

ASSESSMENT OF QUALITY IN FOOD BY INSTRUMENTAL AND CHEMICAL METHODS

Introduction

- Food is made up of chemicals.
- These chemicals are all potentially significant, as they determine the nutritional value, eating properties and suitability for use in particular products and processes.
- Estimation of chemical composition or proximate composition of food will give the details of nutritional value of food.
- The proximate composition of food that is estimated is the moisture, protein, fat, carbohydrate and ash content.
- The quality characteristics of food involve the assessment of changes to volatile bases, nucleotide and lipid oxidation products.

A. INSTRUMENTAL METHOD

1. Analysis of moisture contents (Air-Oven Drying)

- Air-oven drying is the most common and widely used method for routine moisture determination in standard laboratories around the world.
- The principle of oven drying is based on weight loss on heating.
- The sample needs to be thermally stable
- Should not contain a significant amount of volatile compounds

2. Analysis of ash contents (Using Muffle furnace)

- Ash is the inorganic residue from the incineration of organic matter
- The ash content is determined from the loss of weight that occurs during complete oxidation of the sample, at a high temperature (usually 500-600°C) in a muffle furnace through volatilization of organic materials.
- Total ash content is a useful parameter of the nutritional value of many foods and feeds.
- High levels of acid-insoluble ash indicate the presence of sand or dirt in the sample

3. Determination of protein content in foods

- The quantification of total protein in food products can be performed
- directly, or
- by determination of the total organic nitrogen followed by conversion of total nitrogen to crude protein content using a suitable conversion factor.

i) The Lowry's Method (Spectrophotometer)

The reduction of the Folin-Ciocalteu reagent, composed of a mixture of phosphomolybdic and phosphotungstic acid, by oxidation of tyrosine, tryptophan, and to a lesser extent, cystine, cysteine, and histidine residues on the polypeptide side chains of protein.

Copper chelates in the peptide structure, which facilitates the electron transfer from the amino acid functional groups to the mixed acid chromogen.

The oxidation-reduction reaction is accompanied by the formation of a characteristic blue color with absorption maximum at 745-750 nm.

The intensity of the colour, depends on the amount of these compounds in the protein, which is then measured using a spectrophotometer.

ii. The Kjeldahl Method (Kjeldhal distillation apparatus)

Organic nitrogen is transformed to ammonia, which is retained in solution as ammonium sulfate. Ammonia is liberated from the digest by distillation for 10 min. in the presence of alkali.

The liberated NH_3 is collected in a boric acid solution.

It then is titrated against a very dilute H_2SO_4 and the Nitrogen content is determined.

The protein content is then calculated by multiplying the N value with 6.25 (protein conversion factor).

4. Estimation of lipids from foodstuffs (Soxhlet apparatus)

- Lipids should first be extracted either for total lipid estimation or for further analysis to be performed.
- The analysis of crude fat content present in food sample involves refluxing extraction of fat using ether in a Soxhlet apparatus.
- After evaporating the ether, the crude fat obtained will be dried and weighed

B. CHEMICAL METHODS

Chemical methods that may measure, freshness quality by the estimation of

- (1) Total volatile base nitrogen (TVB-N)
- (2) Trimethylamine (TMA)
- (3) Nucleotide degradation products (Inosine mono phosphate (IMP), hypoxanthine etc.)
- (4) Lipid oxidation products (peroxides, FFA value, aldehydes etc.).
- (5) Other chemical methods: Estimation of indole, biogenic amines and histamine.

1. TOTAL VOLATILE BASE NITROGEN (TVB-N)

- Spoilage organisms convert many nitrogenous compounds into volatile bases.
- Urea - in elasmobranchs is degraded to ammonia by bacterial action.
- The TVB-N developed during the storage of unfrozen fish is ammonia and trimethylamine, which are characteristics of flavor and odour changes.
- Volatile bases are liberated when the sample is treated with K_2CO_3 .
- The liberated NH_3 is absorbed in acids and the quantity absorbed is determined by the titration of excess of acids against a standard alkali.

