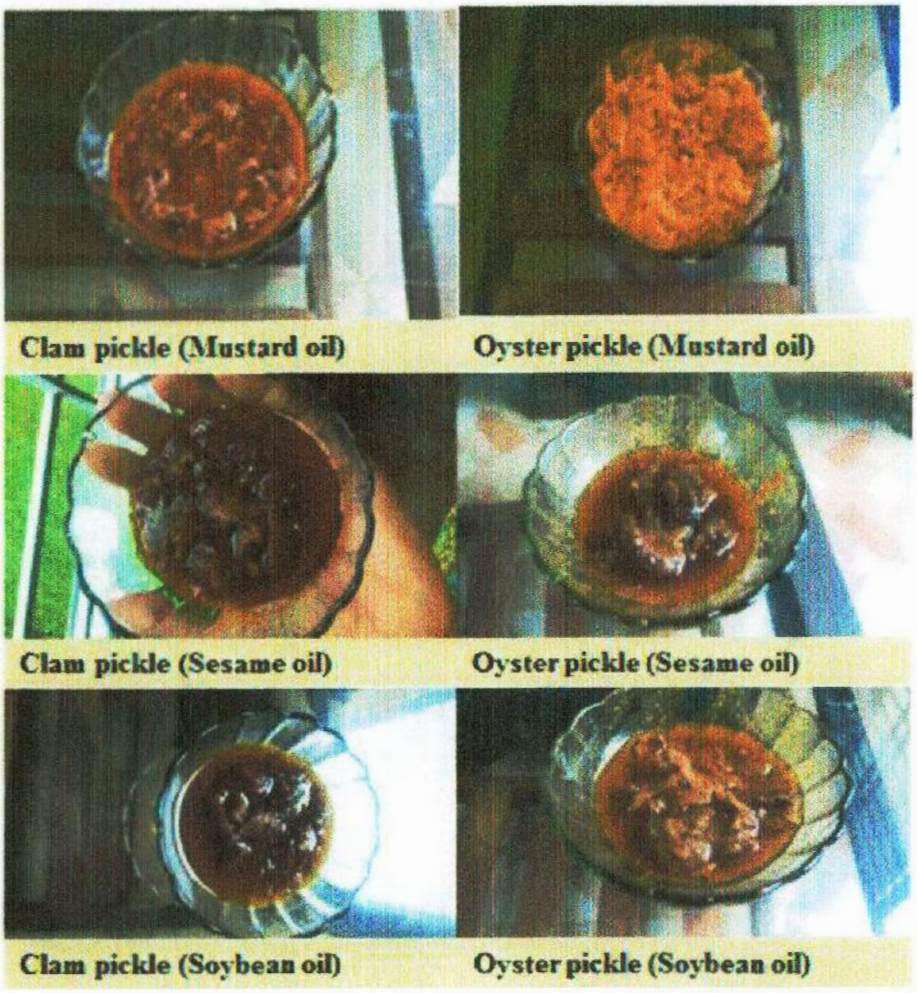


Figure 3.1: Pickles prepared from blood clam (*Anadara granosa*) and oyster (*Crassostrea madrasensis*) using different oils.

3.2.3 Sample storage

Prepared pickle samples were stored at room temperature at 35–37°C in 18 plastic jars (Made from polyethylene terephthalate or PET, has plastic code 1). Each of the jars contained 300g pickle. Among these 18 bottles 9 jars contained blood clam pickles in such way: pickle prepared

from blood clam with mustard oil were stored in 3 jars, pickle prepared from blood clam with soybean oil were stored in another 3 jars and pickle prepared from blood clam with sesame oil were stored in 3 jars. Pickle prepared from oyster also stored in 3 + 3 +3 separate jars for mustard oil, soybean and sesame oil.

3.2.4 Selection of best pickled products prepared from blood clam (*A. granosa*) and oyster (*C. madrasensis*)

Pickles prepared from blood clam (*A. granosa*) and oyster (*C. madrasensis*) with 3 different types of oil were undergone biochemical, microbiological and organoleptic evaluation to find out the best recipe of blood clam (*A. granosa*) and oyster (*C. madrasensis*) pickles.

