MANUAL
ON
QUALITY ASSURANCE OF FISH AND FISHERY PRODUCTS
(CC-FSP 438)

A Course of Second Year Fourth Semester
(As per ICAR Approved 5th Deans committee's Report for Four Year B.F.Sc. Professional Degree Programme)

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LIST OF EXPERIMENTS

Chapter 1

Sensory Quality Analysis

Sensory evaluation provides rapid measurements of freshness. Sensory evaluation is defined as the scientific discipline used to evoke, measure, analyze and interpret reactions to characteristics of food as perceived through the senses of sight, smell, taste, touch and hearing. Sensory characteristics of whole fish are clearly visible to consumers and so, sensory methods are still the most satisfactory way of freshness assessment. There are two types of sensory methods: subjective and objective. Subjective assessments response is based on the assessor's preference for a product. They are estimated using adjectives such as like/dislike or good/bad, which require subjective decisions. This method can be applied in the fields like market research and product development. Objective assessments are based on organoleptic changes that occur in fish on storage. Objective scoring schemes require trained expert judges and the panel. These assessors individually use their appropriate senses (sight, smell, taste and touch) to determine the level of each sensory characteristic in the defined grade standard appropriate for the seafood. Sensory methods are also fast and nondestructive unless fish is cooked; and is the most commonly used method in quality control.

I. EU Freshness Grading (EU Scheme)

EU freshness grading was introduced for the first time as per the Council Regulation No. 103/76 (for fish) and 104/76 (for crustaceans) and updated by decision No. 2406/96 (for fish, some crustaceans, and only one cephalopod, the cuttlefish). EU scheme is commonly accepted in the EU countries for freshness grading of market fish within the Union and generally carried out by trained personnel in auction site. Whole and gutted fish are assessed in terms of acceptance of skins, eyes, gills, surface slime, belly cavity, odour and texture of fish.

There are four quality levels in the EU scheme, E (excellent). A(good quality), B(satisfactory quality and C (unfit). E is the highest quality and below level B is the level where fish is discarded or rejected for human consumption. However, the method has still some disadvantages like requirement of trained and experienced persons, does not differentiate between species and does not give information on the remaining shelf life of fish.

ii. Quality Index Method

Quality index method (QIM) has been suggested as an alternative to EU scheme. QIM was originally developed by the Tasmanian Food Research Unit in Australia (Bremner, 1985). This method considered to be rapid and reliable to measure the freshness of whole fish stored in ice. It is based on significant sensory parameters like skin, slime, eves, belly, odour, gills, etc. for raw fish (Branch and Vail, 1985). Characteristics may be listed, assessed and appropriate demerit point score is recorded (from O to 3). Scores for all characteristics are summed to give the overall sensor score. Quality index (QI) is close to 0 for very fresh fish, whereas higher scores are obtained for the spoiled fish.

There is a linear correlation between the sensory quality expressed as a demerit score and storage life on ice, which makes it possible to predict remaining storage life on ice. This method is considered to be a relatively fast, non-destructive and specific for species. In addition, the QIM is suitable for early stage of storage of fish, where other instrumental methods are not accurate.

QIM Eurofish published a manual containing QIM schemes for 12 fish species and information on their usage (Martinsdottir *el al.,* 2001). QIM is rapid, easy to perform, non-destructive and requires only short training. It can be used as a tool in production planning and quality warranty work. Rapid PC based QIM is also available on the internet at http:www.dfu.min.dk/QIMRS/ qim_0202.htm.

QIM schemes have been developed for raw gilthead sea bream, farmed Atlantic salmon, fresh cod, common octopus, herring. brill, haddock, plaice, Pollock, redfish shrimp, sole and turbot.

EXERCISE: Develop QIM scheme for Sensory Evaluation of *Catla catla*.

Quality Parameter	Description	Score

iii. Torry scheme

Torry scheme was developed at the UK Tory Research Station for usage by expert and trained judges. This is the most comprehensive scoring scheme to assess fish (Shewan *et al.,* 1953). It has been widely used in its original or modified forms. Torry Scheme, often referred to as the Torry scale is a descriptive 10 point scale. It has been developed for lean, medium fat and fat fish species. In this scheme, panelists evaluate the odour and flavour of cooked fillets. The scores are given from 10 (very fresh) to 3 (spoiled). Average score of 5.5 may be used as the limit for consumption.

iv. Quantitative Descriptive Analysis

Quantitative descriptive analysis (QD*A*) is used by a trained panel to analyse the sensory attributes of products such as texture, colour, and flavour. QDA provides a detailed description of all flavour characteristics in a qualitative and quantitative way. Trained panel is handed a broad selection of reference samples and use the samples for creating terminology that described all aspects of the product. Descriptive words should be carefully selected, and the panelists trained should agree with the terms. Objective terms should be used rather than subjective terms. In QDA, the words for describing the odour and flavour of the fish can be categorized into two groups: positive and negative sensory parameters based on whether fish are fresh or spoiled.

Objective sensory methods are essential for quality control and estimation of shelf life of seafood. However, sensory methods are time consuming, expensive, require trained personnel. Therefore, instrumental methods are also needed to satisfy the need for quality measurements in fish industry.

https://www.youtube.com/watch?v=DDyfzUb3Np4

Chapter 2

Physical Quality

I. Texture analysis

Texture analyses for seafood are extremely important in research, quality control and product development in the seafood industry. Fish muscle becomes soft or mushy as a result of autolytic degradation or tough as a result of frozen storage. Texture includes the most common parameters such as hardness, springiness and chewiness of the food. Hardness is the important textural parameter as it correlates well with the sensory assessments. Texture Profile Analysis is an imitative test in which the sample is compressed twice, mimicking the action of the jaw. The force-deformation curve is analyzed to determine a number of textural parameters.

Hardness measurement by Texture Profile Analyzer

Use a flat-ended plunger or spherical probe for the analysis of whole fish, fillets and mince. Place the probe or plunger first in contact with the sample. The software automatically records the thickness of the sample. Compress the sample to 80% of their original height at 2 mm/sec speed using the cylindrical shaped piston of 3 mm diameter for the first cycle. Compress once again within 5 sec after the first cycle. The instrument records the force deformation curve. Express the hardness using peak forced of the first compression cycle in Newton. Replicate each measurement at least six times.

ii. Torrymeter

Torry fish freshness meter "Torrymeter" was developed at Torry Research Station in Aberdeen, Scotland. Dielectric properties of fish are used for determination of freshness. Dielectric properties of fish skin and muscle alter in a systematic way, as tissue components degrade during spoilage. These changes occurring at micro level are related to alterations in appearance, odour, texture and flavour during spoilage and used as a quality indicator. A linear relationship was found between Tortymeter readings and sensory attributes for many fishes such as cod, Baltic herring, hake, blue whiting, flounder, mackerel, whole iced gillhead sea bream, and farmed Senegalee sole. Tortymeter readings are significantly affected, when fish is washed with seawater, as the ions interfere with electrical properties of skin. Presence of lipid also has an effect on the dielectrical properties of fish. Loss of skin, muscle integrity and deterioration of skin caused by bruising during harvest and packaging give variable values.

iii. Intellectron Fischtester VI

Intellectron Fischtester VI (Germany) measures the electric properties like resistance, conductivity and capacitance of the fish flesh similar to Torrymeter. Electric properties of fish can change after death of the fish due to the disruption of the cell membranes by autolysis. This method is based on conduction through skin and therefore works only on whole fish and fillets with skin. Mechanical abuse and freezing can affect the readings. Readings are used as an objective measure on the state of freshness/spoilage together with sensory data.

iv. RT-Freshtester

RT-Freshtester reflects dielectrical properties of fish like Torrymeter and Intellectron Fischtester VI. Reading decreases with Storage time. It is fast and nondestructive and allows automatic grading of 60-70 fish/min. It needs calibration depending on sample preparation, season, fishing grounds and fish handling procedures. It is unsuitable for frozen or thawed fish, partly frozen such as superchilled fish and fish chilled in RSW.

V. Cosmos

Cosmos instrument was developed by Japanese. It is applied for the evaluation of fish quality by determination of smell intensity. It is handheld, portable as well as rapid and nondestructive. It could be used for evaluation of fresh and chilled fish

in the seafood industry and on fishing vessels. There is strong correlation between the organoleptic and cosmos readings of six species of fish. It can be used for objective quantitative evaluation of fresh and chilled fish quality.

vi. Electronic Nose

Odour is the main indicator of fish freshness. Electronic nose called Freshsense was developed by Element-Badvaki in Ireland. It is a rapid and nondestructive method to measure volatile compounds indicating spoilage odours in seafood. Freshsense is based on a closed, static sampling system and electrochemical gas sensors, which are sensitive to volatile compounds. The most important chemical involved in fresh fish odours are long chain alcohols and carbonyls, bromophenols, and N-cyclic compounds. Apart from this, short chain alcohols and carbonyls, amines, sulfur compounds and aromatic, N-cyclic and acid compounds are produced by microbial activity and lipid oxidation during storage of fish. The concentrations of these compounds are related to the degree of spoilage.

