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National Academy of Agricultural Science (NAAS) Rating : 3.03

Biochemical Analysis of Fortified Freshwater Fish Sausage

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ABSTRACT: *Fish is a nutritious food consisting of high grade protein, vitamins, minerals and relatively low calories. In addition, sea foods are excellent sources of omega-3 poly unsaturated fatty acid which have thetherapeutic value. Therefore sea food is considered as important nutritional sources. To utilize such quality food and to combat acute protein deficiency prevailing among the populations in several parts of the world, scientists have always been trying various methods of processing of fish and fish products. In this study, Tilapia fish were taken for sausage preparation due to its high flesh content, and size of the fish is convenient for manual dressing and also easy separation of flesh by machine. The fortified fish sausage can be prepared with addition of fish oil and potassium iodide in order to increase the nutrient content in the sausage. The quality parameters were analyzed for prepared sausage viz., Protein, PV, FFA which were found to be good for treatments of T_6 (5% fish oil + KI (150 µg)) and T_7 (10% fish oil + KI (150 µg)). The treatment T4 (15% fish oil) overall accepted followed by T_3 (10% fish oil) and T_6 (5% fish oil +KI (150 µg)) has bright red in colour and texture. The prepared sausage was stored in the refrigerator condition for its shelf-life study. The Tilapia fish are acceptable for sausage preparation due to its high flesh content, and also the size of the fish is convenient for manual dressing and for machine separation of flesh.Fortified fish sausage can be prepared with addition fish oil and potassium iodide in order to increase the nutrient content in the sausage. Protein, PV and FFA is found good for the treatments T6 and T7.*

Keywords:fish sausage, treatments, quality parameters.

INTRODUCTION

India is blessed with a coastline of 8,118 km with an Exclusive Economic Zone (EEZ) of 2.02 million sq.km and a continental shelf of 0.506 million sq. km. The inland water resources include 1,91,024 km of rivers and canals, 2.05 million ha of reservoirs, 2.25 million ha of ponds and tanks, 1.3 million ha of oxbow lakes and derelict waters, and 1.24 million ha of brackish waters. India has an estimated fish production potential of 8.4 million tones, of which the marine sector will account for 3.9 million tones and the inland fisheries sector will account for 4.5 million tonnes.Indian fishery is multi-species and consists of a large number of fish species of varying sizes. The catch consists of some very small sized fishes, which often cannot be put to any economic use. Such fish catch is discarded over board or at landing centers. The low value fish constitute a sizable part of the country's total marine catch. Discarding of these fishes, which are rich in protein, is therefore, a loss to the nation where such cheap protein food is very much needed. The producers and processors reject these fishes only because they don't have any commercial value. Therefore, it is necessary to give Emphasis on preparation of value added products which are acceptable to the consumers, such as protein concentrate, fish-wafers, fish-sausages, fish-soup, fish-cutlets, fish-balls, fish-feed, etc., using low value fish. This study will be helpful for grass root fishermen to utilize low value and underutilized fishes, through processing and value addition. Food fortification is the process of adding micronutrients (essential trace elements and vitamins) to food. It can be purely a commercial choice to provide extra nutrients in a food, or sometimes it is a public healthpolicy which aims to reduce numbers of people with dietary deficiencies in a population.

MATERIALS AND METHODS

Preparation of fortified sausage from low value fresh water fish

The method of preparation of fortified fish sausage was according to the standardized procedure. The fishes were dressed to remove scales, head and viscera

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and washed in chilled water excess water was drained. The cleaned fish pieces were fed to the de-boner to separate from the skin and bones.The separated meat was minced in a mincer to reduce the particle size. The minced meat was thoroughly mixed with the other ingredients viz., salt, sugar, chili powder, pepper powder, coriander seeds powder, ginger garlic paste, corn starch, vegetable oil, fish oil and potassium iodide to make a fine paste using silent cutter the paste mixture was stuffed in to the synthetic casing with the help of the sausage filler and the ends were tied off using the thread. The sausage were heat processed at 88+-2C FOR 60 minutes to obtain proper gel strength and were cooled to room temperature. Fish sausage paste was prepared in different composition of fish oil and potassium iodide (KI). The details of the combinations are given below.