3.3 Experiment-2: Biochemical, microbiological and sensory evaluation of pickles prepared from blood clam (*A. granosa*) and oyster (*C. madrasensis*)

The best pickle products prepared from blood clam (*A. granosa*) and oyster (*C. madrasensis*) were stored at room temperature at 35–37°C for six months to evaluate their shelf-life. Initially the samples were evaluated at 15 days interval for first three months and then after 30 days interval up to 4 months and 60 days up to 6 months.

3.3.1 Biochemical and microbiological analysis

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

3.3.2 Organoleptic characteristics evaluation

The sensory evaluation (appearance, color, flavor, odor, taste, texture) of both the pickle were carried out by serving meat pickle to a panel consisting of eight members. The organoleptic scores for the product with a 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.

3.3.3 Economic analysis

Total ingredients which were used for preparing pickles were the operational cost. The total cost of pickle preparation for 500 g of each pickle was calculated.

3.4 Statistical analysis

The statistical analysis was performed using R Studio version 3.6.0 and Microsoft Office Excel 2007. At first the proximate data (percentage data) were transformed by ARCSIN formula and count data, organoleptic scores were transformed by square root formula. Shapiro-Wilk test was performed for normality test and then One Way ANOVA and Tukey test were done to evaluate differences among the treatments. All analysis were performed at 0.05 significance level.

Chapter-4

Results

Chapter-4

Results

4.1 Selection of the best pickled products prepared from blood clam (*A. granosa*) and oyster (*C. madrasensis*)

4.1.1 Biochemical and microbiological analysis

The protein and lipid content of Blood clam (*A. granosa*) pickles using three different oils (mustard oil, sesame oil and soybean oil) were 29.91±0.01%, 22.23±0.04%, 26.43±0.06% and 17.32±0.03, 15.26±0.03, 15.89±0.07 respectively. Pickles prepared from oyster (*C. madrasensis*) using mustard oil, sesame oil and soybean oil contained 17.32±0.03%, 15.26±0.03%, 15.89±0.07% protein and 30.49±0.01%, 27.41±0.06%, 29.23±0.03% lipid respectively. Pickles prepared from blood clam showed higher protein content than oyster pickles ($P < 0.01$) (Table 4). Oyster pickle using different oils contained higher lipid content than blood clam pickles ($P < 0.01$) (Table 4.1). 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 4.1: Proximate composition of pickle prepared from blood clam (*A. granosa*) and oyster (*C. madrasensis*) with different oils

Parameters	Pickle					
	Clam pickle (Mustard oil)	Clam pickle (Sesame oil)	Clam pickle (Soybean oil)	Oyster pickle (Mustard oil)	Oyster pickle (Sesame oil)	Oyster pickle (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 Standard ± deviation

Table 4.2 indicates the differences of biochemical and microbiological quality among the blood clam pickles prepared with three different oils such as mustard oil, sesame oil and soybean oil. The one way anova showed significant differences ($P < 0.001$ and $P < 0.01$) among these three pickles.

Table 4.2: Biochemical and microbiological analysis of *A. granosa* pickle with different oils at initial day

Parameters	Pickles			
	Clam pickle (Mustard oil)	Clam pickle (Sesame oil)	Clam pickle (Soybean oil)	F
TMA-N(mg/100 g)	2.49±0.02	2.74±0.02	2.91±0.04	152.6***
TVB-N(mg/100 g)	4.97±0.04	5.11±0.06	4.93±0.03	13.697**
pH	5.25±0.05	5.45±0.05	5.35±0.05	12**
TPC(log cfu/g)	2.25±0.04	2.41±0.02	2.53±0.02	59.917***

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.

Biochemical and microbiological quality of oyster pickles prepared using mustard oil, sesame oil and soybean oil were also significantly different ($P< 0.001$ and $P< 0.01$) at the initial day of storage (Table 4.3). From this result pickles prepared from mustard oil for both species were selected as the best because of the acceptance of mustard oil is the best for pickle preparation in Bangladesh.

