3.1 Biological material

Rohu, *Labeo rohita* (Ham.) an Indian major carp belonging to the Cyprinidae family was used throughout the study. It is easily available throughout the year in uniform size from aquaculture operations and is popular in the country. Rohu is of uniform size and has a body shape amenable for easy portioning with minimum wastage. Fish for the study was mostly purchased from Yeshwanthpur fish market of Bangalore and brought to the laboratory in iced condition within 2hr. The sample was occasionally harvested from the fish farm of Central Institute of Freshwater Aquaculture and brought to the laboratory in iced condition. The fish usually weighed between 650-800gms with an average length of 25-30cms.

3.2 Processing materials and surfaces

3.2.1 Fish dressing and portioning

Fish were dressed on wooden boards using heavy-duty steel knives and a descaler for removal of scales. Boards, knives & descaler were washed with detergent and tube well water and sanitized with 100ppm chlorine dioxide (contact time 15mins).

3.2.2 Process floors and surfaces

All processing operations were done on terrazzo tile floors previously washed with detergent and tube well water and sanitized by 100ppm chlorine dioxide with a contact time of 15mins.

3.2.3 Fish holding containers

Commercially available plastic trays and tubs of various capacities as required were used for holding fish in process. They were subjected to the same washing and sanitization protocol as described in 3.2.1.

3.2.4 Packing containers and materials

Fish were packed in polystyrene trays / dishes of 17x15x3.7cm size (750ml) with matched lids. Trays were internally lined with two paper napkins
and overlaid with a perforated 100 gauge polypropylene (PP) sheet. Trays and PP sheet were sanitized with 100ppm chlorine dioxide with contact time of 15mins before use. Paper napkins were sanitized by exposure to UV light for 2hrs. Tray/ dishes, PP sheets and paper napkins, LDPE and PP covers of 100 gauge were purchased from local commercial supplies. Polyester LDPE laminate was a kind gift of CIFT, Kochi.

3.3 Chemicals

3.3.1 Acids and alkali
All Acids and alkali were of GR grade and purchased from E.Merck limited, Worli, Mumbai.

3.3.2 Biochemical reagents
Kovac's reagent for estimation of Tryptophan was purchased from Himedia Laboratories Pvt. Mumbai, India

3.3.3 Gel Electrophoresis
Chemicals for Polyacrylamide Gel Electrophoresis were procured from Sisco Research Laboratories, Pvt. Mumbai, India

3.3.4 Indicators
Methyl red indicator, iodine, alpha napthol, Tetramethyl-para-phenylene-diamine were obtained from Himedia Laboratories Pvt. Mumbai, India. Tashiro’s indicator (0.2% solution prepared by dissolving 150 mg methylene blue and 50mg methyl red in 100ml absolute alcohol) and starch (SRL Pvt, Mumbai) were prepared in the laboratory.

3.3.5 Salts and other chemicals
AR grade salts were obtained from E.Merck limited, Worli, Mumbai.

3.3.6 Solvents
Chloroform (GR grade) for free fatty acid and peroxide analysis were procured from E.Merck limited, Worli, Mumbai.
3.3.7 Sanitizers

Chlorine in the form of commercial bleach liquor (Sodium hypochlorite) was purchased locally from wholesale stockists. Chlorine dioxide in the form of a two part commercial preparation consisting of a stock solution and an activator was purchased from M/s Vetcare, Yelahanka Industrial area, Bangalore and used as per manufacturer’s instructions.

Actual chlorine/ ClO₂ concentrations obtained upon dilution of stock solutions were confirmed using a chlorine strength testing kit (Microquant chlorine test kit, E. Merck (India) Ltd) following manufacture’s instructions.

3.3.8 Spices and Spice Oleoresins

Commercial samples of Turmeric and chilli powder (MTR foods, Bangalore), Ginger- garlic paste (Dabur Hommade paste). Clove, Cinnamon and Pepper were procured from the local supermarket. Spice oleoresins of chillies, turmeric and pepper from AVT McCormick Ingredient, ‘Spice enclave’, Panampillynagar, Kochi- 682036.

