D.N.R COLLEGE (A) BHIMAARAM

DEPARTMENT AQUACULTURE

III BSC PRACTICAL MANUAL

PAPER-7 POST HARVEST TECHNOLOGY IN FISH AND FISHERIES

1. Preparation of Isinglass

Introduction

Air bladder (fish maws) usually in the dried form serves as the raw material for making isinglass. Isinglass is probably the most important product from bladder and is chiefly used as a clarifying agent for liquors.

Objectives

After performing this experiment, you will be able to:

- prepare isinglass from air bladder; and
- identify some of its propert

Principle

The hard dried bladder must first be softened by soaking in water. The treatments given either for drying bladders or for converting to isinglass should not adversely affect the quality of the protein, collagen, contained in it. It's this collagen that is responsible for the special properties of isinglass. Air bladder in the softened form is flattened to a low thickness, followed by drying.

Requirements

- Dried fish maws
- Knife, scissors, vessels, polyethylene bags, etc.

- Balance
- Rollers (or roller press with cooling facility, if available)
- Drier
-

Procedure

1) Weigh the dry fish maws.

2) Soak in chilled water for several hours until it become soft.

3) Cut into small pieces or strips.

4) Press the material on a hard surface using rollers. Reduce the thickness in stages down to less than 1/16 inch. Prevent any temperature rise by dipping the material in chilled water in between pressings.

5) Place in drier at 40–45ºC. Dry slowly to a moisture content of less than 10%. The material becomes hard dried as 'leaves'.

6) Weigh and pack in plastic bag.

Test for the ability to form gel*:* Put a sample of isinglass in hot water (above 80ºC). After dissolution, keep the solution in fridge (chilled). Observe for formation of jelly.

Observations

Weight of dry fish maws (x) =

Weight of isinglass (y) =

Yield of isinglass (y/ x × 100) = ……%

Solubility in hot water : soluble/insoluble

Gel formation upon chilling : gel formed/remains as liquid

Results

The yield of isinglass from fish maws is ..... %

The isinglass prepared is capable of ..... in hot water and forming

a ..... upon chilling.

Precautions

- Take care to keep the temperature low throughout processing.

Preparation of Isinglass3 Precautions

Q.2 PREPARATION OF SALTED AND DRIED FISH

INTRODUCTION

Salting is a traditional method of processing and preservation of fish in many countries of the world, using common salt. The process involves a group of operations, wherein the fish is subjected to washing, gilling, gutting, mixing with salt and packing in a suitable container. Salting is also a sort of preliminary process in the preparation of other products like canned fish, smoked fish, frozen fish, etc., wherein the shelf life of the product is extended by:

1). Dehydration of fish due to removal of moisture content.

2). Exerting osmotic pressure on the microorganisms, leading to plasmolysis of bacterial cell.

3). Reducing the action of proteolytic enzymes of fish, due to high salt concentration.

4). Salt on ionization yields chloride; which is harmful to the microbes.

Objective

After performing this experiment, you will be able to Precautions

prepare salted and dried fish from the given fish sample.

EXPERIMENT

Principle

The experiment enables you to understand the basic methodology of preserving fish in the traditional methods.

Good quality cleaned fish

Requirements

Good quality cleaned fish

Salt

Preservatives

Packaging materials

Electronic weighing balance

Procedure Raw material : Good quality fresh fish.

Washing : Wash the fish with clean fresh water, to remove dirt, slime, etc.

Weighing : To calculate the yield using weighing balance.

Butterfly style : The fish is split through the dorsal line from the root ofdressing the tail to the tip of the snout and the gills and viscera removed. The flesh may be scored, as far as the skin and not through the flesh. The fish may be scaled before splitting.

Washing : Wash in clean water to remove blood, slime, etc.

Draining : Drain the excess moisture on the surface of the fish.

Mixing : Mix the fish with salt and preservatives in the ratio of 1:4 (Salt: Fish) in a shallow box. More salt is required in the thick parts of the fish than in the thin parts. Salt is applied to the cut portion and

rubbed over the surface. Salt and fish are arranged in alternate layers. Small amount of salt is added at the bottom and the quantity can be increased as the height increases. At the top more quantity of salt is added. The fish are kept in layers, in such as way that their skin face the bottom and the top layer fish, with skin side up and kept for 12-48 hours, depending on the fish, market preference, weather and working conditions.

Washing : Wash the salted fish in 10% brine or clean sea water to remove large salt crystal from the surface of fish.

Weighing : To calculate the loss of moisture due to salting.

Drying : The salted fish are dried under sun for 1 week or in hot air oven for 5-10 hours (at 70ºC) till the moisture content reduces to about 30-40%.

Weighing : To calculate the dried fish yield.

Storage : The dried fish are stored in polyethylene gunny bags.

Preservatives added to salt used for dry salting of Fish:

1) Sodium Benzoate (0.25%) - To prevent halophilic bacteria.

2) Sodium Dihydrogen Phosphate (1.5%) - To enhance the activity of sodium benzoate. 3) Potassium Sorbate (0.5%) - To prevent mold growth.

Observations

Shelf life: The shelf life of salt dried fish, stored at room temperature ranges from one week to around six months, depending in the salt content, fat content, moisture content, packing and the storage temperature of the product.

Calculations Weight of Raw Material : ‘a’ g

Weight of Dressed Fish (Butterfly style) : ‘b’ g

Dressed Fish Yield (%) : ‘b’/’a’ × 100

Weight of Salt used : ‘z’ g

Weight of Fish after Salting : ‘c’ g

Weight of Fish after Drying : ‘d’g

Quantity of Moisture Loss due to Salting : ‘b’ – ‘c’g

Quantity of Moisture Loss due to Drying : ‘c’ –‘d’ g

Percentage of Moisture Loss due to Salting : ‘b’ – ‘c’/’b’ × 100

Percentage of Moisture Loss due to Drying : ‘c’-‘d’/’c’ × 100

Dried Fish Yield (%) : ‘d’/’a’ × 100

Results

Dried fish yield of given sample is ..... %

3. PROCESSING & FLOW- CHART

Introduction

Fish is found abundantly in all natural waters and is a valuable source of food. Out of about 25,000 species of fish, 250 species of fish are used for edible purpose. Edible fishes are classified into two major categories as fishes having vertebrae with fin appendages are termed as finfish and fishes without skeleton but covered with hard shell are termed as shellfish. Edible shellfishes are mainly salt water fishes.