Different electronic noses have been employed for measurement of fish freshness. They are metal-oxide semi conductor gas sensors, electrochemical sensors (CO, H_2O. NO, SO_2, and NH_3), thickness shear mode quartz resonators, semi conductor dimethylamine (DMA) gas sensor and prototype solid state based gas sensor called the Fish nose. There is a good correlation between the response of CO sensors and QIM methods. Data analysis becomes important in electronic nose measurements to determine the relation between sensor output patterns and properties of the sample. The most frequently used methods are artificial neural works (ANNs). chemometric analysis such as principle component analysis (PCA) and partial least square regression (PLS-R).

vii. Near Infrared Reflectance Spectroscopy

Near infrared reflectance spectroscopy is used in various analytical applications for its speed, simplicity and ability to measure numerous samples within a short time. It is non-destructive, easy to handle and requires little training of operators. This method is applied for quality assessment of frozen minced red hake and cod caught by long line and gillnet and also, thawed chilled MAP cod fillets. Fourier transform infrared spectroscopy (FTIR) is another technology that is a rapid, nondestructive and suitable for online industrial production chain. It requires too much handling of samples, causing changes in protein and muscle structure. Diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy has advantages, as it is fast, simple, sensitive, non-expensive and requires a small amount of sample. It is found useful in assessing the freshness and quality of sardine during iced storage.

viii. Water activity meter

Water activity is the measurement of the energy status of water in a system. It indicates the tightly structurally bound water in a substance. It is a relative humidity of air in equilibrium. Concept of a_w is of particular importance in determining product quality and safety. The a_w influences colour, odour, texture

and shelf life of many products, including dry fish products. It predicts safety and stability with respect to microbial growth, chemical and biochemical reaction rates and physical qualities. Water activity is estimated by the use of AquaLab LITE water activity meter, which is a dielectric humidity sensor to measure the water activity of the sample. This meter is also provided with infra red sample temperature sensors. In this method, a special hydroscopic polymer is placed between the electrodes in a head space of a scaled chamber. Electrical properties of the polymer change depending upon the humidity in a closed chamber. The signal is then translated and displayed as water activity on the instrument's display panel. At equilibrium, relative humidity of air in the chamber is the same as water activity of the chamber, having an accuracy of 0.015 a_w. Do the measurement within 5 min. Calibrate the instrument using the following calibration standards before testing the water activity of the samples.

Procedure

Switch on the instrument and allow it for 10 min. Fill the sample cup with comminuted dry fish sample upto 50% of the space. See that the minimum sample should cover the bottom of the sample cup. Ensure that the inside and outside of the sample cup are clean. Ensure sample temperature is not more than 4°C above the chamber temperature. Place the sample up inside the cup holder. Press down on the front top of the case to seal the cup inside. Press the center button to start measurement.

Note down the readings.

https://www.youtube.com/watch?v=zjqvadmr2_A

Chapter 3

Biochemical Quality Analysis

Chemical and biochemical methods for the evaluation of seafood quality are more reliable and accurate. These objective methods should correlate with sensory quality and the chemical compound that is determined should increase or decrease as microbial spoilage or autolysis progresses. Currently, the most used method to evaluate fish freshness is to combine several measurements obtained from different methods and correlate the finding with sensory analysis.

3.1. ATP and its breakdown products

Rigormortis occurs in postmortem muscle tissue and is associated with stiffness of muscle or flesh. This process results from breakdown of adenosine triphosphate (ATP), which is the main energy source for metabolic activity. Nucleotide breakdown reflects both action of autolytic enzymes and bacterial action. It has been indicated that there is a correlation between nucleotide catabolism and loss of freshness. Initial stages of the reaction catalyzed by endogenous enzymes take place quickly leading to the accumulation of adenosine diphosphate (ADP) and inosine monophosphate (IMP). Oxidation of hypoxanthine (Hx) to xanthine and uric acid is slower and is the result of endogenous enzyme activity or microbial activity. The IMP is associated with fresh fish flavour, whereas inosine and hypoxanthine reflect poor quality.

Concentration of ATP and its breakdown pro been used as indicators of freshness in many fish species. K value proposed by Saito (1959) is the biochemical index for fish quality assessment based on nucleotide degradation. K value includes intermediate breakdown products and varies within species of fish.

Several methods have been proposed for the analysis of single or a combination of nucleotide metabolites, but the HPLC method is the most reliable to calculate the K value.

i. K-value determination by HPLC method (Ryder, 1985)

Reagents

1. Perchloric acid, 0.6M
2. KOH, 1 M
3. Phosphate buffer solution (pH 6.8): Mix equal volumes of 0.04 M KH,PO, and 0.06 MK,HPO, in distilled water.

Procedure

Take 5 g of fish muscle tissue and homogenize with 25 ml of chilled 0.6 M perchloric acid at low temperature for 1 min. Centrifuge the homogenate at 6000 rpm for 20 min at 4°C and filter. Take 10 ml of the supernatant, neutralize to pH 6.8 with chilled IM KOH immediately (visualized by formation of KCl precipitate). Allow to stand at 0°C for 30 min, filter through a syringe filter (0.45m m) and store at -30°C for subsequent HPLC analysis.

HPLC analysis

Use reverse-phase C 18 column. Separate the nucleotides using isocratic elution with filtered phosphate buffer solution (pH 6.8). Maintain the flow rate at 1.5 ml/ min throughout the chromatographic separation. Inject 50ml of the sample into the HPLC. Identify the peaks of the sample by comparing with the ak of chromatogram of the mixture standard solutions. Quantify each nucleotide breakdown products by comparing the peak area the samples with the peak area of the standards.

ii. Hypoxanthine determination

Hypoxanthine (Hx) is formed in fish due to autolytic degradation of ATP is also an useful indicator of fish freshness. Hx content increases with time of storage to about 5 m moles/g wet weight and subsequently declines or remains at that level. Content of hypoxanthine correlates well with sensory assessment, the flavour in particular. Limit of acceptability differs for each fish species ie. cod - 2 to 3 µ mol/g; herring-2 to 2.5 µ mol/g; mackerel – 1 to 1.5 µ mol/g; shrimps - 2 µ mol/g; squids- 2 to 4 u mol/g.

A protein free extract of fish muscle is made using perchloric acid. The extract is neutralized and the perchlorate is removed as its insoluble potassium salt. The hypoxanthine in the neutral solution is converted by the enzyme, xanthine oxidase to uric acid, which has an absorbance maxima at 290 nm.

Reagents

1. Perchloric acid, 0.6M

2. NaOH, IM

3. KOH, IM

4. Potassium hydroxide – phosphate buffer

   Dissolve 27.2 g of KH_2PO_4 in about 250 ml water and add 170 ml of 1.0M NaOH. Check that the pH is 7.6 ± 0.5 and adjust, if necessary. Add 557 ml of IM KOH to the buffer solution and make up to 1 L with distilled water.

5. Phosphate Buffer, pH (7.6),0.25M

   Dissolve 17.0 g of KH_2PO_4 in about 250ml distilled water. Add 107 ml of 1.0 M NaOH and check the pH. Adjust pH 7.6±0.5, if necessary. Make the volume up to 500mL with distilled water.

6. Phosphate buffer, pH (7.6) 0.05M

   Dilute 0.25M phosphate buffer five folds with distilled water.

7. Xanthine oxidase working solution

Commercially available stock enzyme solution has an activity of 3-4 EU/ml. Dilute this stock solution 50-folds with chilled 0.05 M phosphate buffer (Prepare fresh each day)

8. Hypoxanthine standard solutions

S*tock solution (100mg/ml)*: Dissolve 10 mg of hypoxanthine in about 70 ml of boiling water. Cool and make up to 100ml with distilled water. Make up fresh each week and store in a refrigerator.

Working solution: Dilute the stock with distilled water to get working standards containing 5, 10, 15, 20, 25 mg hypoxanthine in 1.0 ml. Check the concentration by measuring the optical density of the 10 m*g*/ml solution at 250 nm such that it should be 0.786. (The molar extinction coefficient of hypoxanthine molecular weight 136.1 is 10.7×10^3).

Procedure

Weigh out accurately 5.00+0.05 g of fish sample and homogenize with 50 ml of chilled 0.6M perchloric acid. Centrifuge at 5000 g for 15 min and filter it through Whatman No. 1 filter paper. Take 5.0 ml of the filtrate and add 5.0 ml of KOH-phosphate buffer, chill and filter again.

Solution A (Reaction mixture): Pipette out 0.5, 1.0 and 2.0 ml of filtrates into the test tubes and make up to 2.0 ml with distilled wa*t*er. Add 2.0 ml of 0.25 M phosphate buffer and 0.5 ml of enzyme solution.