Table 4.3: Biochemical and microbiological analysis of *C. madrasensis* pickle with different oils at initial day

Parameters	Pickles			
	Oyster pickle (Mustard oil)	Oyster pickle (Sesame oil)	Oyster pickle (Soybean oil)	F
TMA-N(mg/100 g)	2.89±0.04	3.02±0.06	3.59±0.03	210.94***
TVB-N(mg/100 g)	4.81±0.02	5.09±0.06	5.45±0.03	197.08***
pH	5.45±0.05	5.65±0.05	5.6±0	16**
TPC(log cfu/g)	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 = Trimethylamine nitrogen; TVB-N = total volatile base nitrogen; TPC = total plate count; CFU = colony forming units.

4.1.2 Sensory evaluation

The results of sensory evaluation of blood clam and oyster pickles using three different oils at initial day were showed in Table 4.4 and Table 4.5. The sensory evaluation was done by the panelists. In case of both species pickles prepared with mustard oil showed much better result than sesame oil and soybean oil. The sensory scores of pickles using different oils were significantly different ($P< 0.001$ and $P<$

0.01). From this result pickle prepared from blood clam and oyster using mustard oil were selected for their shelf-life analysis for six months.

Table 4.4: Sensory evaluation of *A. granosa* pickle with different oils at initial day

Organoleptic characteristics	Pickles			
	Clam pickle (Mustard oil)	Clam pickle (Sesame oil)	Clam pickle (Soybean oil)	F
Appearance	9±0	8.1±0.8	8±0.3	233.01***
Color	8.9±0.3	8±0.3	8±0.3	167.39***
Odor	8.8±0.3	6.5±0.1	7.5±0.5	78.129***
Taste	8.8±0.3	6.2±0.3	7.2±0.2	286.7***
Texture	8.2±0.4	6±0.2	6.5±0.1	89.3***
Flavor	8.8±0.3	7±0	7.2±0.2	58.917***
Overall acceptability	8.8±0.3	6.9±0.9	7.4±0.6	153.67***

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

Table 4.5: Sensory evaluation of *C. madrasensis* pickle with different oils at initial day

Organoleptic characteristics	Pickles			
	Oyster pickle (Mustard oil)	Oyster pickle (Sesame oil)	Oyster pickle (Soybean oil)	F
Appearance	8.9±0.3	7.5±0.5	7.5±0.5	211.94***
Color	9±0	8.2±0.4	8±0.3	187.9***
Odor	7.8±0.1	7±0.3	7±0.3	74.01***
Taste	7.5±0.5	6.7±0.2	6.5±0.1	149.7**8
Texture	7.9±0.7	6±0	6±0	238.7***
Flavor	8.5±0.1	6.5±0.1	6.5±0.1	69.19***
Overall acceptability	8.3±0.6	6.9±0.8	6.9±0.7	345.89***

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

4.2 Shelf-life assessment of pickles from blood clam (*A. granosa*) and oyster (*C. madrasensis*)

Pickles prepared from blood clam (*A. granosa*) and oyster (*C. madrasensis*) using mustard oil were selected as the best. Pickles prepared from blood clam (*A. granosa*) and oyster (*C. madrasensis*) using mustard oil were stored for six months to assess their shelf-life.

Table 4.6: Biochemical and Microbiological quality of *A. granosa* pickle using mustard oil

Parameters	Initial	15th day	30th day	45th day	60th day	75th day	90th day	120th day	180th day	F
TMA-N (mg/100 g)	2.49±0.03	2.88±0.05	3.49±0.09	3.96±0.07	4.81±0.02	5.08±0.04	6.7±0.12	8.21±0.04	11.29±0.03	2877.8***
TVB-N (mg/100 g)	4.97±0.05	5.19±0.01	5.84±0.04	6.63±0.07	8.48±0.08	10.52±0.06	12.32±0.01	15.27±0.05	22.44±0.07	9180.3***
pH	5.25±0.07	4.9±0.14	4.65±0.07	4.45±0.07	4.4±0.14	4.15±0.07	4.05±0.07	4±0	3.8±0.07	114.69***
TPC (log cfu/g)	2.25±0.04	2.37±0.02	2.59±0.04	2.83±0.03	2.9±0.03	3.19±0.06	3.69±0.05	3.9±0.08	4.2±0.05	416.88***

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.