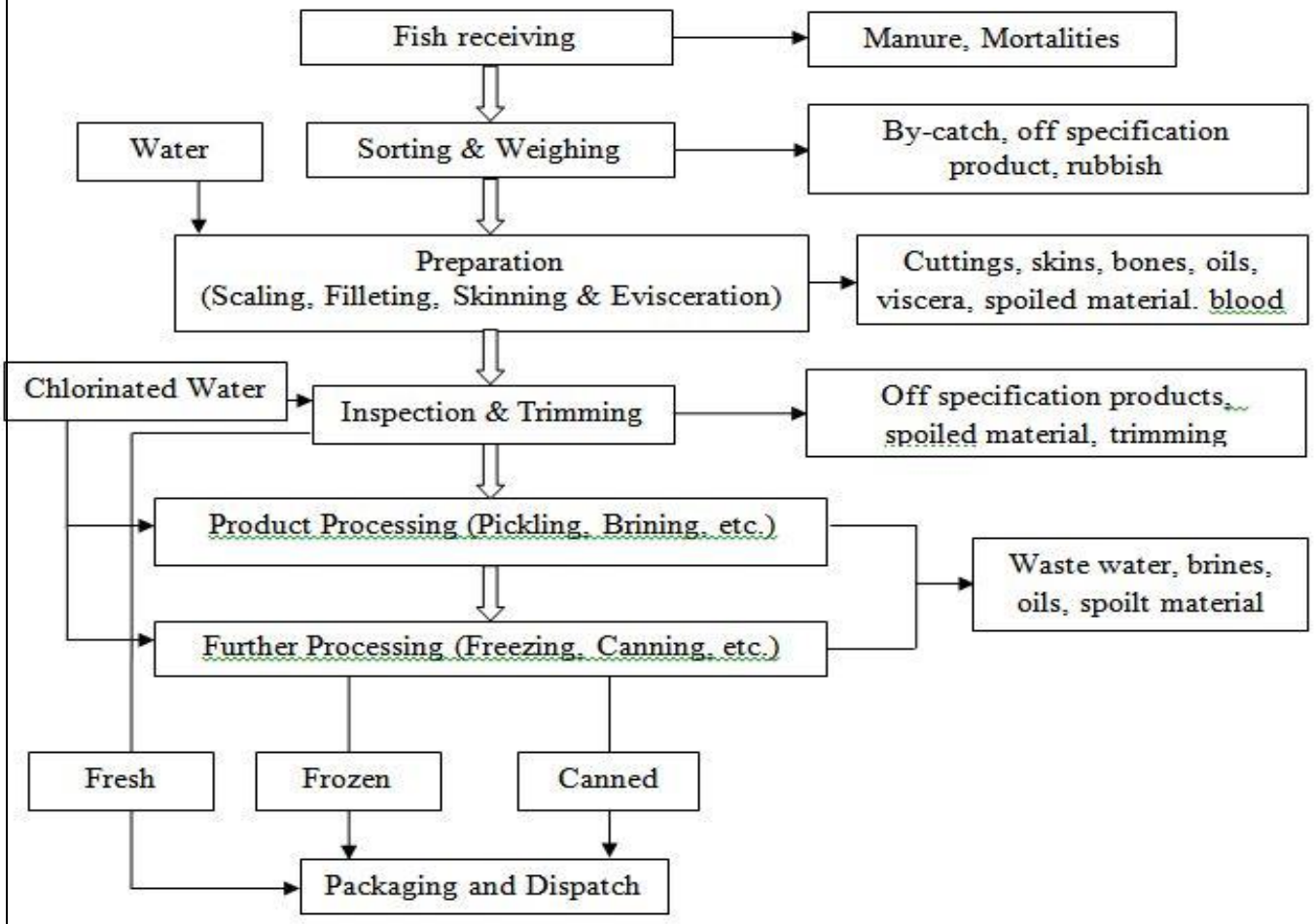
Primary Processing of Fish and Sea Food

Pre-treatment: Fish are kept on ice in boxes before delivery to the processing plant. At the time of arrival, the fish may be re-iced and placed in cold storage until required for further processing. Pretreatment involves ice removal, washing, grading according to size and de-heading. Large fish may also be scaled before additional processing. Some fish are skinned by immersing in to a warm caustic bath. The effluent from this process has a high organic load and has to be neutralized before discharge.

Filleting: The filleting areas are generally separated from the pretreatment department to prevent workers and materials from non sterile pretreatment from contaminating the sterile filleting area. Filleting is performed by machines with mechanical knives that cut the fillets from the backbone and remove the collarbone. Some fillets may be skinned at this step in this process.

Trimming and Inspection: In the trimming section, pin bones are removed and operators inspect the fillets. Any defects and any inferior parts found are removed. Off cuts are collected and minced. Depending upon the final products, the fillets can be cut in to portions according to weight or final product requirements.

Storage/Packaging (Fresh): Fresh products are packaged in boxes with ice which is separated from the product by a layer of plastic. Fillets or pieces can be individually frozen and wrapped in plastic. The blocks are typically frozen and kept in cold storage.



Flow Sheet of Fish Processing

Preservation Methods of Fish

Fish is a perishable commodity. It can not be store in normal condition for longer period. There are some in practice to preserve fish which is explained hereunder.

Canning of Fish

Canning is performed by two methods: precooking and raw pack. Precooking begins with thawing of the fish which are then eviscerated, washed and cooked. Canning retains the natural flavor of the fish usually oily fish are most suitable for canning. Cooking occurs with steam, oil, hot air or smoke for up to 10 hours, depending upon the fish size. The cooked fish are then cooled. After cooling, the head, fins, bones, and undesirable meats are removed and remainder is chopped / cut and placed in cans. Additional fish or vegetable oil, brine, and/or water are added to the cans which are sealed and pressure-cooked before shipment.

The raw pack method begins with thawing and weighing of the fish. Fish are then washed and brined as well as "nobbed," which is the removal of the head, viscera and tails. The fish are placed in cans, then cooked, drained and dried. After drying, liquid (brine, oil, sauce, water) is added to the cans. Finally, the cans are sealed and sterilized with hot water or steam and then stored.

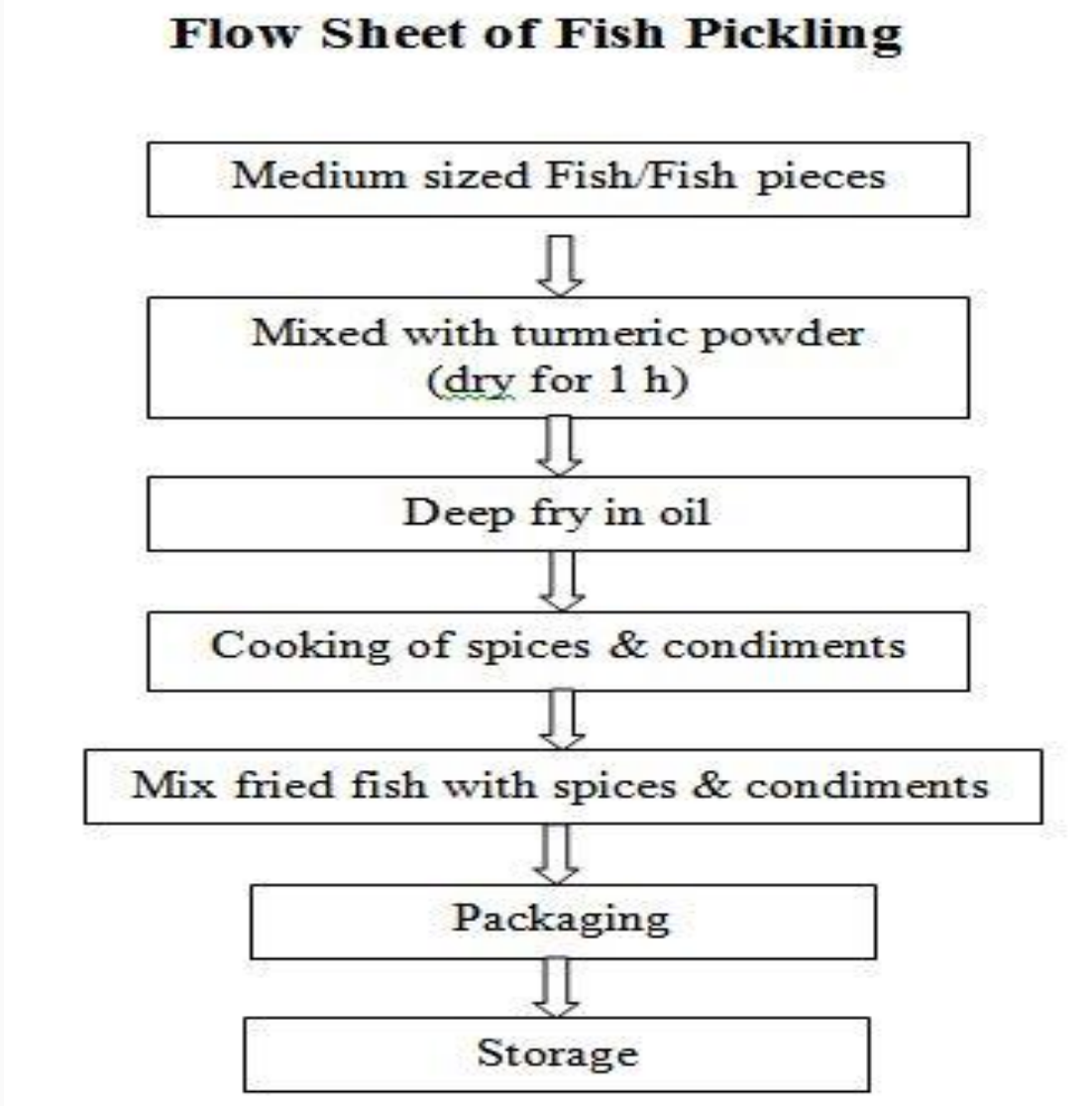
Pickling (Salting) of Fish

The dry salting or wet salting method of pickling is widely used for fish processing in India. In dry salting method, first fishes are rubbed with salt powder and then packed in tubs with dry salt powder sprinkled in between layers of fishes. After 15-20 hours, the fishes are removed from tubs, washed in brine and then followed by sun drying for 2-3 days.