3.4 Microbiological media

All the microbiological media listed were purchased from M/s Himedia laboratories (p) Ltd, Mumbai unless otherwise mentioned

3.4.1 Baird parker agar (Himedia Cat. No M043) was used for confirmation of the positive MPN tubes of Staphylococcus aureus.

3.4.2 Christensen Citrate agar (Himedia Cat. No M 143) was used for the biochemical characterization of E. coli from other members of Enterobacteriaceae

3.4.3 Hugh Leifson Glucose medium was used for biochemical test (carbohydrate hydrolysis test) for characterization of bacteria isolated during sampling. Media was prepared using chemicals available in the laboratory.
3.4.4 **KF agar with 10% NaCl** (Himedia Cat. No. M1007) was used for enumeration of Fecal Streptococci.

3.4.5 **MRS media** (Himedia Cat. No M641) was used for enumeration of Lactic acid bacteria.

3.4.6 **Muller Hilton agar** was used for characterization of antibiotic sensitivity of isolates.

3.4.7 **Penicillin discs** (Himedia Cat. No SD028) was used to determine the antibiotic sensitivity of the isolates.

3.4.8 **Peptone water (1%)** (Himedia Cat. No M028S) was used for the serial dilution of the samples.

3.4.9 **Plate count agar** (Himedia Cat. No M091A) was used for total aerobic plate count of mesophiles, psychrophiles as well as for initial microbial load of the skin, gills and viscera.

3.4.10 **Starch hydrolysis agar** (Himedia Cat. No RM198) was used to test the ability of isolates to produce amylase.

3.4.11 **Tryptone soy broth** (Himedia Cat. No M1229) was used for enumeration of *Staphylococcus aureus* of the samples by the MPN method.

3.4.12 **Violet red bile agar** with MUG overlay (Himedia Cat. No M049) was used for enumeration of *Escherichia coli*.

### 3.5 Analytical

#### 3.5.1 Determination of aerobic plate count (TAPC)

Total aerobic plate count (TAPC) is intended to indicate the level or bacterial load of microorganisms in a product. The detailed procedure for determining the TAPC was as per Chapters 1 and 2 of US FDA Bacteriological Analytical Manual online (2001). A 50g analytical sample was weighed using a balance from M/s Scales India Ltd, Bangalore with an accuracy of ± 1 (Model No. PSM/VAO2) to determine the aerobic plate count value and most probable number of coliforms. 450ml of 1% peptone water containing 50g analytical sample was blended for 2mins using a electronic blender (M/s Johnson,
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Bangalore) giving $10^1$ dilution. Decimal dilutions as required were prepared by transferring 10ml of dilution to 90ml of diluents using air displacement pipettes (E. Merck. Mumbai). The dilutions were shaken 25 times and 1ml sample from required dilution was pipetted into each appropriately marked petriplates in duplicate. Cooled molten media, sterilized in an autoclave (PSM Scientific Instruments, Bangalore. Model No. PSM/VA02) and maintained in a serological water bath (PSM Scientific Instruments) at $45\pm 1^\circ$C and were pour plated under laminar air flow station (M/s Alpha linear, Bangalore) with in 15min of the original dilution.

3.5.1.1 Determination of Mesophilic TAPC

Sample preparation was as described in 3.5.1. The solidified plates were inverted and incubated at $35^\circ$ C for 48± 2hrs in a bacteriological incubator (PSM Scientific Instruments, Bangalore. Model No. PSM/1B-02, 400W). Mesophilic counts were evaluated as per the FDA guidelines and reported in terms of log cfu units using a colony counter from PSM Instruments, Bangalore.