Table 4.7: Biochemical and Microbiological quality of *C. madrasensis* pickle using mustard oil

Parameters	Initial	15th day	30th day	45th day	60th day	75th day	90th day	120th day	180th day	F
TMA-N (mg/100 g)	2.89± 0.04	4.14± 0.06	4.46±0.09	5.88± 0.04	6.09±0.05	7.34±0.06	8.98±0.08	10.85±0.04	13.53±0.08	5278.3***
TVB-N (mg/100 g)	4.81±0.02	6.16±.06	7.39±0.06	8.19±0.05	11.32±0.06	11.5±0.01	13.94±0.12	16.87±0.03	27.93±0.03	12649***
pH	5.45±0.05	5.25±0.05	4.95±0.05	4.7±0.1	4.5±0.1	4.35±0.05	4.15±0.07	4.05±0.07	3.9±0.1	260.11***
TPC (log cfu/g)	2.38±0.04	2.62±0.01	2.72±0.08	2.91±0.03	3.18±0.05	3.33±0.02	4.02±0.05	4.22±0.07	4.4±0.04	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.

Table 4.8: Changes in organoleptic quality of *A. granosa* pickle

Organoleptic Characteristics	Initial	15th day	30th day	45th day	60th day	75th day	90th day	120th day	180th day	F
Appearance	9±0	9±0	9±0	9±0	9±0	8.9±0.3	8.9±0.3	8.5±0.2	8.2±0.1	25.05556**
Color	8.9±0.3	9±0	9±0	9±0	9±0	9±0	9±0	8.7±0.2	8.4±0.1	9.91667ND
Odor	8.8±0.3	8.8±0.3	8.7±0.5	8.5±0.1	8.3±0.3	8.2±0.4	8±0.3	7.8±0.1	7.4±0.2	27.61905***
Taste	8.8±0.3	8.9±0.2	8.8±0.3	8.7±0.4	8.4±0.6	7.9±0.7	7.5±0.5	7.3±0.1	7 ±0.2	25.3871**
Texture	8.2±0.4	8.5±0.1	8.3±0.3	7.8±0.1	7.5±0.2	7.2±0.3	6.9±0.6	6.6±0.2	6.1±0.3	93.08***
Flavor	8.8±0.3	8.9±0.2	8.8±0.3	8.4±0.2	8.2±0.4	7.5±0.5	7.2±0.8	7±0.1	6.6±0.3	39.5**
Overall acceptability	8.8±0.3	8.9±0.2	8.8±0.3	8.6±0.5	8.4±0.6	8.1±0.7	7.9±0.9	7.65±0.6	7.28±0.5	8.066667**

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

Table 4.9: Changes in organoleptic quality of *C. madrasensis* pickle

Organoleptic Characteristics	Initial	15th day	30th day	45th day	60th day	75th day	90th day	120th day	180th day	F
Appearance	8.9±0.3	8.8±0.3	8.7±0.4	8.7±0.4	8.5±0.1	8.5±0.1	8.2±0.4	7.7±0.3	7.2±0.2	25.0778***
Color	9±0	9±0	8.8±0.3	8.5±0.1	8.5±0.1	8.4±0.2	8.2±0.4	7.8±0.2	7.4±0.1	55.1164***
Odor	7.8±0.1	7.2±0.3	6.8±0.3	6.5±0.1	6.1±0.3	5.8±0.3	5.5±0.6	5.2±0.1	4.9±0.2	61.4321***
Taste	7.5±0.5	7.2±0.3	6.9±0.1	6.4±0.2	6.3±0.1	5.9±0.3	5.7±0.2	5.4±0.3	5±0.3	21.2344***
Texture	7.9±0.7	8.0±0.3	8±0.3	7.8±0.1	7.5±0.5	7.2±0.3	6.6±0.3	6.2±0.3	5.8±0.3	35.001**
Flavor	8.5±0.1	8.5±0.1	8.2±0.4	7.9±0.9	7.8±0.1	7.5±0.5	7.2±0.4	6.9±0.2	6.2±0.3	19.005**
Overall acceptability	8.3±0.6	8.1±0.8	7.9±0.9	7.6±0.9	7.5±0.4	7.2±0.7	6.9±0.8	6.5±0.3	6.08±0.4	33.1023***

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

4.2.1. Biochemical and microbiological analysis

The changes in the pH during storage of both pickles are given in Table 4.6 and Table 4.7. 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 increasing ($P< 0.001$) in pH with the increasing storage days of both the pickles.