In wet salting cleaned fishes with longitudinal silts (cut) are packed in large container which containing concentrated salt solutions and stirred

daily till properly pickled. Generally, up to 8-10 days after pickling, salty water is allowed to drain off. Wet salted (pickled) fish is sold into market without drying. During salting ethylene oxide and sodium benzoate may be added to avoid some of the common defects like color changes, mold growth, attacks by insects, case hardening, rancidity development etc.

Freezing of Fish



Freezing, greatly extend the period of storage. In freezing, if the fish is gutted and frozen down to -28 to -30^{0}C within two hours of its catch gives effective keeping quality as similar to that of fresh fish. Large fish are frozen by sharp freezing, while small fishes are usually frozen as fillets (lengthwise cuts), steaks (crosscut section) or sticks (lengthwise or crosswise cut from fillet or steaks) are quick frozen. During freezing some undesirable changes may occur, if proper care is not taken. Slow freezing can result in protein de-naturation due to increases in salt concentration in muscle tissue during freezing and also making it tough and rubbery. Frozen fish undergo oxidative changes and fatty fish become rancid more quickly so oxidation can be prevented by properly protecting or covering of fish with suitable packaging materials (wrappers) before freezing.

Drying of Fish

Sun drying is the most widely used method for drying. Drying removes moisture from tissues and arrests the bacterial and enzymatic growth. This method is not hygienic and also there is appreciable percentage of loss due to spoilage and putrefaction. Dried fish develops a peculiar odor. The fish can be dried in the covered solar dryer with natural draft or forced draft.

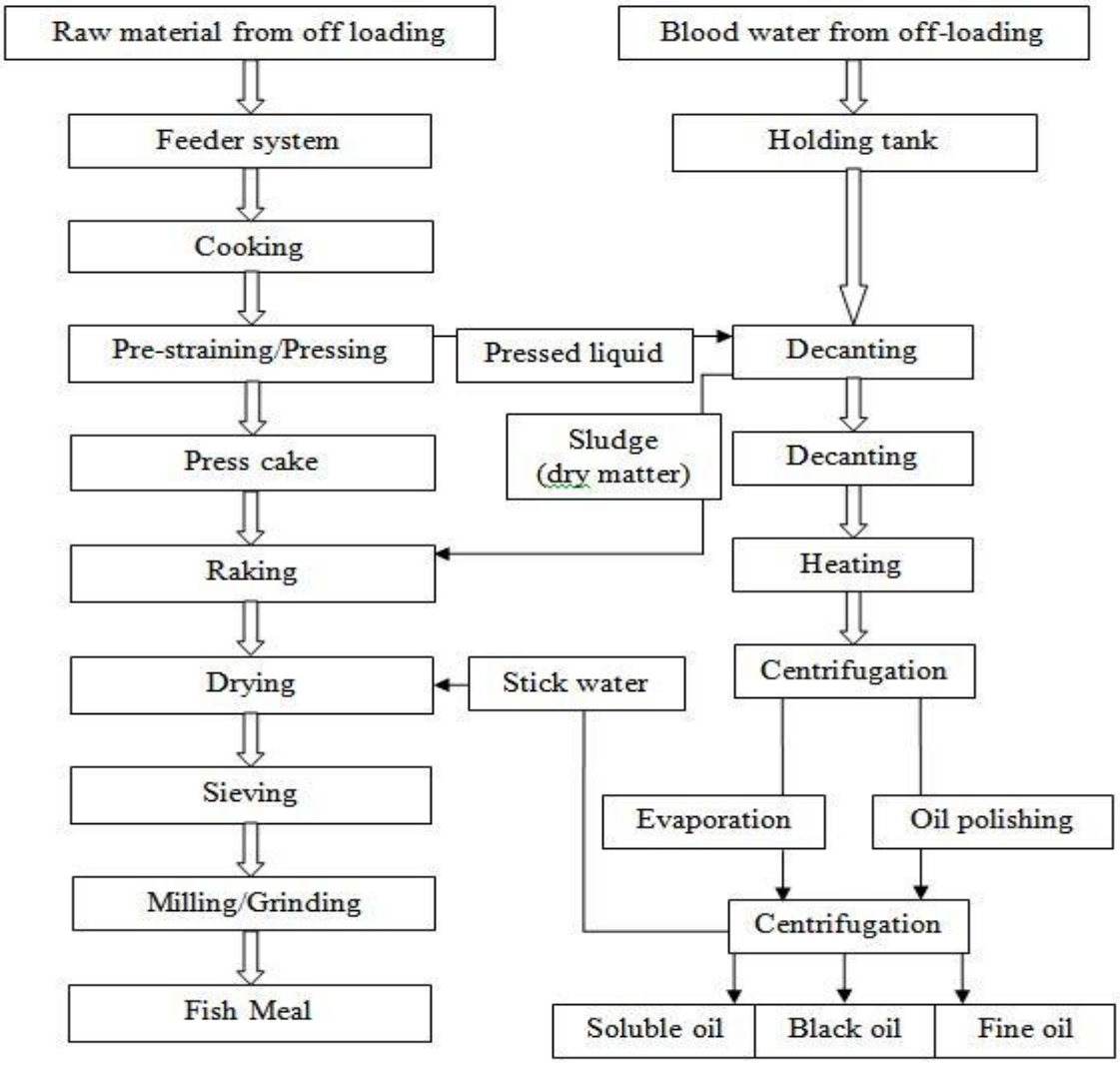
3. Manufacturing of Fish Products

Fish Meal and Fish Oil Production

Fish meal is delivered from the dry components of the fish and the oil from oily component. The water that makes up the remainder of the fish matter is evaporated during the process. The fish by-products are cooked in a process that coagulates the protein and releases the water and oil. The mixture is screened and the liquid from the mixture is squeezed out through a perforated casing. The pressed cake is shredded and dried with steam or direct flame dryers. The meal passes through a vibrating screen

and to a hammer mill where it is ground to the desired size. The meal is used in animal and pet feed due to its high protein content.

The oil is further processed by passing through a decanter to remove sludge which is then fed back in to the meal dryer. Oil is separated from the liquid by centrifuge and is polished by using hot water washes and additional centrifuging. The removed water is evaporated to concentrate the solids and the remaining oil is refined to remove any impurities.



Flow Sheet of Processing of Fish Meal and Oil

Fish Flour (Fish Protein Concentrate)

Fish flour contains 85 to 90 per cent of high quality protein. The extraction of oils and fatty substances from ground fish tissue by solvent extraction till no fishy odor or flavor remains. The fat extracted tissue is dried after evaporation of solvent by dehydration process using dryer. After dehydration, milling gives a bland highly nutritious powder rich in high quality protein and minerals. This product, when processed under sanitary conditions is good for use as human consumption.

4.Microbiological and chemical quality of a traditional salted fermented fish

Introduction

The aim of this study was to study and identify the micro flora associated with salted fermented fish and to determine the proximate composition, salt, total volatile basic nitrogen (TVB-N) content, thiobarbutric acid number (TBN) and pH, of the salted fermented fish product of Jazan

Materials and methods

Preparation of salted fermented fish

The whole, fresh fish is cleaned, gutted and covered with salt and arranged in alternate layers either in wooden boxes and then left to ferment on fishing boat for month or more, depending on ambient temperature. Liquid exudates from the fish during fermentation time are allowed to drain off.