Uses

It is an indirect method of assessment of freshness quality of seafood.

It is not capable of identifying the early stages of deterioration of freshness quality because TVB-N values are not linearly related to the length of time (days), if the species being evaluated was stored in ice and it cannot be used to predict the storage life of that species.

Useful for assessing quality during later stages of storage -can be routinely used as a standard method for assessing the quality changes in chilled, frozen, dried and canned seafood.

Levels of TVB-N permitted in fish and fishery products

Item	Maximum admissible limit
Fresh fish	35-40 mg/100 g
Frozen fish	Not greater than 30 mg / 100g
Salted & sun dried fish	Not greater than 100 –200 mg / 100g
Canned seafood	Not greater than 20 mg / 100g

2. Trimethylamine (TMA)

Trimethylamine (TMA) is formed from trimethylamine oxide (TMA-O) which in turn is converted into dimethylamine (DMA) and formaldehyde

Values of TMA depend upon

Fish species, the time of year at which caught, location of the catch, stage of spoilage, type of processing and / or storage and method of analysis.

Estimation is suitable for evaluating samples of medium to poor freshness quality of fish stored in ice and in frozen condition.

Method of estimation

Conway micro diffusion method and distillation method.

Limits of whole round and chilled fish: 10-15 mg
TMA-N / 100 g

Indication of spoilage of fish Levels: <15 mg TMA-N
or 1.08m molesTMA/100g.

The fishy odour of TMA plus lipid is generally detectable when TMA level reaches **4 - 6 mg n/100 g**, which is before the actual spoilage point.

3. NUCLEOTIDE DEGRADATION PRODUCTS

- The ATP of fish muscle breakdown either during the death struggle or subsequently.
- Liberation of **inosine 5'-monophosphate (IMP)**
- Converted into **hypoxanthine** during storage- in a progressive degradation by dephosphorylation, and the action of ribonuclease enzymes.
- Concentration of free **hypoxanthine** increases in the chilled stored muscle of most species
- **IMP** - contribute to the pleasant flavour of fresh fish.
- Its degradation to hypoxanthine is a factor in the progressive loss of desirable flavour and in the development of bitter 'off' flavour.

- **Estimation of Hypoxanthine**

- A protein extract of fish muscle is made using perchloric acid.
- A portion of it is neutralised and the perchlorate is removed as its insoluble potassium salt.
- The hypoxanthine in an aliquot of the neutral solution is converted to uric acid by the enzyme xanthine oxidase
- Measured by its absorption at 290nm.

Estimation of nucleotides

Degradation of ATP

ATP → adenosine diphosphate (ADP) → adenosine monophosphate (AMP) → inosine monophosphate (IMP) → inosine → xanthine (HXR) → hypoxanthine.

These nucleotides - separated by means of anion-exchange chromatography - measured using a spectrophotometer – k value – calculated- used to assess the quality of fish.

$$\text{K value (\%)} = \frac{\text{HX} + \text{HXR}}{\text{ATP} + \text{ADP} + \text{AMP} + \text{IMP} + \text{HXR} + \text{HX}} \times 100$$

K % of maximum **20** is acceptable for consumption of raw fish.

4. Indole

- Indole - has a fecal odour - highly volatile- an useful index of the freshness quality of shrimp during its non-frozen storage.
- Indole -the decomposition product formed by the break down of tryptophan in the protein of muscle by microbial enzymes.
- **Estimation** : Steam distillation - extraction of indole in CHCl_3 - colour development using Ehrlich's reagen - stripping down colour formed in acidic phase - measuring in spectrophotometer or spectrofluorometer at 290nm.
- Used to determine if raw shrimp had undergone high temperature abuse.
- Shrimps with less than 25micrograms/100g indole are organoleptically accepted.