3.5.1.2 Determination of Psychrophilic TAPC

Sample preparation was as described in 3.5.1. The solidified plates were inverted and incubated at 10-12$^\circ$ C for 48-72 ± 2hrs in a BOD incubator (M/s ICT ltd). Psychrophilic counts were evaluated as per the FDA guidelines and reported in terms of log cfu units.

3.5.1.3 Enumeration of Escherichia coli/ Coliforms

E. coli and coliforms are gram-negative, rod shaped facultative anaerobic bacteria. Sample dilutions were prepared as in Sec. 3.5.1. The lower dilutions were pour plated with violet red bile agar and overlayed with VRBA media containing 100µg of methyl- umbelliferyl- β -D- glucuronide (MUG) per ml. The plates after solidifying were incubated at $35^\circ$ C for 48-72 ± 2hrs and observed under long wave UV light for fluorescent colonies to identify E.coli among coliforms.
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3.5.1.4 Enumeration of *Staphylococcus aureus*

Presence of *S. aureus* or its enterotoxin in processed foods or on food processing equipment is generally an indication of poor sanitation and can lead to severe food poisoning. Sample dilutions were prepared as in 3.5.1 and 1ml from lower dilutions was added to 9ml Tryptone soy broth with 10% NaCl and incubated at 35°C for 48-72 ± 2hrs. The positive tubes indicated by growth (turbidity) were streaked on to Baird parker agar and incubated at 35°C for 48hrs. Further confirmation included transfer of black pinpoint colonies positive for *S. aureus* to Brain infusion broth for isolation, enumeration and ancillary tests.

3.5.1.5 Enumeration of Fecal Streptococci

*Streptococcus fecalis* are enterococci involved in toxin production and food poisoning. Sample dilutions were prepared as in 3.5.1 and enumerated by plating 1ml of lower dilution on to selective KF Streptococcal agar base with 10ml of 1% TTC (2,3,5- triphenyltetrazolium chloride) and incubated at 35°C for 48hrs. Colonies showing pink/purple colour were taken as positive.

3.5.2 Determination of moisture

AOAC (1984) method was used for the determination of moisture in a hot air oven (PSM Scientific Instruments, Bangalore. Model No. PSM/HAO-02).

3.5.3 Determination of pH

pH was measured by taking 10gms comminuted muscle sample with 100ml distilled water and homogenizing the same by a mortar and pestle (M/s Vasa Scientific, Bangalore). pH of the homogenate was recorded using a pH meter model 340i of WTW Gmb H, Germany.

3.5.4 Determination of Peptide nitrogen

Samples of comminuted muscle sample were blended with 2.5 volumes of 10% Trichloro acetic acid (TCA) using mortar and pestle for 10mins and filtered through Whatman no.4 filter paper. TCA extracts were analyzed for
peptides by the Biuret reaction using a spectrophotometer of UV1 thermo electron corporation, USA according to the method of Gornall et al (1949).

3.5.5 Determination of Total volatile base nitrogen

TCA extract samples for determination of Total volatile base nitrogen (TVBN) were prepared as explained in 3.5.4 and estimated by the Conway Microdiffusion method (Conway, 1962) using conway dishes from Superfit Continental Pvt. Ltd. India.

3.5.6 Determination of water soluble proteins

One gm of comminuted sample was homogenized in 10 volumes of ice cold water using the Sonicator (Model No.VCX-130PB, M/s Sonics and Materials, USA) and centrifuged (Kendro Laboratory Products, Model No. D35200) at 14000 rpm for 15mins. Protein in the clear supernatant was estimated by Biuret reaction (Gornall et al. 1949) against a standard curve using bovine serum albumin as standard.

3.5.7 Determination of peroxide value

Peroxide value was analyzed by the method- 28.023 of the AOAC (1975).