TMA-N contents of both meat pickles were initially low such as 2.49±0.03 and 2.89±0.04 mg/100 g (Tables 4.6 and 4.7) and then gradually increased to 11.29±0.03 and 13.53±0.08 mg/100 g during the storage period. The statistical analysis showed that there was a significant difference ($P< 0.001$) between the days of storage in TMA-N contents of both the meat pickles.

The TVB-N values were found to be significantly increased with storage period in both the meat 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* meat pickle (Tables 4.7) and 4.81±0.02 to 27.93±0.03 mg/100 g in *C. madrasensis* meat pickle (and 4.7) during storage period.

The microbial qualities of both pickles are shown in Tables 4.6 and 4.7. The initial bacterial load (TPC) in *A. granosa* and *C. madrasensis* meat pickles was 2.25±0.0.04 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.

4.2.2 Organoleptic characteristics evaluation

The organoleptic characteristics of both the meat pickles are given in Tables 4.8 and 4.9. 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* meat pickle were significantly

changed during storage ($P< 0.00$ and $P< 0.01$).The appearance and color of blood clam pickle was very good throughout the storage period. However, appearance and color of oyster pickle were good throughout the storage period. The odor of blood clam pickle was remained good while the oyster pickle showed very fishy odor. Blood clam pickle remained satisfactory taste throughout the storage period. However taste of the oyster pickle was very fair at the end of storage. The texture of both pickled products became more spongy at the end of storage. No rancid flavor was found during the storage period of both pickles.

4.2.3 Economic analysis

The economoic analysis of pickle prepared from *A. granosa* and *C. madrasensis* was showed in table 4.10. 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.

4.10: 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
	Pach foron	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		98.7

Labor cost	40
Equipment cost	64
Utility charge	3
Total cost	**205.7**

Chapter-5

Discussions

Chapter-5

Discussion

5.1 The best pickled products prepared from blood clam (*A. granosa*) and oyster (*C. madrasensis*)

The present study revealed that pickles prepared from blood clam (*A. granosa*) and oyster (*C. madrasensis*) using mustard oil were the best pickle than pickles with sesame oil and soybean oil. Shikha *et al.* (2018) reported that thai pangus pickle prepared with mustard oil had higher acceptability than thai pangus pickle with soybean oil. The high acceptability of mustard oil for both pickles was due to the traditional taste and use of mustard oil in Bangladesh.

5.2 Shelf-life assessment of pickles from blood clam (*A. granosa*) and oyster (*C. madrasensis*) with mustard oil

5.2.1 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 (Gopal *et al.*, 1985). 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 both the pickles. Similarly decreasing trend of pH in Blood clam pickle by Gupta and Basu was 4.50 to 4.36.Chandrashekar *et al.* 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 Gopal *et al.* (1985) and Vijayan *et al.* (1989).

Trimethylamine (TMA) is one of the major components contributing to the odor of decomposing fish product (Hebard *et al.*, 1982). This volatile amine is produced by bacterial degradation of trimethylamine N-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 in both pickles. 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 & Patterson (2013). Chandreshekar *et al.* (1978) reported 2.38 to 6.72 mg/100g TMA-N for

Nemipterus japonicas pickle. Yellappa and Chandrasekhar (1989) 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 (NH_3) that are produced by both bacterial and endogenous enzyme (Lannelongue, 1980). The increase in TVB-N in the later stages of storage may attribute to the bacterial spoilage (Joseph *et al.*, 1998). The production of TVB-N resulted in the reduction of organoleptic scores too. The release of TMA and NH_3 produces some off odors in the food products. The present study showed 22.44±0.07 and 27.93±0.03 mg/100 g TBV-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 and from 9.80 to 32.20 mg/100g in fish pickle of *Nemipterus japonicus* is reported by Chandreshekar *et al.* (1978). Renitta & Patterson (2013) have reported 5.2 to 24.62 mg/100g 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 Yellappa and Chandrasekhar (1989).