Solution B (Extract blank): Pipette out 0.5, 1.0 and 2.0 ml of filtrates into the test tubes and make upto 2.0 ml with distilled water.

Solution C (Enzyme blank): Prepare a blank containing 2.0 ml of 0.25 M phosphate buffer, 2.0 ml of distilled water and 0.5 ml of enzyme solution.

Solution D (Buffer blank): Prepare a blank containing 2.0 ml of 0.25 M phosphate buffer and 2.5 ml of distilled water.

Incubate all the mixtures at 35°C for 30 min. Measure the absorbance at 290 nm in the spectrophotometer. Calculate the NIL absorbance due to uric acid after correcting for the blanks as follows.

AB = (A-D) - (B-D) - (C-D)

= (A-B) - (C-D)

Calculation

Molar extinction coefficient of uric acid at 290 nm is 12.4×10^3. One mole of uric acid is equivalent to one mole of hypoxanthine in 4.5 ml of the reaction mixture is:

AB x 49.4 mg

3.2. Biogenic amines

Concentration of biogenic amines has been reported to a reliable method of measuring the quality of fish, depending on species. Formation of biogenic amines results from microbial degradation during the later storage of fish, and their concentration *i*ncreases with storage time. They are generated by microbial *d*ecarboxylation of specific free amino acids in fish or shellfish tissue. The most significant biogenic amines formed during postmortem in fish and shellfish products are histamine, putrescine, cadaverine, tyramine, tryptamine, 2-phenylethylamine, spermine, spermidine and agmatine. Since the amines are produced by spoilage bacteria towards the end of shelf life of a fish, their levels are considered as indices of spoilage rather than freshness.

Among the biogenic amines, histamine is potentially hazardous and causative agent of histamine intoxication. Others especially putrescine and cadaverine are reported to enhance the toxicity of histamine. Hazardous concentrations of histamine are 5 mg/100g and 20 mg/100g fish as the legal limits set by USFDA and EU, respectively. Biogenic amine content depends on fish species, free amino acid content, presence of decarboxylase positive microorganisms, moment of capture and stomach contents at death.

By means of decarboxylation reactions, tyrosine produces tyramine, histidine yields histamine, arginine leads to putrescine, lysine produces cadaverine, tryptophan forms tryptamine and phenylalanine yields 2-phenylethylamine. Putrescine is also an intermediate of a metabolic pathway that leads to spermidine and spermine. Quality index (QI) and biogenic amine index (BAI) are proposed by Mietz and Karmas (1977) and Veciana-Nogues *et al.* (1995) for determination of quality of fish.

QI = histamine + putrescine+ cadaverine /1 + spermidine + spermine

BAI = histamine + putrescine + cadaverine+ tyramine

QI is based on the increases in putrescine, cadaverine and tyramine and decreases in spermine and spermidine during storage of fish, whereas BAI is based on increases in histamine, putrescine, cadaverine and tyramine.

There are various analytical techn*i*ques used to determine the concentration of biogenic amines such as thin layer chromatography (TLC), HPLC, GC, E*LI*SA, capillary electrophoresis and biosensors. Among these techni*qu*es, HPLC is mostly performed because of its sensitivity, reliability and reproducibility.

i.TLC method (Shakila *et al., 1*995)

Amines extracted in trichloro acetic acid are converted to dansyl derivatives using dansyl chloride. The fluorescent dansyl amines are visualized under UV and quantified using densitometer at 356 nm.

Reagents

1. TCA, 5%

2. NaOH, 4 N

3. Phosphate Buffer (pH 9, undiluted)

4. Dansyl reagent

Dissolve 50 mg of dansyl chloride (5-dimethyl amino naphthalene 1-sulphonyl chloride) in 10 ml of acetone

(Prepare fresh before use)

5. Biogenic amine standard

*Stock (*2mg/ml): Dissolve 0.20 mg of each amine (histamine dihydrochloride, cadaverine dihydrochloride, putrescine dihydrochloride and tyramine dihydrochloride*)* in 100 ml of 5% TCA

W*orking (*0.2mg/ml): Pipette out 0.5 ml of the stock and make up the volume to 50 ml with 5% TCA

Procedure

Dissolve 10 g silica gel G *(*Gypsum binder) in 30 ml of distilled water to prepare one 20 x 20 cm plate. Make slurry with a glass rod and pour on the glass plate. Allow for air-drying and activate at 110°C for 1 h before use. Take 10 g of fish sample, add 30 mL of hot 5%(80-90°C) TCA and homogenize well for 2 min. Centrifuge at 3000 rpin for 10 min and filter the supernatant through Whatman No. 1 filter paper. Take 1 ml of the sample TCA filtra*t*e in a stoppered test tube, to which add 1 drop of 4 N NaOH, 1 ml of phosphate buffer and 2 ml of dansyl reagent. Close the test tubes, mix well for 30 sec, wrap the tubes with aluminum foil and place in thermostatic oven set at 50°C for 1 h. Similarly, derivatize 1 ml of each amine working solutions to their respective dansyl derivatives. Mix equal quantities of dansyl standards to obtain standard amine mixture. Place individually 25 ml of sample dansyl amines as spots on the activated silica gel plate. Simultaneously, place 25 ul of standard amine mixtures as a spot along side of the sample in the silica gel plate. Develop the chromatogram using chloroform : triethylamine (100:20) to separate the amines. After development, spray the plate with triethanolamine: isopropanol (8:2) to enhance the fluorescence. View the plate under UV light at 254 nm and calculate the Rf values and compare with the intensity of fluorescent spots of the authentic standards. Scan the TLC plates in a TLC scanning densitometer set at 356 nm. Calculate the concentration of individual amines with the help of area intensities of the authentic standards.

3.3. pH

The pH is also a important parameter that shows depletion in fish tissue during storage. Postmortem pH varies from 5.5 to 7.1 depending on season, species and other factors. Low initial pH is associated with higher stress before slaughtering due to the depletion of energy reserves, mainly glycogen with the production of lactate. Since the activity of enzymes depends on pH, it affects reactions taking place during storage of fish. A relatively low ph may cause a decrease in water binding capacity of myofibrils, affect light scattering and appearance of fish; and promote oxidation of myoglobin and lipids. The pH is normally determined by a pH which has a glass electrode and calomel electrode. Most of the pH meters have combined single electrode for measuring the pH. The pH of a solution is defined as the negative logarithm of the hydrogen ion activity.

$$pH = -\log_a(H^+)$$

Reagents

1. Buffer solution 1: The pH of this solution is 4.0.
2. Buffer solution 2: The pH of this solution is neutral (7.0)
3. Buffer solution 3: the pH of this solution is 9.2.

Procedure

Prior to the pH estimation, standardize the electrodes with suitable buffer solutions. Use pH 4.0 and pH 7.0 standard buffer solutions as standards for low pH foods. Use minimum two buffers to standardize the pH meter. Weigh 10 g of fish sample and homogenize with 50 ml of distilled water (pH 7.0). Dip the pH electrode in the sample solution and note down the readings.

https://www.youtube.com/watch?v=6BJlmA0M4Jo

3.4 Total volatile base nitrogen

In seafood, total volatile base nitrogen (TVB-N) primarily includes trimethylamine (TMA, produced by spoilage bacteria), ammonia (produced by deamination of amino acids and nucleotide catabolites) and DMA (produced by autolytic enzymes during frozen storage). Analyses of these indicators are considered unreliable because they reflect later stages of spoilage rather than freshness. However, the European Commission (Council Regulation No. 95/ 149/EC of March 1995) on Fish Hygiene specifies that if the organoleptic examination indicates any doubt as to the freshness the fish, TVB-N should be used as a chemical check.

Level of TVB-N in freshly caught fish is generally between 5-20 mgN/100g. However, the levels of 30-35 mg N/100 g muscle are considered the limit of acceptability for ice stored cold water fish.There are three different methods for estimation of TVB-N: EC reference method, Conway microdiffusion method and steam distillation method. EC reference method involves preliminary deproteinization with perchloric acid. Distillation method involves direct distillation of fish after addition of magnesium oxide. Conway method uses trichloric acid instead of perchloric acid for deproteinzation. Although there is a good correlation between three methods, direct distillation method has been recommended as rapid routine method.

Distillation method

Volatile bases liberated during distillation are absorbed by the acids, which are then quantified by back titration of excess acid against a standard acid.

Reagents

1. Perchloric acid, 6%
2. Boric acid, 2%
3. Mixed indicator

   Mix equal volumes of 0.066% of alcoholic methyl red and alcoholic bromocresol green solutions
4. HCl, 0.05N

Procedure

Weigh 10g of fish muscle tissue and homogenize with 50 ml of perchloric acid to precipitate protein. Centrifuge the homogenate at 4000g for 15 min at 5°C and filter. Take 5 ml of supernatant and distill in Kjeldhal apparatus (as for protein analysis). Collect the distillate in 10 ml of boric acid containing 2-3 drops on mixed indicator in a conical flask. Titrate the contents against 0.05 N HCl until pink colour appears. Simultaneously, distill 5 mL distilled water in Kjeldhal apparatus to set as a blank and proceed as above. Difference in the titer values of sample and blank gives the actual titer value of sample.