1. Control (Without addition of fish oil and KI)
2. 5% fish oil
3. 10% fish oil
4. 15% fish oil
5. Control + KI (150 μg)
6. 5% fish oil +KI (150 μg)
7. 10% fish oil +KI (150 μg)
8. 15% fish oil +KI (150 μg)

Storage condition – Refrigerated storage at $6 \pm 2^{\circ}$ C

Control was a sample without fish oil and potassium iodide. Fish sausage was prepared hygienically as per the standard procedure. The experiments were conducted to study the shelf life of the prepared fortified fish sausage to examine their suitability for human consumption. These samples are stored under refrigerated condition for three months. The different parameter of fish sausage like proximate composition, quality parameters, microbial characteristics and sensory evaluation was done atan interval of 30 days.

Bio-chemical parameters of fish sausage

Moisture measurement

The moisture content of the fresh fish, fish sausage paste and fish sausage was determined by following hot air oven method (AOAC, 1995)[1]. About 10g of fish was weighed into a weighed moisture box and dried in an oven at 100+-1°C for 16 hours and cooled in desiccators. The weights of the dried samples were recorded. The moisture content of the sample was calculated by using the formula.

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_1} \times 100$$

Where, W_1 = Initial weight of the sample (g) and W_2 = Final weight of the sample after drying (g)

Crude protein

The crude protein in the fresh fish, fish sausage paste and fish sausage was determined using micro kjeldahl method (AOAC 1995) [1].A 0.5 g of fresh fish, fish sausage paste and fish sausage was transferred to a digestion tube to this 0.5 g of digestion mixture and 10 ml of concentrated sulphuric acid were added . The sample was digested in a unit till it became colourless. Then the tubes were cooled and transferred to the distillation unit. Forty ml of 40% NaOH solution was allowed into the tube. Liberated ammonium was absorbed in 2% boric acid solution contacting mixed indicator. The pink colour of the boric acid solution was turned to green and this was titrated against 0.01N HCL until the colour was obtained. The protein in percent was obtained by using the following

$$\text{Protein (\%)} = \frac{\text{TV} \times 0.014 \times 100\,(\text{ml}) \times 0.01 \times 100 \times 6.25}{\text{Weight of the sample (g)}} \times \text{Aliquot used for distillation (ml)}$$

Where, TV = Titre value

Total minerals

The total mineral content of a fresh fish, fish sausage paste and fish sausage was determined by Muffle furnace method (AOAC 1995) [1]. Accurately 5 g of sample was weighed into a crucible (which was previously heated to about 600 ° c and then cooled. The crucible was placed on a clay pipe triangle and heated first over a low flame till all the material was completely charred, followed by heating in a Muffle furnace for about 3 to 5 h at 600°c. It was then cooled in desiccators and weighed. The percentage of ash was calculated by using the following expression.

$$\text{Total ash (\%)} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample (g)}} \times 100$$

Free Fatty Acid (FFA)

The free fatty acid (FFA) content in the lipid extract was determined by the improved titrimetric method as explained by Ke *et al.*, (1976) [2]. 8ml chloroform extract was added to conical flask of 250ml followed by 4 ml of methanol and 8 ml of isopropanol in the ratio of 2:1:1. To this of 3 drops of 0.5% of met cresol purple was added as an indicator. The sample was titrated to purple end point with 0.05 N aqueous

sodium hydroxide. The percentage of free fatty acid was calculated (as oleic acid percentage) by using the following equation.

$$\text{FFA (\%)} = \frac{\text{Titrated value of sample} - \text{Titrated value of blank} * N \text{ of NAOH} * 28.2}{\text{Weight of the sample}}$$

Peroxide value

10g of sample was taken and ground well with 15 g of an hydrous sodium sulphate and chloroform and then they obtained extract was transferred to 100 ml stopped volumetric flask and made the volume up to 100ml using chloroform. 10 ml of chloroform extract was put into 250 ml conical flask followed by 25 ml of solvent mixture (2 volume of glacial acetic acid and 1 volume of chloroform), 1 ml of KI solution and 35 ml of water were added and placed in dark for 15 m. The liberated iodine was titrated against standard sodium thiosulphate solution and expressed as mili mole of oxygen/Kg of lipid.

$$\text{PV} = \frac{\text{Titrated value of sample} - \text{Titrated value of blank} * N \text{ of } NAS_2)_3}{\text{Weight of the lipid}}$$

The PV of lipid was determined from lipid extract according to Jacobs (1958) iodamatrically.