Sample collection

Twenty-four salted fermented fish samples were purchased from fish market in Jazan and Abu-Arish at different times of the year. Samples were transferred to the Laboratory at King Saud University in Riyadh for analysis.

Microbiological analysis

Samples for microbiological analysis were prepared by homogenizing 10 g of fillet-salted fermented fish in 90 ml of 0.1% peptone water (oxoid) using stomacher lab – Blender 400 (Seward Medical, London, U.K) for 30 s at a normal speed. Decimal dilutions were prepared using sterile 0.1% peptone water solution. 1 ml of the homogenate was used for enumeration of microorganisms. Counts were done by using pour plate and spread techniques as described in the Compendium of Methods for

Microbiological Examination of Foods and Food and Drug Administration .

Total aerobic bacterial count

Total aerobic bacterial count was determined using the pour plate technique. Plate Count Agar (Oxoid, CM463) was used as medium. Plates were incubated at 32 °C for 48 h.

Total coliform count

Total coliform count was determined on violet red bile agar (Oxoid) using pour plate technique and an overlay with the same agar after solidifying. Plates were aerobically incubated in inverted positions at 37 °C for 24–48 h.

Staphylococci *spp.*

Staphylococci spp. were determined on Baird Parker Agar (Oxoid CM 275) supplemented by egg yolk tellurite at 37 °C for 24–48 h. Typical black colonies with zones around and atypical black colonies were considered as Staphylococcus spp.

Yeasts and molds

Yeasts and molds were enumerated on acidified potato dextrose agar (Oxoid). Plates were incubated aerobically upright at 25 °C for 5 days.

Halophilic bacterial count

Halophilic bacterial count was done using Plate Count Agar medium (Oxoid-PCA) with 10% NaCl. Plates were incubated at 37 °C for 3 days.

The presence of pathogenic bacteria

The presence of pathogenic bacteria such as vibrio spp., Campylobacter spp, and Listeria monocytogenes was checked using methods described in .

Microbial identification

Representative colonies were selected from plate counts according to shape and color. Selected colonies were isolated by transferring and streaking on nutrient agar until culture purity was established and further examined by Gram stain.

Characterization and identification of staphylococcal species was carried out using API ID 32 Staph and API CH50 kit was used for identification of bacillus species (API system–BioMeurix, Marcy l'etoile, France).

Chemical analysis

Proximate composition

The proximate analyses of 24 salted fermented fish samples were carried out in duplicate. The moisture, crude protein (Nx6.25), ash, and fat contents were determined according to AOAC (1990).

Determination of salt

The percentage of salt was determined according to the procedure described by Pearson (1973). One gram of sample was weighed accurately and mixed with 50 ml of distilled water. The mixture was titrated with 0.1 N silver nitrate using 0.5–1 ml of 5% potassium chromate to the first appearance of a slight orange color against the yellow color of the indicator. The percentage of NaCl in the sample was calculated as follow: 1 ml of 0.1 $AgNo_3$ = 0.005845 g of NaCl.

Determination of total volatile basic nitrogen (TVB-N)

The determination of TVBN was conducted according to the procedure of Pearson (1973). 10 g of sample was blended with 50 ml of fresh tap water; the blender was washed with 250 ml of fresh tap water into the distillation flask and 1–2 g of MgO was added to the mixture. TVB-N was released by boiling the mixture with magnesium oxide, which prevented

volatile acids from distilling over into the boric acid. The distillate of volatile nitrogen was received in 25 ml of boric acid 2.0% then titrated by 0.1 N sulfuric acid .The result was calculated as follow: TVB-N (mg N/100 g) = Titration (ml of 0.1 N acid) × 14.

Determination of thiobarbituric acid number (TBA)

The method of Pearson (1973) was used for determining the TBA in salted fish. Ten gram of sample was mixed with 50 ml of water in a mechanical blender then the mixture washed by 47.5 ml of water into a suitable distillation flask and 2.5 ml of 4 N HCl was added to bring the pH to 1.5. After distillation about 50 ml in 10 min exactly from the boiling time commences. Five milliliter of distillate was boiled with 5 ml of TBA reagent (0.2883 g of thiobarbituric acid in 100 ml of 90% acetic acid) for about 35 min exactly. The absorbance was recorded at 538 nm and the number of TBA was calculated as follow:TBA number(mgmalonaldehyde/kgsample)=7.8×absorbance.

Measurement of pH

The pH of the samples, was measured using a digital pH-meter (JenwayPH10 flested UK), after calibration using standard buffer solutions of pH 4 and pH 7.

Results and discussion

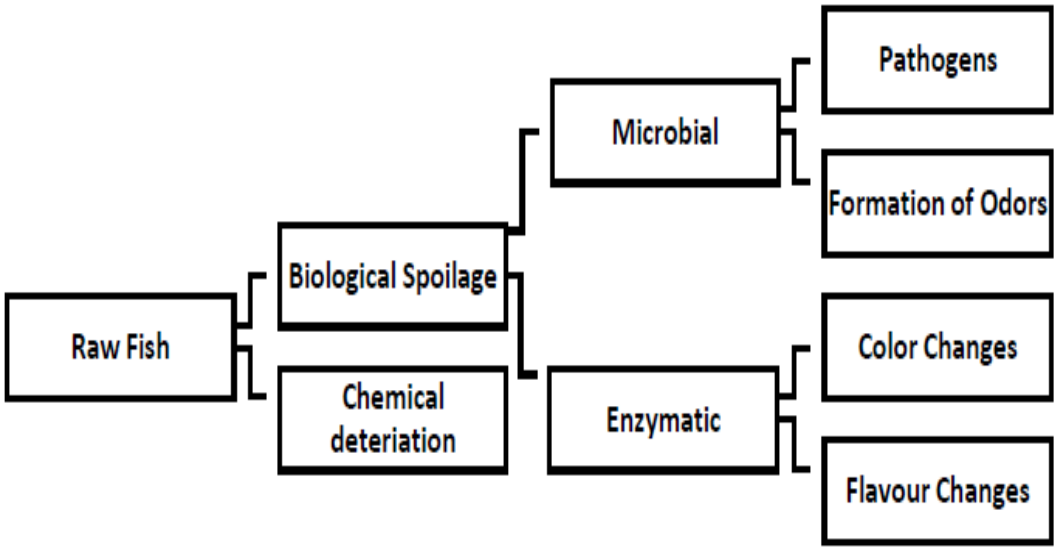
Microbiological analysis

Results of microbiological analysis are given in The microflora of salted naturally fermented fish consisted of various species of micro-organisms such as aerobic, halophile, and staphylococcal bacteria, yeasts and molds

5. Evaluation of fish / fishery products for organoleptic, chemical and microbial quality

Fish being a highly perishable product, the quality of the fish starts to deteriorate after the fish is brought to the landing sites. The deterioration of the fish occurs due to:
(1) Bacterial invasion and putrification,
(2) enzymatic autolysis,
(3) chemical oxidation and
(4) Mechanical damage and environmental optimization aggregating growth of microbe