Calculation

TVBN (mg %) = T.V x 14 x N x 100 x D.F/ W

Where, T. V.-Titer value of the sample, ml: 14 - mg of N_2 in 1N HCl. N - Normality of HCl (0.05); 100 - percentage conversion, W - Wt. of the fish, g, D.F - Dilution factor.

https://www.youtube.com/watch?v=vPdzvCe3Bmo

3.5. Trimethylamine

Fishy odour is caused due to the decomposition of trimethylamine oxide (TMAO) via the enzyme TMAOase demethylase. TMA is produced by the decomposition of TMAO due to bacterial spoilage and enzymatic activity after the death of fish. TMAO appears to be part of the fish and used in osmoregulation. Marine fish have 1-100 mg TMAO/100 g tissue, whereas freshwater fish generally contain only 5-20 mg/100g. Marine teleosts contain 15 – 250 mg TMAO/100 g tissue, while marine elasmobranch contain 1000-1800 mg TMAO/100 g. Fresh fish has a very low amount of TMA with values less than 1.5 mg, TMA/100 g in fresh cod, but values increase during spoilage. Fish is considered stale when the TMA production is higher than 30 mg/100 g cod. Hence, TMA can be used as a microbial spoilage indicator and not as an index for freshness. The permissible limit for TMA is hence, 10-15 mg/100g fish.

Many analytical methods have been developed for the measurements of TMA, DMA or TVB-N contents. They are steam distillation, Conway microdiffusion and titration, colorimetric method, photometry, HPLC method, GC method, injection gas diffusion method, biosensor using flavin containing monooxygenase type-3 and solid state sensors based on bromocresol green.

Conway micro diffusion method (Conway, 1950)

Volatile bases are liberated, when the fish is treated with potassium carbonate (K_2CO_3). Liberated NH3 is absorbed in acids and the quantity absorbed is determined by of titration of excess acids against a standard alkali.

Reagents

1. Sulphuric acid, N/70
2. Sodium hydroxide (NaOH), N/70
3. Trichloroacetic acid (TCA), 20%

4. TCA. 2%

5. Saturated potassium carbonate

6. Neutral formaldehyde solution or 20% formaldehyde

Shake well 100 ml of commercial formalin (i.e. 40%) with 10 g of $MgCO_3$ until it becomes colourless and filter it. Then, dilute with 100 ml of distilled water to make it up to

20% formaldehyde.

7. Tashiro's Indicator

Add 20 ml of 1% alcoholic solution of methyl red to 5 ml of 1% alcoholic methylene blue solution. Mix one volume of this stock solution, one volume of alcohol and 2 volumes of distilled water to get the indicator.

Procedure

Take 10 g of fish sample, add 20 ml of 20% TCA and homogenize well. Filter using a Whatman No. 1 filter paper in 50 ml volumetric flask. Repeat the extraction once again and make up the volume to 50 ml. Pipette out 1 ml of N/70 H2SO4 into the inner chamber of the Conway unit and add 1 drop of Tashiro's indicator. In the outer chamber, add 1 ml of TCA extract, 0.5 ml of neutralized formalin and cover the unit with the greased cover glass leaving little gap for adding potassium carbonate. Then, add 1 ml of K2CO3 in the outer chamber by gentle rotation and cover the unit. Keep the unit overnight at room temperature for 90 min. Then, remove the lid and titrate the contents of the chamber against N/70 NaOH using a micro burette till the of colour. Conduct a blank simultaneously using mi 2% TCA solution.

Calculation

TMA (mg/100g) = V x 0.2 x 50 x 100/W

W here, V - Difference in the titer values of blank and sample, ml; 0.2- Nitrogen conversion factor; 50 - Dilution factor; 100 - Percentage conversion; W- Wt. of the fish, g

3.6. Dimethylamine

During chilled or frozen storage of fish, when bacterial growth is inhibited, the TMAO is slowly converted by an enzyme to DMA and formaldehyde. Formation of these products may cause severe quality changes or spoilage during prolonged frozen storage. Amount of DMA produced depends on species, storage temperature, and time. DMA can be used as a spoilage index during frozen storage of some species like hake.

3.7. Formaldehyde

Formaldehyde content in seafood products is generally considered as non-toxic, but it can react with a number of chemical compounds such as amino acid residues, terminal amino groups and low molecular weight compounds and cause denaturation and cross linkage with proteins as well as reduce the solubility of myofibrillar proteins. Formaldehyde content of frozen seafood is generally used as a spoilage index, especially in gadoid fish. It is determined in fish using Nash reagent (Castell and Smith, 1973).

Protein free extract made with TCA is first neutralized using alkali and treated with Nash reagent containing of ammonium acetate and acetyl acetone that forms a coloured complex with an absorption maxima at 415 nm.

Reagents

1. TCA, 10% or perchloric acid, 6%
2. Sodium hydroxide solution, 45% and 1.0M
3. Double strength Nash reagent(DSNR)
   Dissolve 150 mg of ammonium acetate and 2 mL of redistilled acetyl acetone in 500ml distilled water (This solution is stable for 6 months at 0°C).
4. Formaldehyde standard solution, 38%
   Stock: Dilute 5 ml of formaldehyde in 1000 ml with distilled water
   Working (3.8 ppm/ml): Dilute 5 ml of the stock solution to 500 ml with distilled water

Procedure

Weigh 10 g of fish muscle tissue and homogenize with 30 ml of 10% TCA for 1 min. Centrifuge at 5000g for 15 min at 4°C and filter. Pipette out 5 ml of the supernatant to 15 ml of distilled water taken in a beaker. Neutralize to pH 6 using 45% NaOH first and then with 1.0 M NaOH solution and make up to 25 ml with distilled water. Mix and filter, Pipette out 5 ml of the neutralized filtrate in a test tube. Pipette out 0.5, 1.0, 1.5, 2.0, 2.5. 3.0 and 3.5 ml of the working standard in a series of test tubes and make up to 5 ml with distilled water. Pipette out 5 ml of distilled water in another test tube to serve as a blank. Add 5 ml of DSNR solution to all the test tubes, shake vigorously, heat in a water bath set at 60°C for 5 min. Cool the test tubes and measure the absorbance (O.D) at 415nm in a spectrophotometer.

Calculation

Formaldehyde (in ppm) = CxD.F/W

where, C - Conc. of formaldehyde, ppm; D.F - dilution factor, W - Wt. of the fish, g

3.8. Indole

Indole is a decomposition product formed by the breakdown of tryptophan, amino acid present in shrimps. Indole is 2,3-benzopyrolle having a fecal odour, highly volatile and soluble in hot water, alcohol, ether and benzene. Indole is an useful spoilage indicator in shrimps and crabs and its content should not exceed 25mg/ 100g. Indole can be estimated by two methods: extraction and distillation methods, of which distillation method gives good recovery.

i. Extraction method (Cheuk and Finne, 1981)

Indole is extracted using trichloroacetic acid and allow*ed to react* with *Ehrlich's reagent (p-dimethyl amino* benz*aldehyde) to form a red colour compound having absorption maxima at 570 nm.*

Reagents

1. TCA,10%

2. Petroleum ether (60-80°C)

3. Ehrlichs's reagent

Dissolve 1.8 g of p-dimethyl amino benzaldehyde (p-DMAB) in 9 ml of conc. HCl in 50 ml volumetric flask and dilute with ethanol. (Prepare fresh daily)

4. Indole standard

Stock (1 mg/ml): Dissolve 100 mg indole in 100 ml of petroleum ether

*Working (*5mg/ml): Pipette out 0.5 ml of the stock and make up the volume to 100 ml with petroleum ether in volumetric flask

Procedure

Homogenize 10 g of sample with 20 ml of 10% TCA solution in a homogeniser for 1 min. Centrifuge the homogenate for 10 min at 10,000 rpm. Filter the supernatant through Whatman No. 1 filter paper under slight suction. Transfer the filtrate into 250 ml separating *T*unnel and add 8 ml of petroleum ether. Shake well and allow the two layers to separate. Transfer the petroleum ether layer to another separating funnel. Extract once again with 8 ml of petroleum ether. Combine the extracts in the second separating funnel. Then, extract *the indole* with 5 ml of Ehrlich's reagent by vigorously shaking for 1 min. Take 1, 2, 3, and 4 ml of the indole working standard with blank (petroleum ether) in separate test tubes and adds Ehrlichs's reagent. Read the colour at 570 nm. Draw the standard graph by plotting the concentration of the indole (mg) in X axis and the optical density (OD) on the Y axis. *C*om concentration present in the sample extract from the standard graph.