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 & Patterson (2013) have reported similar increasing trend for *C. ramosus* and *H. pugilinus* pickles. However, Yellappa and Chandrasekhar (1989) have reported that the total counts were in the range of 10^3 to 10^4cfu/g in the clam pickle. Similar increase in TPC was encountered in the range of 10^3 to10^4 cfu/g in prawn pickle (Kumar & Basu, 2001).

5.2.2 Organoleptic characteristics evaluation

The present study revealed that *A. granosa* meat pickle had good appearance, dark brown color and no rancid flavor until the end of the storage period while and *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 liberation of total volatile bases by the action of spoilage bacteria. This correlates well with the mean panel scores and

became unacceptable. Renitta & Patterson (2013) 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.

5.2.3 Economic analysis

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 are 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 farmer 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 lime 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. Preparing pickle from blood clam and oyster can be a great earning source for the local lime farmer of southwestern part of Bangladesh.

Chapter-6

Conclusion and recommendation

Chapter-6

Conclusion and Recommendation

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 smell. These findings indicated that mustard oil is better than sesame oil and soybean oil for preparing mollusk pickle because its flavor is well known for any kind of pickle at this region. Both of the pickles had a longer shelf-life and were safe for human consumption up to six months. The longer shelf life may due to higher amount of spices and ingredients were added for the pickle preparation and higher amount of oil on the top layer of the pickle or due to proper sealing of the cap. The costs of preparing these pickles were also very 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.

Chapter-7

References

Chapter-7

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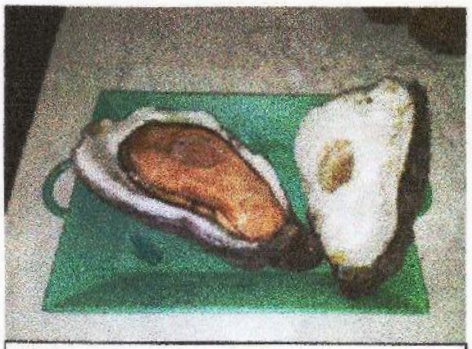
Appendix

Appendix

Photo Gallery

Processing of raw material

Oyster (*Crassostrea madrasensis*)

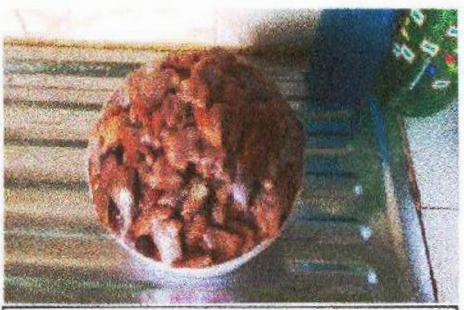


Shell removing

Flesh of oyster

Blood clam (*Anadara granosa*)