Iodine value

Iodine valve of fresh meat, sausage paste and sausage is determined by titration method (AOAC 1995) [1]. Weigh 0.2 g of sample in a 500ml conical flask fitted with round glass stopper. Add 20ml chloroform, shake to dissolve the sample completely and add 25 ml of Hanus iodine solution, mix well, stopper the flask and keep in dark for 30 m. Then add 20 ml of KI solution and mix well. Titrate against 0.1 ml sodium thiosulphate with continues vigorous shaking. Add 3to 4 drops of starch solution as an indicator to extract the iodine from the chloroform layer until the colour is discharged. A blank test sample is carried out under the same conditions.

$$\text{Iodine value} = \frac{A * N * 0.1269 * 100\, g\, I_2 (100g \text{ of oil})/fat}{\text{Weight of oil in (g)}}$$

Where,

A = ml $Na_2 S_2 o_3$ (test blank)

N = Normality of $Na_2 S_2 o_3$ solutions

RESULTS AND DISCUSSION

Moisture content

Fishes are the food having highest moisture content and this is the basic cause for deterioration of food product by fish. Hence it is the one of the most important parameter during storage, which is needed to be taken care of. It was observed from the Fig. 1 that the highest reduction in the moisture content (3.13 %) was observed in the treatment T5 and the lowest reduction in moisture content (2.84) was observed in the treatment T7. It was noticed that the fish sausage were spoiled in the treatments T1, T2, T3, T4, T5 and T8 at 60 days of storage and the sausage in the treatment T6 and T7 were good till 75 days of storage. The reduction in the moisture could be due to the evaporation of moisture from the outer surface of sausage during the storage. This is in line with Balasundari *et al.*, (1997) [3].

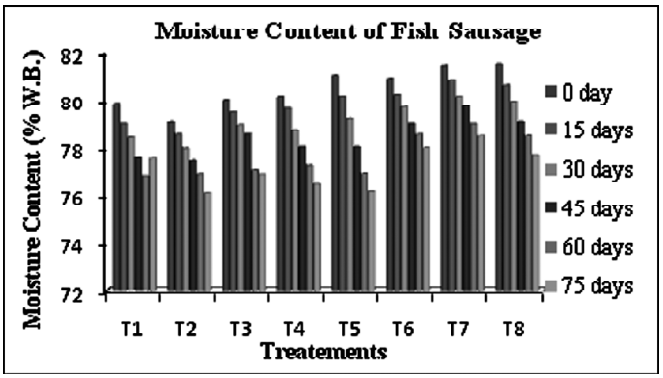

Figure 1: Moisture content of fish sausage during storage

Total minerals

The total mineral content of a fresh fish sausage and fish sausage stored for 90 days at refrigerated condition (6 ± 2 °C) were determined by Muffle furnace method andthe trend was indicated in Figure 2. It was observed from the figure that the ash content retained more (0.45%) compared to all the other treatments and lowest ash content (0.39%) was retained in the treatment T7. The increase or decrease in the total mineral content was dependent on the moisture content. More the reduction in moisture higher values

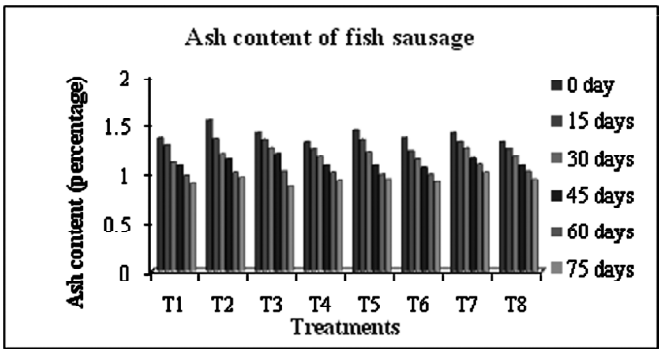

Figure 2: Ash content of fish sausage during storage period

of mineral content. It was observed that sausages were spoiled in the treatments T1, T2, T3, T4, T5 and T8 at 60 days of storage the sausage in the treatment T6 and T7 were good till 75 days of storage.

Crude protein

The crude protein content was considered as a relatively good index for loss of nutritiveness value during frozen storage for meat quality. The Data presented concluded that the protein content of sausage was (19.25%) at zero time while decreased during storage in refrigerated conditions at 6 ± 2 °C. for 75 days to reach (13.99%) at the end of storage period. This decreased of protein due to decomposition of fish flesh by microorganisms which lead to' formation of volatile nitrogenous substances and soluble substances that scared from the tissue as well as the separation of drip during storage Fig. 3. These results are in line with [4].