3.5.8 Sensory evaluation

Bite sized pieces of the steaks were taken and cooked in a microwave oven (Samsung India Electronics Ltd. Model. No. 2739) for 10mins in 3% brine. Later during treatment of steaks with spices the concentration of brine was reduced to 1.5%. The spice treated pieces were also deep fried in sunflower oil for 3-5mins. The boiled /fried pieces were then presented to a panel of 5 trained judges and the scores recorded on a 10 point hedonic scale.

3.5.9 Sodium dodecyl sulfate Gel Electrophoresis (SDS-PAGE)

Discontinuous SDS-PAGE was performed on protein samples extracted for water soluble proteins as in 3.5.6 and salt soluble proteins as in 3.5.4
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3.6 Statistical analysis

Results were analyzed by two way ANOVA (with replication) using Microsoft Excel XP software. Treatments with significant ‘F’ ratios were further analysed for critical difference as per Snedcor and Cochran (1967).

3.7 Experimental

3.7.1 Microflora of processing surfaces and packaging materials

3.7.1.1 Process floors and surfaces

The processing surfaces including terrazzo tiles and wooden boards were sampled for the initial microbial load just before commencing the actual processing. Five random samples were taken by swabbing an area of 2sq cm with sterile cotton swab of the process floors, boards and pooled. The pooled samples were serially diluted, plated and incubated at 35°C for 24-48h before recording the counts.

3.7.1.2 Fish holding containers

Fish purchased from the market were transported in plastic tubs and plastic trays, which were also used to treat the fish portions / steaks. The tubs and trays were sampled by taking swabs as described in 3.7.1.1 before they were used for transportation and treatments for fish. The trays and tubs were then sanitized with 100ppm chlorine dioxide and allowed to dry overnight before sampling again.

3.7.1.3 Packing containers and materials

Polystyrene trays / dishes (17x15x3.7cm) with matched lids used for storage of fishes were sampled for microbial load by rinsing with sterile water, which was plated as in 3.5.1. Another set of polystyrene trays /dishes with matched lids were thoroughly washed in tube well water and sanitized by dipping in 100ppm chlorine dioxide solution with contact time of 20mins and
dried overnight and sampled as before. One set of paper napkins used for internal lining were cut into suitable strips and suspended in 100ml sterile water and sampled for the microbial load, the other set of napkins were sanitized by exposure to UV light for 2 hrs and sampled. The perforated polythene sheets were tested for the bacterial load where one set of sampling was done by taking suitable sized samples in sterile water and another set sampled after it was sanitized by dipping in 100ppm chlorine dioxide for 20mins and allowed to dry overnight.

3.7.1.4 Fish dressing, processing, preparation of fish steaks
determination of microbial load:

Rohu (Labeo rohita) of 25-30cms size and weighing between 650-800 gms were purchased from the local Yeshwanthpur fish market and brought in an hour under iced condition and processed under laboratory conditions. The microbial load of farm reared and market fish were evaluated to decide the dosage of sanitizers required in preparation of the final product. Bacterial isolates were chosen at random by swabbing and plating for total aerobic counts. Characterization was performed on the samples collected from the carton box and cart used for selling the fish, the balance used for weighing; hands of the vendor, along with skin, gills, visceral and flesh samples of the fishes. The treated and untreated steaks in the later stages were also sampled for isolates. The characterization of microflora was performed as reported by Surendran and Gopakumar (1981) flow chart 3.1.

Bacteriological swab samples of samples were taken. During the experiment, skin area of 4-5 fish was sampled by swabbing an area of 2cm² with sterile swabs and templates. Swabs were transferred into 90ml of sterile phosphate buffer saline, shaken thoroughly, dilutions made, plated and results recorded. The fishes were then descaled, definned and eviscerated using boards and knives, pre sanitized with 100ppm chlorine dioxide with a contact time of 20 minutes and sampled for the microbial load. The gills and viscera
were collected separately and aseptically into sterile stainless steel containers and cut into small bits using sterile scissors. Two grams of these pooled samples was added to 200 volumes of sterile PBS in sterile polypropylene pouches separately. Serial dilutions were made as required, plated as described in sec. 3.5.1.
Flow chart 3.1. Scheme for classifying isolated cultures