Existing Tecchniques For Quality Control Attributes for

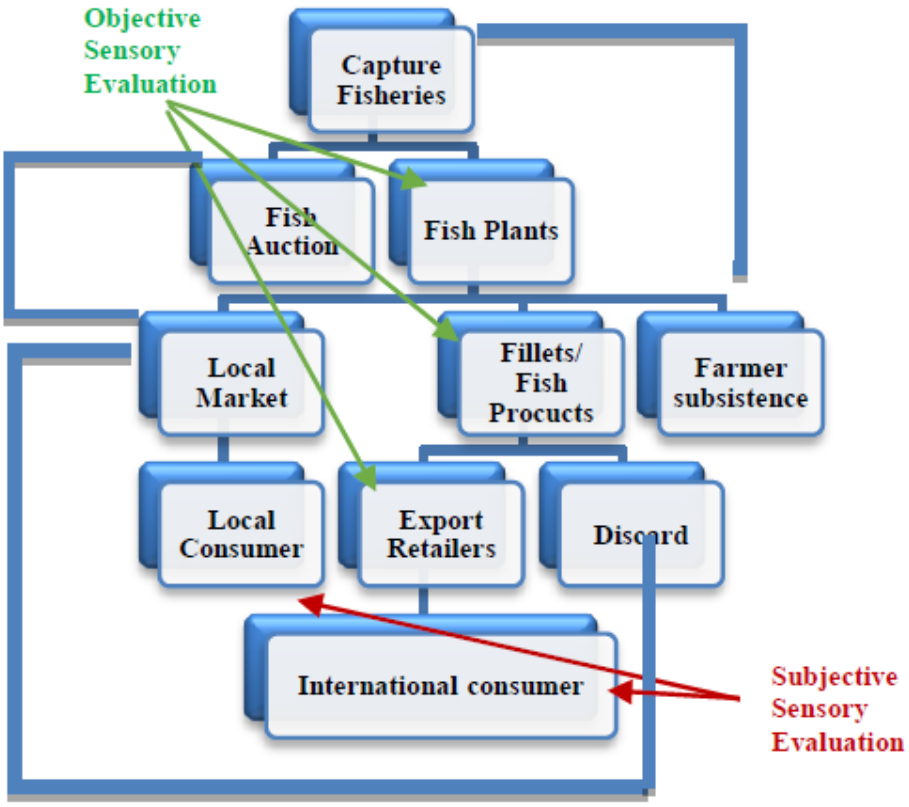


Attributes for assuring the quality of fish

Sensory evaluation measures the freshness of fish and fish products with respect to the five distinct senses including taste, smell, feel and appearance. Sensory evaluation of freshness are widely used attributes for ensuring quality of fish. Sensory assessment can either be objective or subjective but for successful marketing, both are considered. In case of objective sensory assessment, trained personnel are used to classify freshness, whereas, in subjective sensing, one is based upon consumer satisfaction and market analysis of fish markets.

Fish Supply Chain and Sensory evaluatio

ORGANOLEPTIC METHODS



Fish Supply Chain and Sensory evaluation

MICROBIAL ASSESSMENT/BIOLOGICAL EVALUATION:

Fish decomposition starts after catch starts and changes occur in pH, atmosphere, nutrient composition have effects on micro flora. Raw fish consists of its own unique flora, determined by the microbial content of the surrounded water, which still remain despite food processing and subsequent cooling. Categorization of fish with similar microflora is considered when assuring the quality of Fish and its products. Microbes play a vital role in the shelf life of fish as gram-negative, fermentative bacteria (such as *Vibrionaceae*) spoil unpreserved fish, whereas psychrotolerant gram negative bacteria (*Pseudomonas* spp. and *Shewanella* spp)can continue growth in chillers. Several standards are utilized to judge its freshness and adequacy.EU advised Bangladesh Government to implement the Hazard Analysis Critical Control Point (HACCP) in the processing of frozen fishes.

MICROBIAL EVALUATIONS:

Total plate count (TPC): Total number of microbial flora is changed with the time in fish or fish products. The numbers per gram of fish or fish products or per square centimeter of the surface area indicate the quality of fish from the microbiological view point. Total plate count or viable bacterial count is determined by the culture of bacteria present in fish sample using a suitable bacteriological media that could recover maximum number of bacteria in fish tissue. A known weight of fish sample is minced aseptically and serial decimal dilutions are pour-plated with the media. For marine fish agar agar and for processed fish products tryptone glucose beef extract agar media are commonly used. Inoculated plates are incubated
at 370 C for 24 hours and the bacterial colonies are counted.
From the colony counts, TPC is calculated by multiplying with appropriate dilution factor. TPC does not strictly indicate the edibility of the fish. Fish with low TPC may bear pathogen that would have more dangerous if consumed.

Biochemical methods

A variety of chemical compounds or groups of compounds accumulate post-mortem fish
muscles. These chemicals are either intermediate or end products of biochemical changes
occurring in the muscles of fish after death.

Proximate composition: Because of influence of chemical composition on keeping quality, proximate chemical composition like moisture, lipid, protein and ash contents of fish samples from the time or day of harvest to different storage periods or conditions are often investigated. Proximate composition may vary with species, sex, season, place of harvest, feeding condition, etc. So, conclusive results are very difficult to obtain.

Hypoxanthin value: As a consequence of post-mortem changes, breakdown of ATP to ADP, AMP, IMP and finally to hypoxanthin takes place. Hypoxanthin content of muscle increases on storage of fish. Estimation of hypoxanthin is an objective test of freshness of fish. However, the estimation of hypoxanthin is too cumbersome and it is seldom employed in practice. Fish with a hypoxanthin value of 7-8 micro moles/g is considered spoiled.

Histamine content: Histamine develops in freshly caught fish after 40-50 hours of death, if the fish is not properly iced. To avoid histamine formation in tuna, skipjack and mackerel, care is taken to ice or freeze fish as quickly as possible. Histamin is a major problem in warm water pelagic species that causes a form of food poisoning known as scombroid poisoning, as the name derived from the family name of tuna and mackerel, scombroidae.

pH: Change in pH of the fish muscle is an usual good index for freshness assessment.

6. DETERMINATION OF MOISTURE

INTRODUCTION

there are various methods to determine the moisture content such as drying methods, distillation method and kahr-fischer titration method. the determination depends on the following criteria:

a) The form in which water is present
b) Nature of product analysed
c) Rapidity of determination
d) Accuracy desired
e) Availability and cost of equipment required.

in the drying method the amount of moisture in foods is the difference between the weight before and after drying. it is simple and is used (as a standard method) for many kinds of foods. the process of drying is caused by the difference of the relative humidity between a food and the atmosphere, so that the higher the temperature, the faster the drying. some fermented products are unstable and decompose at high temperature.

such fermented products are dried at 40-70° under vacuum. on the other hand cereals are stable at high temperature and are dried at 135°c under normal atmosphere.

fish and fish products are normally dried at 100-110°c. simple and rapid drying methods by oven, infra-red balance and microwave moisture checker are used for the drying of fish products.

Apparatus

method 1 : oven of temperature range 100-150°,
aluminium dish with lid,
chemical balance (100g),
desiccator with some moisture absorbent (silica gel, calcium-chloride, concentrated sulfuric acid etc).

Method 2 : Infra-red balance (Kett, model F-1A).

Method 3 : Microwave moisture checker (Anritsu, model K377C).

Sample Preparation

Collect meat sample (≤ 100g) and pass 2-3 times through food mincer, or chop very finely and mix thorough

PROCEDURE AND CALCULATION

METHOD 1 : OVEN METHOD

1. Dry the empty dish and lid in the oven at 105°C for 30 min and transfer to the desiccator to cool (30 min). Weigh the empty dish and lid to 3 decimal places.
2. Weigh about 5 g of sample prepared to the dish. Spread the meat with spatula. Replace the lid and weigh the dish and contents to 3 decimal places.
3. Place the dish with its lid partially covered in the oven. Dry for 16 hrs or overnight at 105°C.
4. After drying, transfer the dish with partially covered lid to the desiccator to cool (about 45 min). Reweigh the dish and its dried content.