Lipid oxidation products

- **Free fatty acids**

- The free fatty acid value is an indication of the extent of hydrolytic rancidity.
- During storage, fat or fatty material may be hydrolysed by lipases to liberate free fatty acid and glycerol. Lipases may be present in sources from which fat is extracted or produced by microorganisms during storage.
- The fat is extracted using chloroform, methanol and isopropanol in the ratio of 2:1:2 and then titrated with a standard alkali using an indicator.
- The amount of free fatty acid present is neutralised by the alkali and then calculated as the acid number. Acid number is defined as the number of mg of potassium hydroxide required to neutralise the free fatty acid present in 1g of fat of the sample.

- **Peroxide value**

- The peroxide value gives an indication of the extent of oxidative rancidity. It is a measurement of the amount of oxygen absorbed at double bonds in unsaturated fatty acids contained in fish oil, and is defined as the number of milli-equivalents of peroxide per kilogram of oil.
- The unsaturated fatty acids present in fish fat undergo oxidation during storage and form peroxides.
- Peroxide value is used as an indicator of quality of fat.
- Fresh oil usually has peroxide values of less than 1 meg/kg and may increase upto 10meg/kg. Values of the order of 10 to 20 meg/kg usually indicate rancidity.
- The most common method is based on iodometric titration that measures the iodine liberated from potassium iodide by the peroxide present in the oil.

• Thiobarbituric acid value

- The TBA test measures the malonaldehyde produced during fat oxidation. It gives an indication of the extent of oxidative rancidity. As spoilage progresses, there is a steady increase in the TBA number.
- The malonaldehyde and its derivatives can be measured through reaction with thiobarbituric acid that produces a red chromogen, which can be measured spectrophotometrically at 538 nm.
- Fish of good quality will have a TBA value of less than 2, and poorer quality than 2 will probably smell and taste rancid.
- Heat processing, such as canning, causes a large decrease in TBA value, so it is not a useful indicator of rancidity in canned products.

- **Iodine value**

- The iodine value is a measure of the degree of unsaturation.
- The glycerides of unsaturated fatty acids react with a definite amount of iodine, which adds across the double bond.
- The iodine value is often used to classify an unknown oil or fat into a particular class by determining the degree of unsaturation for example, cod liver oil have an iodine value between 155 and 170.

- **Saponification value**

- This value refers to the number of milligrams of potassium hydroxide necessary to completely saponify 1 gram of fat. This value gives an estimate of the average molecular weight of the fat.

5. Biogenic amines

- Biogenic amines- low-molecular weight organic bases -in animals, plants and microorganisms as a result of metabolic processes.
- Biogenic amines are biologically active.
- Psychoactive (acting directly on the central nervous system) or
- Vasoactive (acting directly or indirectly on the vascular system)
- Vasoconstriction:e.g. tyramine and
- Vasodilaton e.g. histamine may be distinguished.
- Normal constituents in foods, but are present only in small amounts.
- Higher levels in aged, fermented or putrefied foods- due to the action of decarboxylating microorganisms- convert amino acids into their corresponding amines.

Estimation

Biogenic amine index (BAI) -a spoilage indicator of fish.

Biogenic amines are derivatised to dansyl amines using the dansyl reagent (5 - (dimethyl amino) naphthalene 1 - sulphonyl chloride)

The dansyl derivative's fluorescence characteristics are examined under UV light at 254 nm.

BAI is based on the levels of histamine, putresceine, cadaverine, spermine and spermidine (in ppm)

$$\text{BAI} = \frac{\text{histamine} + \text{putresceine} + \text{cadaverine}}{1 + \text{spermine} + \text{spermidine}}$$

6. Histamine

Histamine -produced during the storage of pelagic species e.g. tunas, mackerels, carangid species, etc. at elevated temperatures.

Bacterial enzyme decarboxylates free histidine in the muscle to histamine.

Concentration of histamine-an indicator of bacterial spoilage.

Estimation:Extraction of histamine by perchloric acid - followed by n-butanol- under alkaline condition - collection in acid- aqueous phase.-Histamine in the acid- aqueous phase – on condensation with o-phthalaldehyde under alkaline condition – emits fluorescence - read at an excitation wavelength of 340 nm and emission wavelength of 425 nm in a spectrofluorometer.

The maximum concentration permitted is 20 mg / 100 g fresh fish.