- Gram Stained organisms
  - +ve
    - Rods +ve
      - Micrococaceae
    - Cocci
      - Lactobacillus
      - Arthrobacter
    - Rods catalase -ve
      - Sensitivity to penicillin
        - 2.5 IU/disc
  - -ve
    - Pigmentation cytochrome oxidase test
      - No pigment cytochrome oxidase +ve
        - Alcaligenes or Moraxella
      - Yellow/orange pigment cytochrome oxidation -ve
        - Flavobacterium or Cytophaga
      - Fermentative
        - Enterobacteriaceae
      - Non-fermentative
        - Pseudomonas
        - Acid & gas
          - Acid, no gas
            - Aeromonas
      - Reaction in H & L Glucose media
        - Fermentative
          - Acid & gas
            - Acid, no gas
              - Aeromonas
Eviscerated fish were washed thoroughly with tube well water and cut into 1-1.2cm of around 40-50g sized steaks on wooden boards using knives pre-sanitized with 100ppm chlorine. Processing of fish into cut steaks was completed within 30mins. Typically about 15 fishes were used in an experiment. The steaks were then soaked in 5x their body weight in chilled water for 20 mins and drained for 5 mins before packing them into 750ml sized polystyrene display trays (17x15x3.7cm) with lids. The trays were internally lined with paper napkins and samples for analysis were drawn on the 0\textsuperscript{th}, 4\textsuperscript{th}, 6\textsuperscript{th}, 8\textsuperscript{th}, 10\textsuperscript{th} and 14\textsuperscript{th} day of refrigerated (Samsung India Electronics Ltd, India. Model No. SR-25NMB) storage at 4-6°C and 1-2°C. The steaks so obtained were subjected to various treatments as described later.

Fig. 3.7.1.4. Preparation of fish steaks
3.7.1.5. Residual chlorine analysis

Chlorine dioxide was found to be a better sanitizer and this was used at different ppm levels for sanitation of the processing area, the materials involved during processing and the fish itself. The residual chlorine analysis is of utmost importance as the residue can be carcinogenic on ingestion of processed product. The experiment was performed by taking different ppm levels of chlorine dioxide in proportion to a liter and analyzed with chlorine analysis test kit (procured from E.Merck, India).

3.7.1.6. Microbiological quality of salts

Salt used in food processing should be free from both microbial and chemical contaminants. 15% brine solutions of solar and iodised salt were prepared and boiled and allowed to cool overnight and plated for enumeration of total microbial counts the following day. Subsets were performed to ascertain the procedure for sanitizing and optimizing the concentration and grade of salt to be used.

a) 15% brine of AR grade, solar and iodized salts were prepared, filtered through bolting silk and treated with 100ppm ClO₂ and left overnight. The treated samples were then plated and enumerated.

b) 2% brines of solar, iodized salts and 15% brine solution of AR grade were prepared and treated with 30ppm ClO₂ and left overnight. The treated brines were tested for mesophilic counts.

c) Saturated iodized salt brine was analysed for residual chlorine and also for total mesophilic count.

3.7.2 Sanitation of fish steaks stored at 4-6°C or 1-2°C

Chlorine, generated in situ or ex situ in the form of Sodium hypochlorite has been used as a disinfectant or sanitizing agent in the food industry for long, while chlorine dioxide in comparison is a more recent sanitizer. As the initial bacterial load on the steaks affects their course of spoilage, it was sought to be reduced through sanitization. Chlorine and chlorine dioxide were tested at two concentrations on fish steaks and their effect on shelf life quality
parameters assessed. A generalized flow chart for processing of fish steaks is shown in flow chart 3.2.