CALCULATION

$$\text{Moisture (\%)} = \frac{W1 - W2}{W1} \times 100$$

7. DETERMINATION OF SALT

Introduction

sodium chloride (food grade) is an important additive for the production of fish jelly products. its main function is to extract the salt soluble protein to give the gel strength of the final product.

the amount of sodium chloride present in such products can be determined by titrating the extract containing the chloride ion with silver nitrate, agno3. potassium chromate (k2cro4) is used as the indicator and the end point is indicated by the change in colour from yellow to reddish brown.

preparation

collect fish jelly products sample (≤ 100 g) and pass 2-3 times through food *mincer, or* chop very finely and mix thoroughly.

reagents

all reagents should be of gr grade or ar grade:-

a) 0.1n silver nitrate (agno3) solution

dissolve 17 g of agno3 in distilled water and make up to 1 litre in volumetric flask. keep it in a brown colour glass bottle in the dark.

b) potassium chromate indicator, k2cro4

dissolve 5 g k2cro4 in distilled water and dilute to 100 ml.

procedure

1. weigh accurately 25 g sample into a 400 ml beaker.
2. add 200 ml hot boiled water and stir for 60 mins.
3. filter through the glass wool. collect the filtrate in a 250 ml volumetric flask. make up to the volume and shake well.
4. transfer 10 ml filtrate with bulb pipette into 100 ml conical flask. add 50 ml distilled water using the measuring cylinder and 1 ml k2cro4 indicator.
5. Titrate with 0.1N AgNO3 (S ml). At the end point, the colour changes from yellow to

brownish red.
6. Carry out a blank determination using 60 ml distilled water and 1 ml K_2CrO_4 indicator (Bml).

Calculation

$$\text{Salt (\%)} = \frac{250 \text{ ml}}{10 \text{ ml} \times 25 \text{ g}} \times (S-B) \times F \times 100$$

where S = Titration volume of sample (ml)
B = Titration volume of blank (ml)
F = Conversion factor of 1 ml 0.1N $AgNO_3$ to 0.005844 g NaCl

8. Estimation of different types of spoilages on afish

Introduction

fish is a highly perishable commodity; spoilage of fish begins as soon as fish
dies; in tropical conditions fish spoils quite rapidly, within a few hours of landing
if it is not properly cooled. the spoilage rate of fish may be reduced by following
good handling practices and effective temperature control from the very beginning.
three type of spoilage occurs in fish:
1) physical spoilage
2) chemical spoilage
3) microbiological spoilage

Objective

after performing this experiment, you will be able to:

- Identify the different types of spoilages in a fish sample.

Principle

the main idea behind is to understand and evaluate the different types of spoilage
in fish.

Requirements

- Fish in adequate quantity
- Chemicals needed for estimation of chemical indices of spoilage
- Bacteriological laboratory for microbial examination

Procedure/observations

i) estimation of physical spoilage by organoleptic or sensory evaluation
method
many attempts have been made to establish a reliable test for determining the
degree of freshness in sea food. no objective test has been agreed upon as yet,
that consistently correlates with the subjective taste-panel method.
procedure – through observation of the various organs and then estimation of
freshness using the following key.

sl.no. parameter fresh fish spoiled fish

1) Gills red brown or grey, slime formation
2) Eyes protruded sunken, opaque, cloudiness is seen
3) Belly portion firm soft, yellow colour is seen
4) Scales sticking comes out easily
5) Odour good bad

ii) Estimation of chemical spoilage by following methods

after the death of fish, the spoilage bacteria invade the fish muscle, and produce
many chemical compounds, these compounds gradually accumulate in flesh;
hence their determination provides a measure of spoilage. these are known as
quality indicators and their level in fish muscle is often correlated well with the
sensory evaluation.

1) total volatile base nitrogen (tvbn)
2) trimethyle amine nitrogen (tman)

*note: procedures of these two tests are already given in experiment no. 3

iii) estimation of bacteriological spoilage by using total bacterial count method

1) Aseptically collect 10 g of sample in a sterile sample dish.
2) transfer the sample into a sterile mortar or sterile stomacher bag.
3) homogenize with 90 ml of sterile phosphate buffer.
4) depending upon the products tested, dilute the homogenized sample as given
below, so that the number of colony in a petri dish is between
30-300.
5) transfer 1 ml each from the required dilutions to separate sterile petri dishes.
6) add to each petri dish 10-15 ml of sterile tryptone glucose beef extract agar (cooled to 40oc); mix well and allow solidifying.
7) incubate the plates at 37ºc for 48 hours and count the colonies.

calculations number of colonies × dilution

$$\text{tpc per g} = \frac{\text{number of colonies} \times \text{dilution}}{\text{weight of the sample}}$$

9. FISH PROTEIN EXTRACTIBILITY & ITS DETERMINATION

INTRODUCTION

Fish proteins gradually become denatured in cold storage. The rate of denaturation depends largely upon storage temperature.

Badly frozen stored fish are easily recognizable. The appearance of the thawed product, instead of being glossy and translucent, is dull and opaque and the texture, no longer firm and elastic, becomes soft and spongy. The cooked flesh loses its succulence and becomes dry, fibrous and tasteless.

The main proteins of fish flesh are called myosin and actin. They are responsible for the mechanism of contraction and relaxation of muscles and are called myofibrillar proteins. Muscle also contains many other proteins, the sarcoplasmic protein which are soluble in tissue fluid and in any salt solution. During freezing and cold storage, the proteins are affected, especially the myofibrillar protein, resulted in the textural changes of flesh.

The myofibrillar protein extractibility, therefore is used as a quality index for the assessment of frozen fish. It is expressed as follows:-

where: MPN = myofibrillar protein-nitrogen (N mg/100 g sample)
SPN = sarcoplasmic protein-nitrogen (N mg/100 g sample)
TN = total nitrogen (N mg/100 g sample)
NPN = non proteinous nitrogen (N mg/100 g sample)

The protein extractibility is applicable to fish and its product in general and can be used as an indicator of the degree of protein denaturation for demersal and pelagic species during cold storage.

i. sampling and sample preparation

Take a representative sample 22 g or more from the product. Place the sample in polyethylene bag and store in refrigerator or in ice so as to maintain sample integrity before preparation for analysis.

The dark meat, if any, should be removed prior to homogenisation of fish flesh.
Comminute the sample until homogeneous and place the homogenate in a polyethylene bag. Store the sample in a refrigerator or in ice until required. Ensure that the prepared sample is still homogeneous prior to weighing.

ii Apparatus

chopper or mincer
analytical balance, decimal to 0.1 mg
spatula
bottom-drive homogeniser (nihon seiki sn-03) or equivalent
refrigerated centrifuge, capable of centrifuging at 12,500 g
beakers, 100 and 250 ml
bulb pipettes, 10, 20 & 40 ml
glass funnels 0 60 mm

iii Reagents

a) phosphate buffer solution
0.03 m potassium di-hydrogen phosphate, 1 litre.
0.03 m di-sodium hydrogen phosphate, 1 litre.
mix the above solutions into a 2 litre beaker.
adjust the ph to 6.85 using these solutions.

Store in refrigerator.

b) 0.1 m potassium chloride solution
weigh kcl required accurately, use distilled water as solvent.

c) 0.5m potassium chloride buffered solution
accurately weigh kcl required. dissolve the weighed kci in the required phosphate buffer solution.

d) trichloroacetic acid solution (25%, w/v)
dissolve 25 g tca in 75 ml distilled water.

iv. procedure of protein extractibility

1. total nitrogen. accurately weigh a duplicate of 1 g the homogeneous fish sample for protein digestion (refer to protein determination by kjeldahl method b-1.)

2. sarcoplasmic protein nitrogen. accurately weigh 10 g of the homogeneous fish sample. blend the sample with 200 ml of 0.1 m kci solution with the homogeniser for 4 mins (speed set at scale 2). leave to stand in iced water for 2 hrs. centrifuge the blended sample at 12,500 g or 9,000 rpm at 5°c for 20 mins. pipette 20 ml supernatant (sarcoplasmic protein fraction) for digestion (refer to kjeldahl method).

3. myofibrillar protein nitrogen. accurately weigh 10 g of the homogeneous fish sample. blend sample with 200 ml 0.5 m kcl phosphate buffered solution with the homogeniser for 4 mins (speed set at scale 2). leave to stand in iced water for 2 hrs. centrifuge the blended sample at 12,500 g or 9,000 rpm at 5°c for 20 mins. pipette 20 ml supernatant (myofibrillar protein aliquot) for digestion (refer to kjeldahl method).