Calculation

Indole (mg/100g) = C x D.F x 100/W

where, C - concentration of indole, mg; D.F - Dilution factor W - weight of sample, g; 100 - % conversion

ii. Distillation method (Clough *et al.,* 1925)

Sample is steam distilled to release indole, which is then ex*tracted using chloroform and then, allowed to react* with *Ehrlich's reagent (p-dimethyl amino*

benzaldehyde) to form a red coloured compound having absorption maxima at 570 nm.

Reagents

1. Alcohol

2. 5 % Hydrochloric acid: Dissolve 5 ml of HCl in 100 ml of distilled water

3. Saturated sodium sulphate

4. Ehrlich's reagent

Dissolve 0.4 g of p-dimethyl amino benzaldehyde (p- DMAB) in 5 ml of acetic acid, mix with 92 ml of phosphoric acid and make up to 100 ml. (Prepare fresh daily)

5. Indole standard

Stock (1 mg/ml): Dissolve 100 mg of indole in 100 min alcohol

Working (10mg/ml): Pipette out 1.0 ml of the stock and make up the volume to 100 ml with alcohol in volumetric flask

Procedure

Homogenize 5 g of sample with 80 ml of alcohol and transfer into a distillation flask. Distil and collect 50 ml of distillate. Transfer the distillate into a separating flask, add 5 ml of 5% HCL and 5 ml of saturated sodium sulfate. Extract with 25 ml of chloroform and shake well. Allow the layers to separate and collect the lower chloroform layer. Add 10 ml of the Ehrlich's reagent to the chloroform extract, shake well and allow the acid laver to separate. Transfer the acid layer into 50 volumetric flask and dilute to mark with acetic acid. Measure the colour at 590 nm in the spectrophotometer against a reagent blank. Pipette out 1, 2, 3. 5 and 7 ml of the indole working standard into a series of volumetric flask. Add 10 ml of Ehrlich's reagent, shake well and separate the acid layer. Read the color absorbance at 570 nm. Draw the standard graph by plotting the concentration of the indole (mg) in the X axis and the optical density (OD) on the Y axis. Compute the concentration present in the sample extract from the standard graph.

Calculation

Indole (mg/100g) = C x D.F x 100 /W

where, C - conc. of indole, mg; D.F – dilution factor; W - wt. of sample, g; 100 - % co

Chapter 4

Quality Defects In Fish And Fishery Products

The term ‘quality’ means “all those attributes which consciously or unconsciously the fish eater or buyer considers should be present” and which will embrace

intrinsic composition, degree of spoilage, damage, deterioration during processing, storage, distribution, sale and presentation to the consumer, hazards to health, satisfaction on buying and eating, aesthetic consideration, yield and profitability to the producer and middle men. The quality of the fish and fishery products is one of the main indices in the activity of any processor. According to ISO, Quality is defined as the totality of features and characteristics of a product or service that bears its ability to satisfy stated or implied needs

Quality changes during chilled storage

Belly bursting

Enzymatic spoilage causes belly bursting of fish, especially during a period of high food intake. These fish will have large content of digestive enzymes in digestive tract. Such fish will degrade quickly & spoil easily soon after caught. In the dissolved gut components, bacteria proliferate and produces gases such as Co_2 & H_2. This gas production leads to belly bursting after short storage period.

Gaping of fillet

Phenomenon observed in fish which are well fed at the time of capture and are frozen before or during rigor is called gaping. Gaping of fillet occur due to weakening of the connective tissues which bind together the muscle segments in the fish flesh.

Black spots in shell – on shrimp

Black discolouration in shrimp occurs when stored fresh. Bruises and rough handling increase the occurrence of this discolouration, which is caused by polyphenolase enzyme, acting on amino acid Tyrosine. Sulphite preservatives are used to prevent black discolouration. Shrimps are dipped in 0.2-0.5 % sodium bisulphite for one minute. The maximum black spot permitted is 10% by count in shell – on types and 5% by count in peeled type.

Quality changes during frozen storage

Physical changes

The physical changes which occur during freezing and storage of frozen products comprise crystallization of ice with expansion of the volume, and desiccation starting from the surface of the frozen fish.

Ice formation

The crystallization of ice is initiated when the temperature of the fish is lowered about -1°C. At the same time, concentration of various inorganic salts and organic components present in the fluid of the fish occurs and consequently the freezing point falls. There is also an increase in the volume of the fish when the water is converted to ice. The larger part of the water consequently freezes between -1°C to -5°C and it is the rate of cooling and this temperature interval determines the size of the ice crystals. It is well known that slow freezing results

in formation of large ice crystals. These may cause the tissue of the fish to become so porous and perforation of the tissue can often be seen after the fish is thawed. It may even become even spongy. Rapid freezing on the other hand, results in small ice crystals, and the quality of quick frozen fish may be practically equivalent to that of fresh unfrozen fish.

Freezer burn

Freezer burn is a condition that occurs when frozen food has been damaged by dehydration and oxidation, due to air reaching the food. Change in fluctuation in storage temperature influences the desiccation, loss of weight and quality of the fish contributes to a poor appearance or results in "freezer burn". If the desiccation is pronounced, the fish surface may become dry and fibrous. In some cases the skin may change the colour, several other factors influence the loss are, the kind of wrapping, it's sealing and moisture transmission characteristics. The maximum dehydration allowed in shrimp for export from India is 20% by count.

Discolouration

When frozen fish is in contact with air, oxidation of fat or oil in the fish takes place and these results in "rusting" or discolouration of the flesh and development of rancid odours and flavours.

Green/Brown discolouration

Frozen tuna and sword fish may exhibit green and brown discolouration. Uncooked fish meat contains three derivatives of myoglobin. The pigment responsible for the pink colour in normal cooked meat of tuna is hemochrome, derived from the reaction of myoglobin with non-heme constituents. Greening is due to pigments resulting from the oxidation of hemechrome that occurs when the meat is unduly exposed to oxidative condition during and after cooking. Greening of frozen sword fish may be related to up take of H_2S produced by putrefactive bacteria. Proper evisceration and removal of blood immediately after the catch reduce the risk of discolouration. The undesirable discolouration in yellow fin tuna meat can be averted , if the fish is frozen at full rigor, stored at a temperature of -23°C to -27°C,and defrosted by still air at 10°C.

Yellow discolouration

Frozen storage of some fish (cod) may result in yellowing of flesh below skin. Freezing process disrupt chromatophores and release carotenoids and their migration to the S/C fat layers causes yellowing. Cuttlefish muscle has very high phospholipid content and are susceptible to oxidation. Since phospholipids contain amine groups, their oxidation can lead to aldehyde–amine interactions that produce yellow colour. In crustaceans, like lobster, pigment is limited to the surface of the meat, the changes during frozen storage lead to yellow discolourations.

SPOILAGE OF CURED FISHERY PRODUCTS

Moulds and fungi:

Fungus usually grows well on unsalted and salted dried fish, which has high moisture content. Moulds usually grow at the optimum temperature of 30-35°C with a relative humidity above 75%.In salted fish, brownish black or yellow brown spots are seen on the fleshy parts. This mainly caused by the growth or Halophilic mould called *Sporendonema epizoum.* This gives a fish very bad appearance. Some of the fungi isolated from dried fish are *Aspergillus* spp. *A.niger*, A. *flavus, A.candidus, A.amstelodami, A.chevalieri, Rhizopus, Mucor, Penicillium spp., Polypaecilum pisce.*

Spoilage of fish by fungi

1. Colour change due to fungal growth

2. Smell/flavor change

3. Breakdown of fat and protein

4. Production of mycotoxin

Prevention of fungal spoilage

1. Chemical methods

a) Use of preservatives: The main preservatives used are calcium propionate, potassium sorbate, sodium benzoate, parabens, sulphur dioxide, sodium nitrite.

b) Use of fungicides: Probably expensive to use. Broad spectrum fungicide is toxic to humans too.

c) Gamma irradiation: The main problem is recontamination if the product is not sealed properly. High cost of processing and packing is another problem.

2. Physical methods:

a) Control of storage temperature

b) Control of water activity by controlling moisture, salt and fat

c) Keeping storage period as short as possible

d) Adopting insect control measures like fumigation

e) Applying proper curing method viz. drying quickly after brining avoiding contamination from soil,dust etc.

Rancidity

This is caused by the oxidation of fat, mostly in oil rich fishes like mackerel. Oxidation of fat imparts characteristics odour and colour of the fish change to brown. This is known as rust. Certain impurities in salt and traces of copper accelerate this.