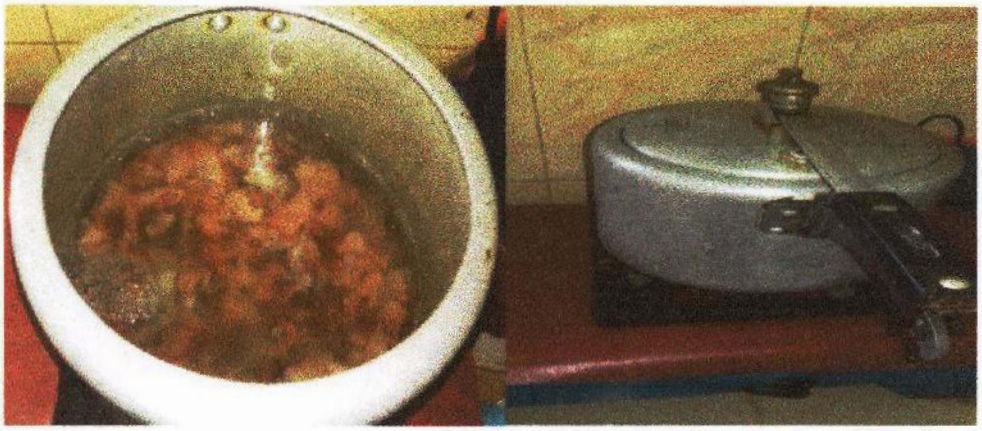
Shell removing



Flesh of blood clam

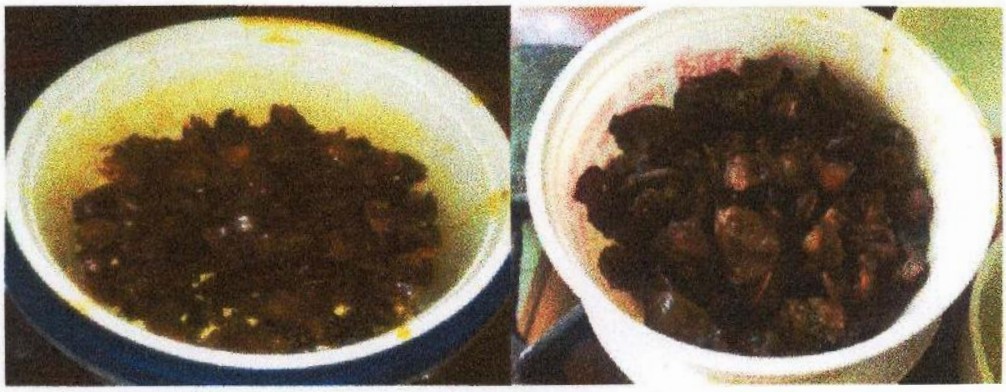
Pickle preparation

Cutting flesh into small pieces



Boiling in pressure cooker

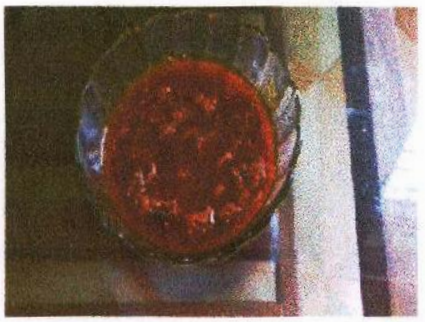
Marination of flesh with spices



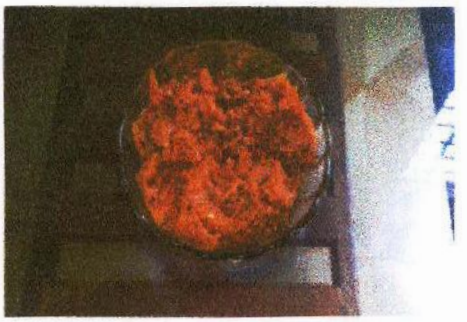
Fried meat

Ingredients used for pickle

Mixture of fried meat and spices



Clam pickle(Mustard oil)



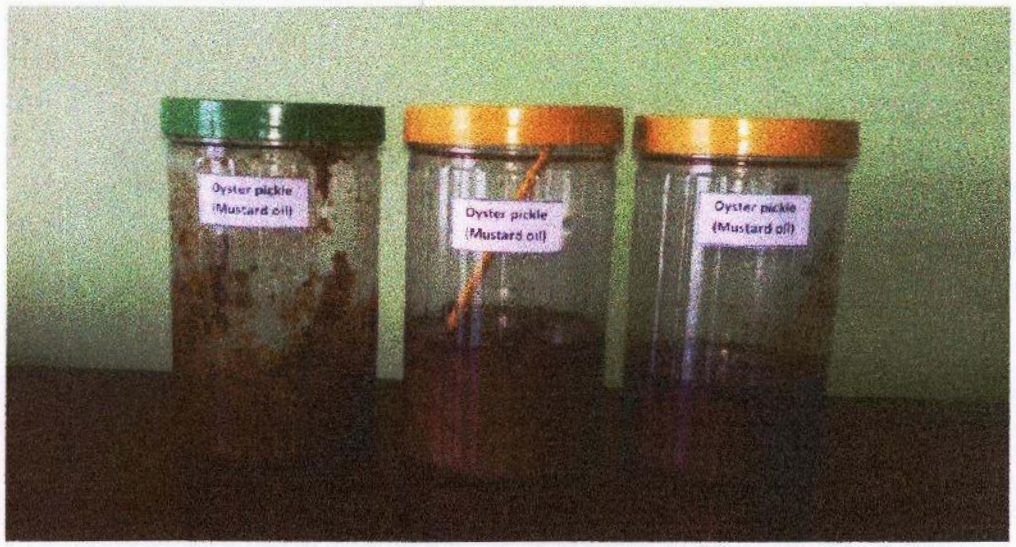
Oyster pickle(Mustard oil)