4. non proteinous nitrogen. pipette 40 mi of sarcoplasmic protein aliquot (see section iv 2) into 100 ml beaker. add 10 ml 25% tca and leave to stand in iced water for 30 mins with occasional swirling. filter the content of the beaker with whatman no. 41 ashless filter paper. pipette 40 ml of filtrate for digestion (refer to kjeldahl method).

V.CALICULATION

Ws in formula (1) in the protein determination by Kjeldahl method (B-1 Section IV) has to be replaced by the meat weight (g) in each of the protein aliquot used as follows:-

$$PN = \frac{W_M}{W_1 \times \frac{20}{W_1 + 200}}$$

V .CALCULATIONS

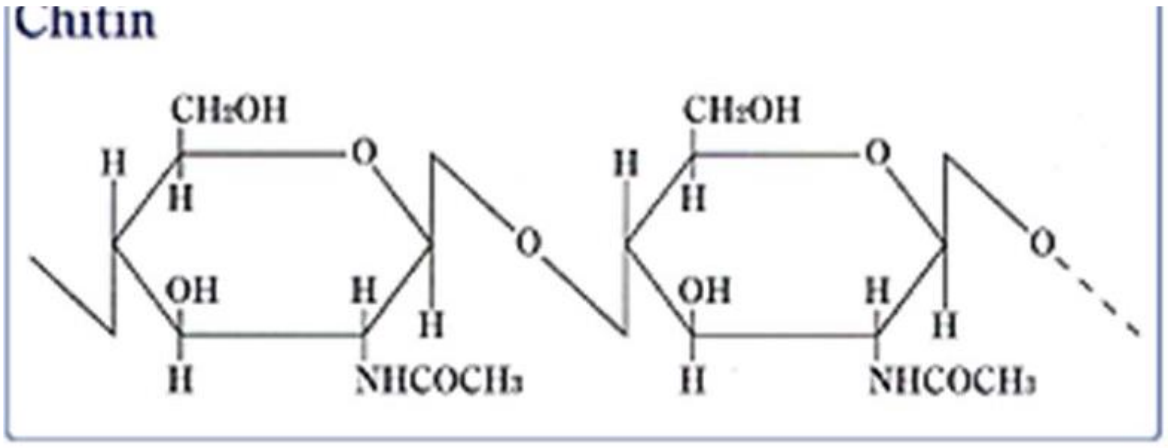
Ws in formula (1) in the protein determination by Kjeldahl method (B-1 Section IV) has to be replaced by the meat weight (g) in each of the protein aliquot used as follows:-

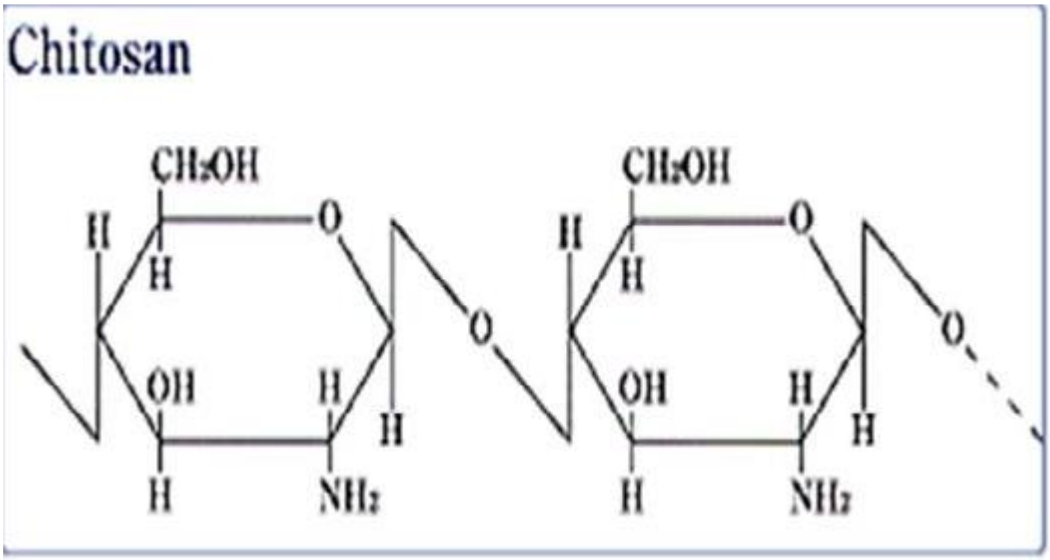
$$W_{MPN} = \frac{20 \times W_1}{W_1 + 200}$$

Q10. Preparation of Chitin and Chitosan

1. INTRODUCTION

Chitosan is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). It is made by treating shrimp and other crustacean shells with the alkali sodium hydroxide. The biopolymer is characterized as either chitin or chitosan according to the degree of deacetyla-tion (DD) which is determined by the proportion of D- glu-cosamine and N-acetyl-D-glucosamine. Structurally, chitosan is a straight-chain copolymer composed of D-glucosamine and N-acetyl-D-glucosamine being obtained by the partial deacety-lation of chitin. Chitosdan is the most abundant basic biopol-ymer and is structurally similar to cellulose, which is com-posed of only one monomer of glucose.
Figure 1: Structure of Chitin





Chitosan solubility, biodegradability, reactivity, and ad-sorption of many substrates depend on the amount of proto-nated amino groups in the

polymeric chain, therefore on the proportion of acetylated and non-acetylated D-glucosamine units.

The amino groups (pKa from 6.2 to 7.0) are completely protonated in acids with pKa smaller than 6.2 making chitosan soluble.

Chitosan is insoluble in water, organic solvents and aqueous bases and it is soluble after stirring in acids such as acetic, nitric, hydrochloric, perchloric and phosphoric .

The typical production of chitosan from crustacean shell generally consists of three basic steps: demineralization, deproteiniza-tion and deacetylation .

Due to its simplicity, relative in-strument availability, and independence of sample solubility, IR spectroscopy is one of the most studied methods for charac-teristics of chitin and chitosan .

Shrimps are in general sold headless and often peeled of the outer shells and tail. About 30-40% by weight, shrimp raw material is discarded as waste when processed shrimp is headless, shell on products .

The main aim of this present work was to prepare chitosan from fishery waste materials which are hazard and toxic for envi-ronment.

2 . MATERIALS AND METHODS:

Chitosan production

Raw materials:

Detailed submission guidelines can be found on the author resources Web pages. Indigenous shrimp shells were collected from Khulna, Bangladesh. Chitosan is easily obtained from crab especially Dungeness crab (*Cancer magister*), shrimp par-ticularly the Pacific shrimp (*Pandalus borealis*), lobster, or craw-fish shells. These are the richest source of chitin and the major sources of crustaceans that are processed into chitin and chi-tosan.

Figure 3: Shrimp shell

Figure 4: Shrimp shell powder

Shrimp shell

Shrimp shell powder

Chemicals and Reagents: All chemicals (NaOH, HCl) were used industrial grade and were purchased from local market.

Preparation of Chitosan: Chitosan preparation is divided into three consecutive steps. Demineralization of Shrimp shells, Chitin processing (Deproteinization) and Chitosan pro-cessing (Deacetylation).Shrimp shells were dried in sun 2 days. After sun drying, the shrimp shells were crispy. Then the shells were grounded into powder. Dried Powder shrimp shells were placed in opaque plastic bottles and stored at am-bient temperature.

Demineralization of Shrimp shells: In this step, finely powdered shrimp shell is demineralized with HCl. At room temperature the shrimp shells were demineralized with 5% HCl for 24 hours with a solid to ratio of 1:6. After 24 hours, the shells were quite squashy and were rinsed with water to re-move acid and calcium chloride and dried in an oven to 60°C. Small amount of treated shell was reacted with again 10% HCl solution, which showed no bubble generation. This test en-sures complete demineralization of shells.

Yield: Weight of demineralized Shell 35.2 gm.