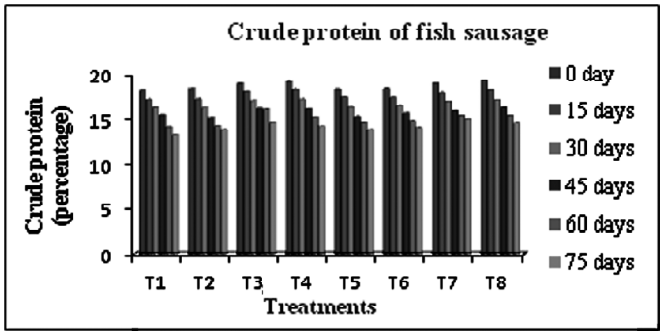

Figure 3: Crude protein content of fish sausage during storage

It was observed from the Fig. 3 that the Crudeprotein retained more T6 (4.33%) compared to all the other treatments and lowest Crude protein (2.92%) was retained in the treatment T3. The loss of protein is due to common phenomenon of protein denaturation. There are various factors which influence the protein denaturation such as effect of ice crystals, binding of fatty acid and lipid oxidation products to proteins, oxidation and interaction of myofibrillar protein in which hydrogen bond, ionic, hydrophobic bond and disulphate bonds are formed. The concentration of solute increases during freezing, resulting in change.

Peroxide value

Peroxide value is a measure of extent of oxidation of lipids and has been used as a criterion for measuring the freshness of fish and shellfish. Oxidative rancidity

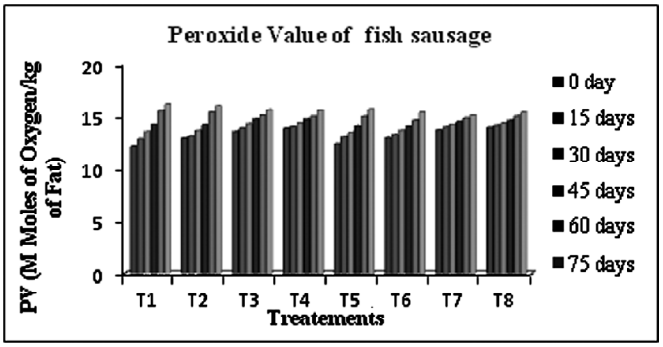

Figure 4: Peroxide value of fish sausage during storage period

is the major limiting factor for the storage life of fish and shellfish products and is a major cause for the off-flavor and odour deterioration in fish. It was observed from the Fig. 4 that the Peroxide valueretained more in the treatment T1 (3.38) and the lowest Peroxide value is found in the treatment T8 (1.06). The observations showed that increase in peroxide value during frozen storage.

Free Fatty Acid

FFA and melanaldehyde formed during storage are known to increase protein cross-kinking reactions leading to the loss of water holding capacity and textural changes. Formation of FFA in frozen stored fish muscle is mainly due to enzymatic hydrolysis of phospholipids and partly due to enzymatic hydrolysis of triglycerides. The rate of change of PV and FFA were low in the early stages of storage, followed by an increased rate of maximum value and then a decrease. It was observed from the Fig. 5 that the FFA valueretained more in the treatment T1 (3.47) and the lowest FFA value is found in the treatment T8 (1.6). The observations showed that increase in FFA content of fish sausage during storage due to enzymatic

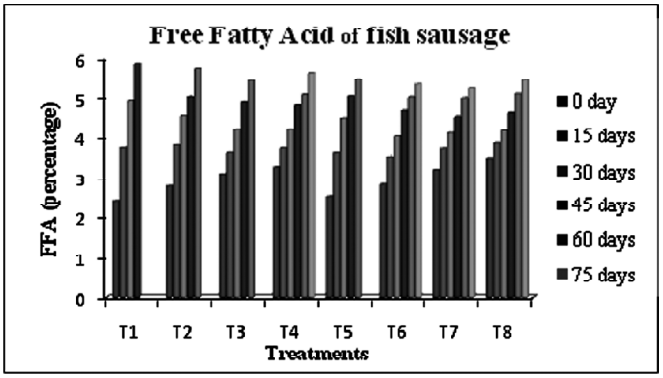

Figure 5: Free Fatty Acid content of fish sausage during storage period

hydrolysis of phospholipids and partly due to enzymatic hydrolysis of triglycerides.

CONCLUSION

The Tilapia fish are acceptable for sausage preparation due to its high flesh content, and also the size of the fish is convenient for manual dressing and for machine separation of flesh. Fortified fish sausage can be prepared with addition fish oil and potassium iodide in order to increase the nutrient content in the sausage Protein, PV, FFA is found good for the treatments T6 and T7.Treatments T7 found good in appearance, T6 has bright color, texture, T5 has good taste, T8 has good aroma.T6 and T7 were found good even after 75 days of storage.

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