Pink /red spoilage

Pink /red spoilage is mainly due to the presence of halophilic bacteria (*Halobacterium salimaria*, *H. cultrirubium*, *Sarcina morrbuae* and *S. litoralis* from the salt. Spoilage appears on the surface as slimy pink patches. They are aerobic and proteolytic in nature, grows best at 36ºC by decomposing protein and giving out an ammoniacal odour

Prevention

 Usage of good quality salt

Insect Infestation

It occurs during initial drying stages & storage of the dried samples. Mainly blowflies belonging to the family are Calliphoridae and Sarcophagidae. Flies come and lay their eggs. These eggs develop into maggots, which bury within the gill region. They develop mainly when conditions are favourable with adequate moisture and intermittent rain. This results in both economic and nutritive loss to the fish processor. Infestation can be reduced by:

 Proper hygiene and sanitation

 Disposal of wastes and decaying matter

 Use of physical barriers like screens, covers for curing tanks etc

 Use of heat to physically drive away the insects and kill them at 45ºC

Fragmentation

Fragmentation occurs due to denaturation and excess drying of fish results in breaking down of the fish during handling.

Prevention

 It is necessary that fresh fish be used as raw material to ensure a good finished product.

Quality assurance of dried fish

 fresh as possible

 The water used for washing and brining should be potable

 Salt used should be of good quality and should not contain high amounts of Magnesium and Calcium chlorides.

Quality changes canned products

Classification of Spoiled Cans

Flipper: This is a can of normal appearance in which one end flips out when the can is stuck against a solid object. The end snaps back to the normal position when very light pressure is applied

Springer: This refers to can in which one end is bulged but can be forced back in to normal position where upon the opposite end bulges

Soft Swell: It is a case in which the bulged ends can be moved by thump pressure but cannot be forced back to the normal position

Hard Swell: This is one in which the ends of the can are permanently and firmly distended

The chief defects and causes of spoilage may be listed as follows:

1. Microbial spoilage: This may result due to

a) Under processing

b) Inadequate cooling

c) Leaker infection/Leakage through seams

d) Pre-process spoilage

2. Chemical spoilage

a) Internal corrosion giving rise to hydrogen swells or pin holing

3. Physical: causes due to

a) Faulty retort operation

b) Under exhausting

c) Over filling

d) Internal vacuum too high (Panelling)

e) Use of cans of inadequate substances

f) Rough handling

4. Miscellaneous like: Rust, Damage etc.

Discolouration

Black discolouration

It is mainly encountered in packing crab, clams, shrimp and lobster .Sulfur compounds in the flesh unite with the iron base of the tin plate to form iron sulfide

Prevention:

 Addition of small amounts of organic acid

 use of parchment-paper can liners

 lacquer containing small amounts of zinc

Copper sulfide discoloration/Blue discolouration in crab meat

It is associated with hemocyanin, a biochemical component in crustacean blood.The degree of blue discolouration depends on the copper content of the meat and average copper level is higher in blue meat (2.8mg% wet wt.) than in normal meat.

Prevention

 Use of metal chelating agent such as citric acid, or ethylene diamine tetraacetic acid (EDTA).

Green discoloration

It occurs in cooked tuna. The TMA content in cooked tuna is closely related to the degree of green colour and that the development of green colour is closely correlated with the TMAO content of raw fish. TMAO content of more than 13mg % N was found to cause greening,a level below this occurrence of greening is unpredictable. It is recommended that tuna with high concentration of TMAO and myoglobin shall not be used for canning

Brown discolouration

Brown discolouration is caused by reaction of protein or amino acid with product of lipid oxidation. It is observed in variety of processed products including white pomfret, sardine etc. Discolouration due to protein-lipid browning is greater in fatty fish than lean fish.

Common defects

Stack burning

Stack burning is caused by over processing. A considerable amount of heat is retained over a long period when canned products are stacked.

Perforation and Corrosion

Perforation and Corrosion in can is prevented by air should be expelled from product and headspace as completely as possible. It is dependent on the temperature. Cans should be thoroughly cooled before packing or stacking to reduce corrosion.

Gaseous Spoilage

Can appears like Swelled or "bulging. It is caused by spore-formers of the anaerobic or facultative anaerobic types. Organisms found quite commonly are *Clostridium welchii* and *Clostridium sporogens* and gas-forming heat-resistant organism is *Clostridium botulinum.*

Non-Gaseous Spoilage

There is no external indication of non-gaseous spoilage. It is caused by aerobic spore formers, *Bacillus cereus, B. mesentericus* and *B. vulgatus.* Storage at temperatures between 40 and 30^0 F will greatly reduce the possibility of flat souring.

Honeycombing

Honeycombing is found in canned tuna meat that is processed from stale raw material. The meat in such cases presents the appearance of honeycomb. During steaming the volume of the meat will contract due to removal of water because of the coagulation of muscle protein that begins at the surface. Production of gas in the flesh expands and make little pockets in the flesh. On cooling, the pockets remain and the flesh seems to be filled with small holes or air paces. It also occur in canned salmon and sardines.

Mush

It is Flabby condition met with some species of pilchards caught at the end of its spawning. This is caused by the invasion of prasitic protozoan *chloromyxum* whish decomposes the fish meat during storage such that it becomes entirely soft during canning.

Struvite formation

Canned marine products such as brine packed shrimp, crab, tuna, salmon are frequently seen to contain some glass like crystals, particularly when the temperature of storage is low. It occurs due to the formation of a chemical compound, magnesium ammonium phosphate hexahydrate,$MgNH_4PO_46H_2O$, called struvite. It can be prevented by adding chelating agent like hexametaphosphate.

Curd and adhesion

'Curd' is precipated protein often found in canned mackerel and salmon. This is more common with salmon, which is generally canned without pre-cooking. The meat coagulated by heat adheres to the inner side of the can ends and presents a poor appearance on opening the can. The lacquer may get peeled off while removing the curd from the can ends. Use of raw fish, which is not very fresh, and, inadequate brining and pre-cooking are some of the reasons responsible for formation of curd. It can be prevented if the raw fish is soaked in 10-15% brine for 20-30 minutes followed by thorough washing before filling.

Quality defects in Coated Fish Products

Coated product is one, which is coated with another foodstuff. Seafood specialties, fish portions (raw and precooked), shrimp, fish fingers, scallops, fish balls, fillets etc are the principal seafood products which are bettered and/or breaded

Shelling

 Separation of breading from substrate due to uncontrolled release of moisture
 Corrective Action –Controlled release of moisture from substrate and breading system

Blow Off

 Breading blows off in the fryer
 Corrective Action –Monitor free water/ice on the surface of the substrate

Poor Adhesion

 Breading system does not adhere to the substrate
 Corrective Action –Increase protein to perform binding

Gummy Interface

 Area between surface of the substrate and breading system is gummy Corrective Action –Ensure product is fully cooked

http://drs.cift.res.in/bitstream/handle/123456789/2257/Quality%20defects%20in%20fish%20and%20fishery%20products.pdf?sequence=1&isAllowed=y

Chapter 5
Estimation of Residual Chlorine in Water

Iodometric method

Principle

Chlorine liberates free iodine from potassium iodide solution in acidic pH quantitatively.# The liberated iodine is determined by titration with standard sodium thiosulphate solution. As chlorine in aqueous solution is not stable, the determination of chlorine must be performed immediately after sampling. Care should be taken to avoid excessive exposure of the water sample to sunlight and agitation.

Requirements

Apparatus / Glassware

Analytical Balance

Burette, 25 ml
Volumetric flask, 1 00 and 250 ml
Conical flask, 250 ml
Measuring cylinder, 500 ml
White porcelain dish, 500 ml
Beaker, 250 ml

Reagents

i) Acetic acid, glacial.

ii) Potassium iodide crystals.

iii) Starch indicator.

iv) N sodium thiosulphate solution: Dissolve 24.8 192 g of sodium thiosulphate ($Na_2S_2O_5$. 5 H_2O) in 200 ml water and transfer to a 1 litre volumetric flask and make up to volume. Standardize the solution with potassium dichromate. Weigh 0.20 to 0.23 g $K_2Cr_2O_7$ and transfer to a 250 ml beaker using about 150 ml water. Add 2 g potassium iodide and mix. Add 20 ml of 1 N HCl , swirl and allow to stand for 10 min. Titrate with the thiosuphate solution adding 1 ml of 1% starch solution towards the end of titration and complete titration where the solution changes from blue green to light green.

Normality of sodium thiosulphate solution = Wt. Of $K_2Cr_2O_7$ (g) X 1000/Vol. of $Na_2S_2O_5$ (mL) x 49.037

Prepare 0.01 N working standard by diluting the 0.1 N thosuphate solution.

Procedure

Take a suitable volume of the water sample into a porcelain dish or beaker. For water containing 1 mg/litre or less chlorine take 1000 ml and for 1 to 10 mg/ litre take 500 ml. The titre value of 0.01 N thiosulphate should not be more than 20 ml. Add 5 ml glacial acetic acid followed by 1 g potassium iodide, stir and titrate with 0.01 N thiosulphate solution until the yellow colour of the liberated iodine is almost disappears. Add 1 ml starch solution and titrate until the blue colour is discharged. Do not carry out the titration in direct sunlight. Blank titration can be carried out by taking equal volume of distilled water.