**Flow chart - 3.2 Processing of fish into steaks and packaging after treatment with salt (PP polypropylene sheet, 200gauge)**
Fish steaks after dressing as described in 3.7.1.4 were dipped in 5x their weight in an ice-water mixture (0.5:1) maintained at 0°C containing effective chlorine concentrations of either chlorine / chlorine dioxide (ClO₂) at 0, 30 and 50ppm for 20mins. The steaks were then drained for 5mins and packed in 750ml polystyrene serving/display dishes (trays) with lids. Tray bottoms were lined previously with paper napkins and overlaid with 100guage polypropylene (PP) sheets. Sanitization of the packaging materials has been described in 3.2.4. About 150g steaks were packed in each tray closed with lids stored at 4-6°C and 1-2°C. Samples were drawn on 0, 4, 7 and 10th d at 4-6°C storage and 0, 4, 6, 8, 10 and 14th d at 1-2°C of storage. The samples were analysed for Total aerobic plate count (Meso and Psychrophiles, Sec.3.5.1.1 and 3.5.1.2), Moisture, pH, Peptide nitrogen and Total volatile base nitrogen (Sec. 3.5.2, 3.5.3, 3.5.4 and 3.5.5). Organoleptic and sensory evaluation was also carried out (Sec 3.5.8)

3.7.3 Effect of sanitation and salting protocol on fish steaks

After optimization in the level of ClO₂, the effect of a combination of sanitization and salt was evaluated. Sodium chloride (NaCl) is a normal ingredient in cooking, and a preservative at high concentrations. But when used at low concentrations encountered in cooking, it can only exert a slight hindrance or selective action on the microflora. The fish were processed as in flow chart 3.2 and the dressed steaks were then tossed with 20%, 15% and 2% GR grade NaCl for 10mins and then packed into display dishes and stored at 4-6°C and analysed for the microbiological and biochemical parameters as described in 3.7.2. 30ppm ClO₂ sanitized steaks were kept as control and sampled.

3.7.4 Effect of sanitation, salting and acidulants on the shelf life of fish steaks

Acidulants are a class of food additives, both organic and inorganic, that exert preservative action principally by lowering pH to unfavorable levels for spoilage microflora. Some acidulants also act as metal chelators and effect
preservation, independent of the pH reduction. Preservative action of different acidulants was evaluated on fish steaks.

Processing was carried out as described in flow chart 3.2. The ClO₂ sanitized steaks were treated by dipping steaks in (1: 1: 0.25, fish: water: ice) of 0.1M solutions of one of the different acidulants viz. Lactic acid, Sodium metabisulfite, Propionic acid, Citric acid, Acetic acid and Sodium benzoate for 10mins maintained at 0°C. Treated steaks were drained for 5mins and packed as before. Storage, microbiological and biochemical parameters were carried out as detailed in 3.7.2 on 0, 4, 6, 8, 10, and 14th d of storage at 1-2°C.

3.7.4.1. Effect of sanitation, salting and sodium acid salts on the shelf life of fish steaks

Among the steaks treated with acidulants, Sodium benzoate gave promising results and hence two other sodium salts were also tested for their effect on shelf life of fish steaks. Sodium acetate and Sodium lactate were tested at 0.1M concentration with one set as control without any acidulant. Processing, treatment method and packaging was carried out as described in 3.7.4. Microbiological and biochemical parameters were analyzed as in 3.7.2 from 0th-14th day of storage.

3.7.5 Optimization of Sodium benzoate level in combination with sanitation on shelf life of fish steaks:

Sodium benzoate was found to be the better acidulant among those tested. Sodium benzoate is an effective antibacterial and antifungal agent with a pKa value of 4.20. The optimum level of Sodium benzoate required for shelf life extension of fish steaks was sought to be determined using various levels of Sodium benzoate.