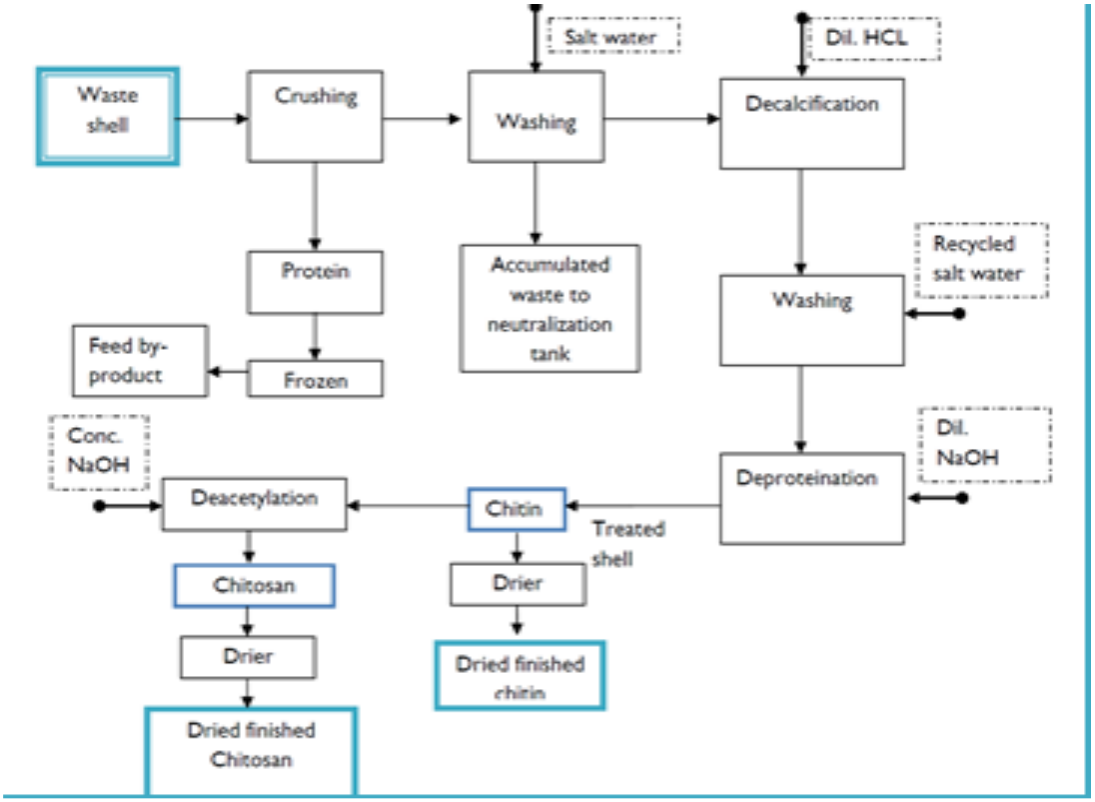
Deproteinization: Demineralized shells was deprotein-ized with 5%NaOH solution for 48 hours at 60-70°C at a solid to solvent ratio of 1:10 (w/v). After processing, the residue was washed with distill water to remove NaOH. Then it is dried for 2 days and the product found is called chitin.

Yield: Weight of Chitin 24.15 gm.

Chitosan processing (Deacetylation): Deacetylation is the process to convert chitin to chitosan by removal of acetyl group. After rinsing with distilled water, the decalcified chitin was transferred to a 60% sodium hydroxide solution. The so-lution was heated in a domestic microwave oven for 2 hour for deacetylation. After rinsing with distilled water and drying at 60ºC, the deacetylated chitin (now known as chitosan) was ready for use.

Yield: Weight of Chitosan 18.97 gm.

Degree of Deacetylation of Chitosan:



Titration methods:

Dried chitosan (0.2 g) was dissolved in 20 cm^3 0.1M hydrochlo-ric acid and 25cm^3 deionized water. After 30 minutes continu-ous stirring, next portion of deionized water (25 cm^3) was added and stirring continued for 30 minutes [5]. When chi-tosan was completely dissolved, solution was titrated with a 0.1 $mol \cdot dm^{-3}$ sodium hydroxide solution using automatic bu-rette (0.01 cm^3 accuracy). Degree of deacetylation (DA) of chi-tosan was calculated using formula:

where: m – weight of sample, V_1, V_2– volumes of 0.1 $mol \cdot dm^{-3}$ sodium hydroxide solution corresponding to the deflection points, 2.03 – coefficient resulting from the molecular weight of chitin monomer unit, 0.0042 – coefficient resulting from the difference between molecular weights of chitin and chitosan monomer units.

For titration volume of 0.1 $mol \cdot dm^{-3}$ sodium hydroxide solu-tion required, $V_2 – V_1 = 10.5$ ml.

Degree of Deacetylation of Chitosan calculated= 87.32%.

3 .RESULTS AND DISCUSSIONS

Characterization of Chitosan:

Chitosan was prepared from shrimp shell discussed in the material and method section. The main difference between Chitin and Chitosan is that Chitosan is soluble in 1% acetic acid. The characterization of Chitosan was confirmed by FT-IR analysis and XRD analysis.

amorphous respectively. It is report-ed that, the characteristics crystalline peaks with slightly fluc-tuated diffraction angles found in WAXD patterns indicated that two types of alpha-Chitosan and gamma-Chitosan exhib-

ited comparable degree of crystallinity and had a consistent peak between 19-20° [10].

Scanning electron microscope (SEM) Analysis:

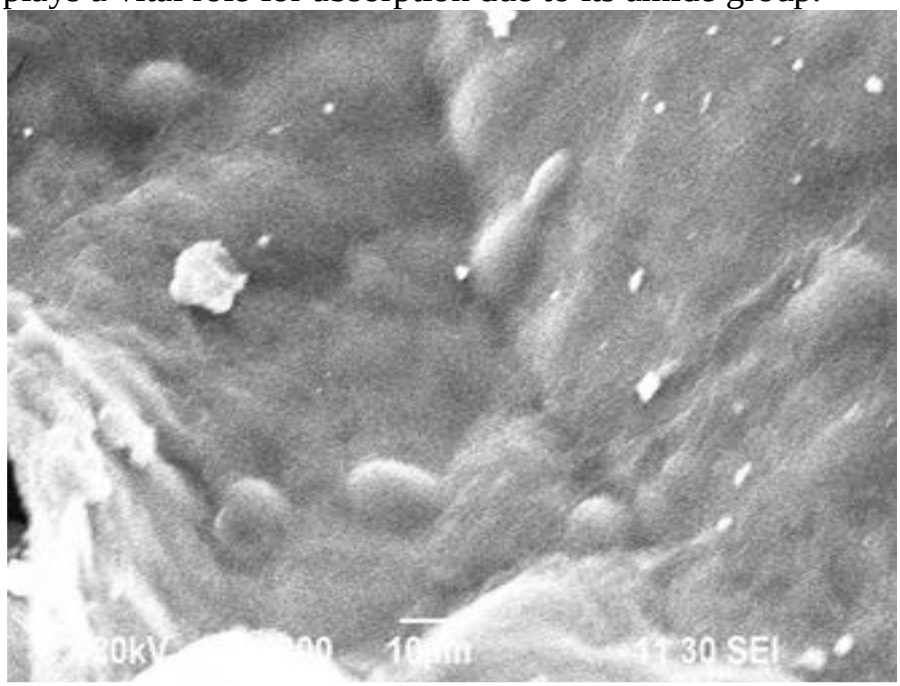
Chitosan prepared from shrimp shell waste was examined by scanning electron microscopy (SEM) having a magnification range of 5,000 and accelerating voltage 20 kV. The SEM micro-graph illustrates the morphology of the prepared chitosan from shrimp shells.

The micrographs showed non-homogenous and non-smooth surface as shown in

Figure 9: SEM image of prepared Chitosan.

4 CONCLUSION

The present observations indicate that the prepared chitosan in this study is soluble in 1% acetic acid solution. The FTIR, XRD, SEM of the prepared chitosan confirmed that the pre-pared material is Chitosan. The preparation of chitosan from shrimp processing waste (shells) would successfully minimize the environmental pollutants. Chitosan Can be plays a vital role for absorption due to its amide group.



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