Sample storage

Clam pickle (Mustard oil)

Clam pickle (Sesame oil)

Clam pickle (Soybean oil)



Oyster pickle (Mustard oil)

Oyster pickle (Soybean oil)

Oyster pickle (Sesame oil)

TMA-N and TVB-N determination

Sample solution preparation

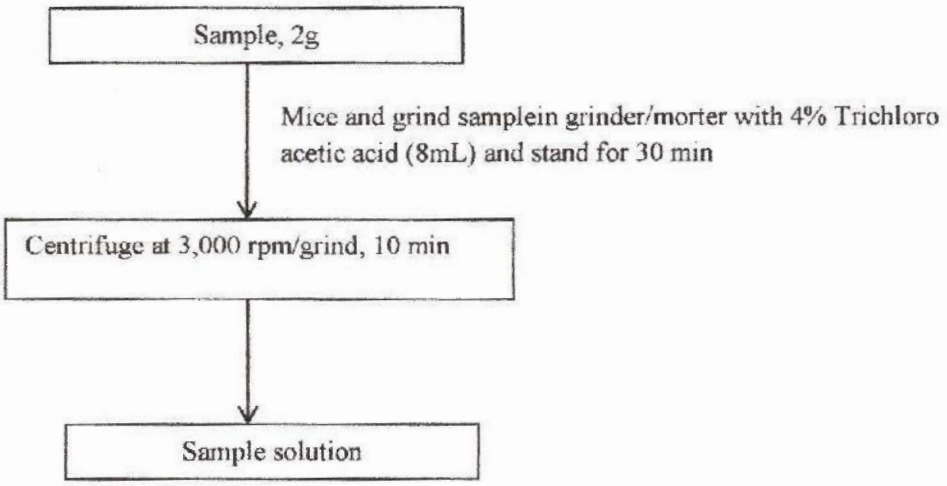
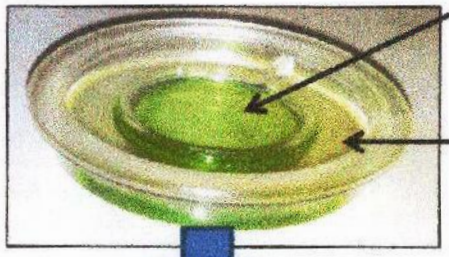


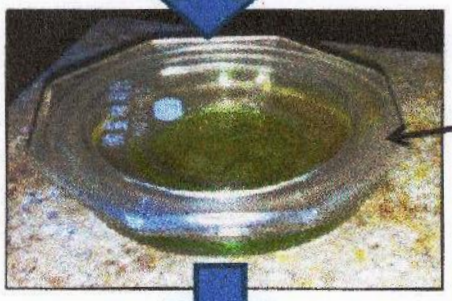
Figure 1: Flowchart of preparation of sample solution

Conway's Microdiffusion method



Inner ring solution 1 ml (1% boric acid with indicator)

Sample solution 1 mL + Outer ring solution 1 ml (Saturated K_2CO_3 and addition of 10 % formaldehyde for TMA-N)



Fixing the lid with tragacanth gum

Diffusion ← 37° C for 60 min in Incubator

Titration ← Inner ring solution against 0.02 N HCl

Calculation of TMA-N, TVB-N (mg/100g)

Figure 2: Flow chart of TMA-N and TVB-N determination

Calculation

$$\text{TMA-N or TVB-N (mg/100g)} = (V_s - V_B) \times (N_{HCl} \times A_N) \times \frac{[(W_S \times \frac{M}{100}) + V_E] \times 100}{W_S}$$

Where,

V_S = Titration volume of 0.02 N Hcl for sample extract (mL)

V_B = Titration volume of 0.02 N Hcl for Blank (mL)

N_{HCl} = Normality of HCl (=0.02× factor of HCl)

A_N = Atomic weight of Nitrogen (14)

W_S = Weight of sample (g)

M = Moisture (%) of sample

V_E = Volume of 4% Trichloro acetic acid

Sensory evaluation

Honorable Professors from Fisheries and Marine Resource Technology Discipline, Khulna University evaluated the sensory characteristics of pickle