Processing of the fish was done as described in flow chart 3.2 till the sanitization of steaks by 30ppm ClO₂. The dressed steaks were then dipped in 0.01 and 0.05M solutions of Sodium benzoate separately for 10mins, drained for 5mins and packed into dishes and sampled for microbial and biochemical
parameters as described in 3.7.2 from 0th - 14th day of storage. One set of 30ppm ClO₂ sanitized steaks was kept as control without any benzoate treatment. The processing has been described in the flow chart 3.3.

Fish

Processed as in flow chart 3.2 till sanitizing with ClO₂

Dip in a mixture of water and ice containing (0.01, 0.05 and 0.1 M) acidulants (Fish: water: ice, 1:1: 0.25) for 10 mins

Drained for 5 mins

Pack as in flow chart 3.2

Stored at 1-2°C

Analysis as at flow chart 3.2

**Flow chart - 3.3 Treatment with acidulants**

3.7.6 Effect of sanitation with Sodium benzoate and salt combinations on the shelf life of fish steaks:

Hurdle technology involves the use of as little concentrations of additives or preservatives as possible to extend the shelf life of any food product. After optimizing the Sodium benzoate concentration with 30ppm ClO₂ sanitization, an additional hurdle was introduced in the form of salt to the sanitizer and benzoate combination and a lower concentration of benzoate was also tested to find out if the combination can reduce the concentration of benzoate required.
Steaks were processed as outlined in flow chart 3.2 till sanitization with 30ppm ClO₂. Chlorine dioxide sanitized steaks were drained and treated with one of the following combinations.

a) Dip treated in 0.05M Sodium benzoate

b) Tossed in a mixture of 1% salt (commercial iodised salt) and 0.005M Sodium benzoate for 10min and

c) Tossed in 1% salt for 10min.

The three treated steaks were packed and analysed for various parameters as detailed in 3.7.2. as also for water-soluble protein fractions (3.5.6).

3.7.7 Effect of sanitation with Sodium benzoate, salt and antioxidant combinations on the shelf life of fish steaks:

With the further extension of shelf life of fish steaks it was noticed that steaks at later stages of storage developed rancidity. Hence a low concentration of antioxidant was incorporated in treatment to assess its effect on shelf life of steaks.

Processing of steaks was done as shown in flow chart 3.4. Packed steaks were stored at 1-2°C and steaks were sampled on 0, 4, 6, 8, 10 and 14d and subjected to microbial, biochemical and sensory evaluation as described at 3.7.6.
Fish

Processed as in flow chart 3.2 till sanitization of steaks with ClO₂

Divide into batches

- Dipped in 1:1:0.25 Vols. Of 0.05M S. benzoate for 10 mins
- Toss in a mix of 1% salt and 0.05M S. benzoate for 10 mins
- Toss in a mix of 0.005M S. benzoate & 0.002% BHA/ BHT for 10 mins
- Toss in a mix of 1% salt +0.005M S. benzoate & 0.002% BHA/ BHT for 10 mins

Drain for 5 mins

Pack as in flow chart 3.2

Stored at 1-2°C

Sampled at periodic intervals and analyzed as in flow chart 3.2

Flow chart. 3.4 Treatment with Sodium benzoate and antioxidants
3.7.8 Effect of Sodium benzoate, salt, antioxidant and chelator on the shelf life of fish steaks:

In the previous experiments it had been observed that blackening of steaks occurred whenever salt was included in treatment. Since addition of salt is necessary in a ready to cook product and cannot be avoided, a low concentration of citric acid was included in the treatment mix as a chelator to assess its effectiveness in minimizing blackening. Processing was done as described in flow chart 3.5. Sampling and quality assessment was carried as described in 3.7.6.