Observations

Volume of water taken for titration = V = - ml

Volume of thiosulphate solution required (titre) = V1 =- ml

Normality of sodium thiosulphate solution = 0.01 N

Calculations

1000 ml 1N sodium thiosuphate = 35.46 g chlorine (ie. 1g mole of chlorine) or 1 ml 1N sodium thiosulphate = 35.46 mg chlorine or V1 ml of 0.1 N sodium thiosulphate =V, x 0.01 x 35.46 mg chlorine Therefore, residual chlorine content of the water (mg per litre) = V1 X 0.01 X 35.46 X 1000/V = V1 X 354.6/V

Result

Residual chlorine content of the water sample = mg per litre or ppm

https://www.youtube.com/watch?v=usTEPoYbRIQ

Chapter 6
Testing of Corrugated Fibre Board Boxes

Significances and Test of Important Parameters :

i) Kraft Liner, Fluting Media

The following tests are performed on liner and fluting medium.

(a) Grammage

Significance : Grammage is a measure of the weight of paper or paper board expressed in gms per square meter. It is also called as substance. Its unit is gms/ sq mt and popularly known as gsm.

Test Method : TAPPI T 410 or IS : 1060 (Part – I) 1987 are the reference for test method. Minimum 10 numbers of specimen of size 10 cm X 10 cm are cut, weighed and calculate the gsm by diving the area.

(b) Caliper of Thickness

Significance : Caliper is the measure of the thickness of a sheet of paper. Its units is mm or microns.

Test Method : The reference of test methods are TAPPI T-441 or IS: 1060- Part-I-1966. Thickness of test specimens are measured by using a calibrated micrometer.

(c) Water Absorptiveness of Nonbibulous Paper and Paperboard (COBB Test)

Significance : Water absorbency is a characteristic pertaining to the sheets ability to resist water penetration and absorption.

The details about test methods are prescribed in TAPPI T 441 and IS : 1060 Part – I – 1966. The test specimens are clamped, poured 100 ml. of water. Allowed to absorb water for 60 seconds. The water is removed after 45 seconds prior to 30 minutes, the additional water, is wiped and weighed. The difference in weight divided by sample area gives the value in gm/ml.

(d) Bursting Strength

Significance : To measure the force required to rupture the board when pressure is applied from one side.
Test Method : The sample is subjected to mullen bursting testing equipment and the force require to burst the paper is measured The test values are expressed as kg/cm2 or pound/sq inch.

(e) Tensile Breaking strength

Significance : It is the maximum tensile force per unit width developed in a test specimen at rupture or break.
Test Method : Test specimen cut to specified size is clamped between two jaws of tensile tester. Then the two jaws move away from each other at a specific speed. The force at which specimen breaks is recorded. This test is conducted five times. The average breaking force divided by specimen width gives tensile strength in KN / m.

ii) Corrugarted Board

The following parameters are assessed in the laboratory for corrugated fibre board.

(a) Caliper

Significance : The thickness of corrugated board is the distance in millimeters measured between the two parallel contact plates of a micrometers between which the specimen is subjected to a pressure of 20 kpa.

Test Method : The reference of list methods are TAPPI T-441 or IS: 1060- Part-I-1966.

(b) Board Grammage by Ply Seperation Method

Significance : To determine the basic weights of the combined board and its components. As per TAPPI, the average readings are considered.

Test Method : The samples are soaked in water to allow the adhesive to dissolve in water. Then the layers of papers are peeled off to separate the layer carefully.

(c) Bursting Strength

Significance : To measure the strength properties of the board in terms of kg/ cm2 .

Test Method : The sample is subjected to mullen bursting strength tester and the force required to rupture or burst the board is determined.

(d) Puncture Resistance

Significance : The puncture resistance is a measure of the energy needed to punch through a material.

Test Method : The reference test method is TAPPI T 803. A pendulum with pyramidical shaped head, selected so as to simulate a corner of a box, is released from a certain height. The freely falling pendulum acquires kinetic energy and the head puncture the board. The energy consumed is expressed in ounce inch per tear inch or kg-cm.

(e) Flat Crush Test (FCT)

Significance : To measure of the resistance of the flutes in corrugated board to a crushing force applied perpendicular to the surface of the board.

Test Method : TAPPI T 825 or IS : 4006 – Part-I are the reference standards. Circular shaped CFB is kept in between the platen and compressed till rupture the flutes. Measure the force is measured in Kg.

(f) Edgewise Compression Test or Edge Crush Test (ECT)

Significance : ECT of a corrugated board is defined as the maximum vertically applied compressive force along the edge of the board without the board bucking.

Test Method : The reference is TAPPI T 811. The sample is kept under horizontal plates with the flutes vertical. The plates are then passed down with a constant speed of 10 ± 3mm/minute so that the load on the edge of the board gradually increases. The load at which the board buckles gives the ECT. SI unit of ECT is KN/m.

(g) Performance Tests of Corrugated Fibre Board Boxes

(a) Box Compression Test (BCT)

Significance : This is the most important and the most common test for corrugated boxes. BCT is the measure of the ability of a corrugated box to take top down loads. It is the measure of stackability of the box and determines how much load can be stacked upon the box without the walls of the box buckling.

Test Method: TAPPI T 804 or IS : 7028 (Part-VI) are the reference test method for BCT. The box is placed in a press between two parallel plates which apply pressure to the box at right angles to its flaps. The bottom platen is fixed and the top plates moves at the speed at 10±3mm/min. Now, run the machine and continue till the box gets crush. Take the reading from the recorder in kgs. This will clearly indicate that how much force is required to compress the box.

(b) Drop Test

Significance: This test is conducted: To determine the ability of the package to stand up to rough handling, the degree of protection offered to the contents by the package and to compare the different types of packaging for the same product.

https://www.youtube.com/watch?v=_JhTQvDlNGw

Chapter 7
Detection Method for Nematodes in Fish Fillets And Fishery Products

The candling procedure is a non destructive method of using various light sources and intensities. The method is often applied in the industry for routine control to remove visible nematodes in fish fillets before further processing. Candling is also recommended as official codex method for the detection of nematodes in quick frozen blocks of fish fillets (Codex, 1992). It is a very simple and inexpensive method and allows a large sample throughput, but fails for certain fishery products like fish sticks and skin-on fillets and does not yield quantitative results.

Description of the pressing method with fluorescence detection

Fresh fillets are skinned and the flesh is placed in the middle of a plastic bag and squeezed between two 12 mm thick acryl glass sheets to a thin layer of 2-3 mm by means of a hydraulic or manual press device. The resulting press cake of the fresh fish has to be deep frozen in the plastic bag for several hours before analyzing.

Deep frozen samples can be analyzed directly after thawing and pressing. The coatings of fish portions and sticks have to be stripped off. The size of the plastic bag depends on the size of the sample. For herring and small saithe fillets, use plastic bags of 700 mm length and 300 mm width .

The pressed sample (in the plastic bag) is examined for nematodes under UV-light at 366 nm, either in a darkened room or in a viewing chamber. Eye protection, appropriate for the type of UV lamp should be used.

All nematodes show a brilliant white-bluish fluorescence and can be counted easily. Additionally, their position can be marked on the plastic bag which allows a later determination of their position in the flesh. As light source commercially available blacklight-tubes were used.

Observation:

Chapter 8
Safety of Water and Ice

Criteria for potable water

Water is used in fish processing both as an ingredient and for cleaning and sanitation. Thus the quality of the water is of great importance. WHO (1993) and EC (1998) have published extensive guidelines on drinking water quality where standards for more than 60 parameters have been elaborated. The microbiological criteria suggested are shown in Tables 8.1 and 8.2.

Very often water needs to go through some form of treatment and disinfection before being suitable for use in fish processing.

Water treatment

Water treatments vary from region to region depending on the water sources available. While groundwater from sedimentary aquifers has undergone extensive filtration the water from hard rock aquifers or surface water sources should be filtered as part of the water treatment in order to decrease the content of particulates, microorganisms, organic and inorganic matter.

Table 8.1 Bacteriological quality of drinking water (WHO, 1996).

Organisms	Guideline value
All water intended for drinking	
E.coli **or thermotolerant coliform bacteria**	**Not detectable in any 100-ml sample**
Treated water entering the distribution system	
E.coli **or thermotolerant coliform bacteria**	**Not detectable in any 100-ml sample**
Total coliform bacteria	**Not detectable in any 100-ml sample**
Treated water in the distribution system	
E.coli **or thermotolerant coliform bacteria**[1]	**Not detectable in any 100-ml sample**
Total coliform bacteria	**Not detectable in any 100-ml sample. In the case of large supplies, where sufficient samples are examined: Not detectable in 95% of samples taken during any 12-months period**

Immediate investigative action must be taken if either E. coli or total coliform bacteria are detected. The minimum action in the case of total coliform bacteria is repeat sampling; if these bacteria are detected in the repeat sample, the cause must be determined by immediate further investigation.