Flow chart 3.5- Treatment with Sodium benzoate, Salt, antioxidants & Chelator
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3.7.9 Effect of sanitation with Sodium benzoate, salt, antioxidant, chelator and spices on the shelf life of fish steaks:

Spices are essential ingredients in most culinary preparations. They not only add flavor, colour and enhance the taste of food, but also have preservative action against microbial spoilage and antioxidant properties. After the previous study (3.7.8) with salt, benzoate, antioxidant and citric acid treatment, the next step was to incorporate spices in the preparation of ready to cook fish steaks. As a prelude to the incorporation of spices, microbiological quality of spices was tested by determination of total plate count (mesophiles) of all the spices following FDA methods (2001). After some preliminary trials a spice mixture of turmeric, chillies, pepper, cloves, cinnamon and ginger garlic paste, tamarind and corn flour (binder) was chosen and combined along with salt, Sodium benzoate, BHA/BHT and citric acid at previously standardized concentrations. This mixture was made into a paste and applied to the steaks and stored at 1-2°C. Prepared steaks were subjected to microbiological, biochemical and sensory evaluation as previously described at intervals (3.7.8). Peroxide value as per AOAC Method # 28.023(1975) was also analysed (sec.3.5.7).

3.7.10 Effect of Sodium benzoate, salt, antioxidant, chelator and spices on the shelf life of fish steaks:

After encouraging results from previous study (3.7.9), the concentration of Sodium benzoate used as acidulant/preservative was sought to be reduced further, since spices themselves have a preservative action. Hence, concentration of Sodium benzoate was reduced to half its previous level (0.0025 vs 0.005M) and its effect on shelf life assessed against the original concentration and untreated control. Other experimental parameters including evaluation criteria were as described in 3.7.9.
3.7.11 Effect of Sodium benzoate, salt, antioxidant, chelator, spices and oleoresins on the shelf life of fish steaks:

Spices are natural products and hence have inherent biological variability in their potency and quality. Hence an attempt was made to replace some of the spice mixture with spice oleoresins. Turmeric, chilli and pepper in the spice mixture used in Sec. 3.7.10 were replaced with their respective oleoresins on an equivalent potency basis based on the oleoresin manufacturer's recommendations. Subsequently the application of spice and spice oleoresin mixture was carried out as detailed in flow chart 3.6 and shelf life and other parameters assessed as before (3.7.9).
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3.7.12 Effect of Sodium benzoate, salt, antioxidant, chelator and spices with window style packaging on the shelf life of fish steaks

Treatment of steaks with various hurdles as done in 3.7.11 gave encouraging results. However, hitherto the treated steaks were packed in polystyrene dishes with opaque but not airtight lids. Thus, although the packaging had only partial barrier like character, the product could not be seen unless the lid was opened. These issues were addressed by providing a see through lid for the tray.

Steaks were treated as detailed in flow chart 3.6. After packing in trays, one set were closed with specially modified lids, which had a rectangular cut window of 2.5x4cm. The cut/ opening was sealed with a clear 100 gauge PP sheet of slightly larger dimensions and bonded to the lid using a commercial...
adhesive (Fevibond). After curing for 24-48hrs the lids were washed thoroughly and sanitized by dip in 100 ClO₂, to provide a see through window to view the packed steaks. Shelf life and other parameters were analysed as mentioned at 3.7.11.

3.7.13 Evaluation of different packaging materials in combination with optimized hurdles on shelf life of fish steaks.

The treatments and packaging were performed as in sec. 3.7.12. The trays were over wrapped in three different packaging materials without the lids. Steaks were treated as detailed in flow chart 3.6. Three sets were packed by over wrapping the trays (without lids) with one of the following packaging materials and sealing the same. Packaging materials used here were Polyethylene/ Polypropylene, Low-density polyethylene and Polypropylene (PP). They were sterilized by exposing to UV light for 2hrs. Steaks processed as described in 3.7.10 were packed in the trays, which were over wrapped in the packaging materials described and stored at 1-2°C. Shelf life and other parameters were analysed as mentioned at 3.7.11.
Fig. 3.7.12 Window style packaged control (A) and spice (B) marinated steaks
Fig. 3.7.13. Overwrapped steaks with different packaging materials