1. *Although E. coli is the more precise indicator of faecal pollution, the count of thermotolerant coliform bacteria is an acceptable alternative. If necessary, proper confirmatory tests must be carried out. Total coliform bacteria are not acceptable indicators of the sanitary quality of rural water supplies, particularly in tropical areas where many bacteria of no sanitary significance occur in almost all untreated supplies.*

Table 8.2 Microbiological criteria for drinking water (EC, 1998).

Parameter	Parametric value	Method of examination
E. coli	**0/100 ml**	**ISO, 9308-1**
Enterococci	**0/100 ml**	**ISO, 7899-2**
Indicator		

Colony count, 22°C	**[No abnormal change]**	**Pr EN ISO 6222**
Coliform bacteria	**0/100 ml**	**ISO, 9308-1**

Former directive 80/778/EC (EC 1980) used 100 cfu/ml as guidelines.

Parasites are removed to a large extent by filtration. The levels of bacteria and virus also decrease markedly and the removal mechanisms are both filtration and adsorption. The cation concentration influences adsorption, i.e. increasing concentrations give rise to increased adsorption. Ca^{2+} and Mg^{2+} seem to be especially efficient. These small cations will decrease the repulsive forces between the soil particles and the microorganisms. Iron oxides also have a high affinity for viruses as well as bacteria. Ferric hydroxide impregnated lignite has even been suggested as a local filtration/adsorption media.
The disinfection efficiency is greatly affected by

- type of disinfectant,
- type and state of microorganism,
- water quality parameters such as turbidity (or suspended solids),
- organic matter,
- some inorganic compounds,
- pH
- temperature.

The "hardness" of the water may indirectly influence disinfection since deposits may harbour microorganisms and protect them from cleaning agents and disinfectants.
By far the most widespread disinfectant is chlorine but also chloramines, chlorine dioxide, ozone and UV are being used in some instances. Chlorine is cheap and available in most places and monitoring the free residual levels is simple. For disinfection WHO (1996) is recommending 5 mg chlorine/litre and for effective disinfection there should be a residual concentration of free chlorine of ^{3}0.5 mg/l after at least 30 minutes contact time at pH <8.0. For disinfection of clean equipment up to 200 mg/l is used. To avoid corrosion a lower concentration of 50-100 mg/l and longer contact times (10-20 minutes) are often used. Current guidelines are shown in Table 8.3.

Table 8.3 Concentrations of chlorine used in fish processing.

Type of water	Residual levels	Recommendation by
Drinking water	**0.5 mg/l**	**WHO 1996**
Water for clean-up	**100 mg/l**	**Reilly 2000**
Water in contact with fish	**10 mg/l**	**Reilly 2000**
Seawater for cooking of shrimp	**20 mg/l**	**Watson and Prout 1996**

Chloramines are more stable but less microbiocidal and much less efficient in killing parasites and virus than chlorine. Chlorine dioxide is, if anything, more microbiocidal than chlorine, especially at high pH, but there is concern with

regards to the by-products. In the case of ozone and UV there is no residual matter to monitor. Ozone seems to be very efficient in killing protozoa. The efficiency of UV disinfection decreases markedly if there is any turbidity or dispersed organic matter and problems are often encountered due to a lack of lamp maintenance. The resistance of the various microbiological organisms varies a lot. In the case of most disinfectants the order of sensitivity in decreasing order is:

vegetative bacteria > viruses > bacterial spores, acid-fast bacteria and protozoan cysts.

The sensitivity varies within groups and even within species. Indicator bacteria are unfortunately among the more sensitive microorganisms and the presence of, for example, faecal coliforms in treated, disinfected water is therefore a very clear indication that the water contains potentially pathogenic microorganisms while the absence of such indicator bacteria does not guarantee pathogen-free water.

Bacteria from nutrient-poor media as well as otherwise stressed bacteria may also exhibit greatly increased resistance. Some of the effects mentioned on the efficiency of free chlorine are illustrated in Table 8.4.

Table 8.4 Inactivation of microorganisms by free chlorine.

Organism	Cl_2 residues mg/l	Temp. °C	pH	Time, min.	Reduction %
E. coli	0.2	25	7.0	15	99.997
E. coli	1.5	4		60	99.9
L. pneumophila (water grown)	0.25	20	7.7	58	99
L. pneumophila (media grown)	0.25	20	7.7	4	99
Acid-fast *Mycobacterium chelonei*	0.3	25	7.0	60	40
Virus					
Hepatitis A	0.5	5	10.0	49.6	99.99
Hepatitis A	0.5	5	6.0	6.5	99.99
Parasites					
G. lamblia	0.2-0.3	5	6.0	-	99
G. lamblia	0.2-0.3	5	7.0	-	99
G. lamblia	0.2-0.3	5	8.0	-	99

If microbes are associated with granular material or other surfaces the effect of a disinfectant such as chlorine decreases drastically. Attachment of *Klebsiella pneumonia* to glass surfaces may, for example, increase the resistance to free chlorine by 150-fold.

Organic matter may react and "consume" disinfectants such as chlorine and ozone and the presence will also interfere with UV light. The chloramines are less susceptible to organic matter.

pH is important in disinfection with chlorine and chlorine dioxide. There is greater inactivation of microorganisms at low pH in the case of chlorine and greater

inactivation at high pH in the case of chlorine dioxide. In general, higher temperatures result in increased inactivation rates.

Use of non-potable water

The use of non-potable water may be necessary for water conservation purposes or desirable because of cost. Non-potable water may, for example, be surface water, sea water or chlorinated water from can cooling. Relatively clean water such as chlorinated water from can cooling operations may be used for washing cans after closing and before heat treatment, for transporting raw materials before processing (after the water has cooled off), for initial washing of boxes, for cooling of compressors, for use in fire protection lines in non-food areas and for fluming of waste material.

Separation of potable and non-potable water

It is absolutely necessary that potable and non-potable water should be in separate distribution systems which should be clearly identifiable.

If potable water is used to supplement a non-potable supply the potable source must be protected against valve leakage, or back-pressure, for example, by adequate air-gaps. Back-flow, due to sudden pressure differentials or blockage of pipes, has unfortunately occurred in many systems.

Potentially contaminated water such as coastal water or surface water, should not be used at the production premises but may, if aesthetically acceptable, be used for removing waste material in places where no contact to food is possible.

Monitoring water quality

The responsible person should have continuously updated reference drawings of the pipe system and the authority to remove dead-ends. Especially in cases where a plant has undergone many changes, the piperuns, may become more and more complicated over the years. The person should also be in contact with the local waterworks and the authorities in order to be informed of special events (repairs, pollution accidents or other changes).

Water may be contaminated due to bad location of source (close to septic tanks, agriculture drainage systems), cracked or improperly sealed off piping systems or even floods and heavy rains. In the plant, contamination of the water may be due to cross-connections or backflow (back pressure or back siphonage). Where necessary, backflow should be controlled by air-gaps, vacuum breakers or check valves.

A quality monitoring scheme could consist of a plan of all the sampling points and a checklist describing what to examine and why, the frequency, who takes the sample, who does the analysis, what is the limit (value, tolerance) and what to do in case of deviation (Poretti 1990). If the water is obviously polluted there is of course no reason to wait for analytical results. The sampling frequency and the range of parameters will vary with the circumstances and a special monitoring program may be needed after repairs, or when using new water supplies, for example. A minimum monitoring program for water quality could be:

- measurement of free chlorine daily
- measurement of total viable count and coliforms on a weekly basis.

The technical procedures describing the analyses for the common indicator organisms are given in standard textbooks. The EC Directive (EC, 1998) specifies some methods and equipment to be used. The values used by the company

should refer to the specific method employed and the recommendations should include how to sample (tap flow, volume, sampling vessel, labelling, etc.) and how to handle and examine the sample. Even though the commonly used methods for detecting, for example, faecal coliforms are standard analyses, faulty handling of the samples often occurs. Samples should be processed within 24 hours or less, be kept cool but not frozen (preferably below 5°C), and be kept in the dark. The impact of sunlight can be very dramatic, causing false negative results.

If chlorination is used for disinfection monitoring of the free chlorine level is the simplest way of checking the water treatment and should be performed most often (e.g. on a daily basis). Simple laboratory methods and commercial dip-sticks are now available for on-the-spot measurements (e.g. Merchoquant Chlor 100 from Merck). The microbiological indicator parameters may be checked less frequently. If disinfection systems that leave no residuals are being used, then checking of equipment should be done regularly. The performance of the systems may be monitored at weekly intervals using indicator bacteria measurements.

https://www.youtube.com/watch?v=LpA_9z2s4Wg