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Fish Protein Dispersion as a Coating to Prevent Quality Loss in Processed Fishery Products

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The conventional technique of water glazing has limitation in protecting the products during prolonged frozen storage. A novel fish protein glaze was devised and its superior protective ability in retarding quality losses of frozen fish items as compared with water glazing is demonstrated. The methodology for fish protein glaze preparation involved soaking of fish meat in pieces in excess cold water followed by soaking in dilute acetic acid to induce hydration of proteins resulting in gel formation. Finally hydrated gel was homogenized in water to get protein dispersion to an apparent viscosity of 1 Pa.s. The fish samples were given a dip in dispersions prepared from the same fish meat. Glazing of frozen mince muscle blocks of Indian mackerel (*Rastrelliger kanagurta*) by a dispersion prepared from mackerel myofibrillar proteins significantly reduced lipid oxidation and dehydration in the product during storage at –17°C. Influence of glazing alone or in combination with low dose gamma irradiation was also examined on the shelf life of seer fish (*Scomberomorus guttatus)* steaks stored in ice. It was observed that while untreated steaks had a shelf life of 15 days, the steaks displayed extension in shelf of 5 and 15 days when subjected to glazing alone or glazing in combination with gamma irradiation at 1kGy respectively.

Key words: Protein dispersion, gelation, irradiation.

Most of the fish products traded in international markets are in frozen conditions. Prolonged frozen storage is known to lead to deterioration in the quality of fish and shellfish items. Quality changes in the fish include lipid oxidation, particularly in fatty fish items, protein denaturation and textural changes due to interaction of proteins with oxidized lipids resulting in toughening of the texture. In addition, long term frozen storage results in dehydration, which causes significant losses in weight and hence problems in commercial trade in fish products. Further, surface dehydration in fish fillets causes loss of their natural appearance and toughening. Conventionally, water glazing is being adopted to control quality deterioration during frozen storage of fishery products (Aitken, 1976).

Due to highly perishable nature of fish products various processes have been developed for shelf life extension of the products. While chilling alone has limitation in appreciable extension of shelf life, combination of chilling with other treatments such as modified atmosphere packaging, gamma irradiation, treatment with permitted food additives etc. have been applied to further augment the shelf life (Leistner & Gorris, 1995; Scott 1989).

During the recent years, there has been interest in development of edible coatings from polysaccharides, proteins, and also lipids to extend shelf life of foods (Gennadios *et al.*, 1994). Such coatings can retain quality of fresh, frozen and processed muscle foods including fish items by retarding moisture loss, reducing lipid oxidation and discolouration, enhancing product appearance in retail packages, and functioning as carriers of food additives such as antimicrobial as well as antioxidant agents (Gennadios *et al.*, 1994; 1997). Edible packaging films based on fish myofibrillar proteins have been

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developed (Cuq *et al.*, 1995; Venugopal 1998). In spite of numerous studies on various applications, technology for large-scale production of edible films remains rather cumbersome, which generally involves drying the ingredient in solution in thin layers and peeling of the film.

In this paper, development of fish-based edible coating from acetic acid induced protein gel for surface coating of frozen mackerel mince blocks and chilled stored seer fish steaks is described. Indian mackerel (*Rastrelliger kanagurta*), a medium fatty fish, was used as a model system. It is known that mincing causes oxidation of unsaturated fatty acids in the fish due to disintegration and exposure of the fish tissue to air (Venugopal, 1992). Frozen storage of the mince further enhances oxidation of the unsaturated fatty acids as a result of concentration of minerals during freezing (Balachandran, 2001).

In case of chilled storage a commercially important fish, seer fish (*Scomberomorus guttatus),* was used. Combination of chilling along with dip treatment with fish protein dispersion on the shelf life of the fish steaks was examined. Influence of low dose of gamma irradiation (1kGy) was also examined in conjunction with dispersion coating on the chilled shelf life of the fresh seer steaks.

Materials and methods

Fresh mackerel (*Rastrelliger kanagurta*) and seer fish (*Scomberomorus guttatus)* were procured from local market and brought to laboratory in ice. The fish were beheaded, eviscerated and cleaned with potable water. The dressed mackerel were passed through a laboratory model belt and drum type deboning machine equipped with a stainless steel perforated drum. The meat obtained was further minced by blending for 45 sec in a food processor ('Singer' model). The mince was spread evenly on an aluminium tray and was cut into rectangular blocks (4 x 4 x 3 cm). The mince was held overnight at -18^0C in a contact plate freezer and the frozen blocks of mince were collected. The beheaded, eviscerated seer fish was cut into steaks of about 2 cm thickness, each steak weighing about 50 g. The steaks were washed in potable water, drained and used for dispersion coating.

A portion of fresh mackerel or seer fish minced muscle (500 g) was suspended in excess of chilled water (0-4^{0}C) and held overnight in a cold room. The muscle was drained, washed once more in chilled water, and the drained meat was homogenized for a period of one min in a food processor in the presence of equal amount of cold water. To the homogenate acetic acid (0.5% v/v) was added with gentle stirring to obtain the gel. The gel was homogenized with twice the amount of water containing 3% (v/v) glycerol and 0.75% (v/v) acetic acid to yield the protein dispersion. The pH of protein dispersion was adjusted to 3.5 with acetic acid. These protein dispersions prepared from mackerel and seer fish mince were used for coating the mackerel frozen blocks and seer fish steaks, respectively.

The viscosity of protein dispersion was measured using Brookfield Synchrolectric viscometer Model RVT (Soughton MA, USA), as described by Venugopal & Shahidi (1994). Before the measurements the dispersion was brought to room temperature by holding in a water bath for 4 h. The viscosity was measured by selecting spindle No. 2 at a speed of 50 rpm. The values were recorded after 30 sec of rotation of the spindle in the dispersions. The viscosity values were obtained from conversion factor provided by the manufacturer. The values of viscosity were designated as apparent viscosity expressed in terms of Pascal sec (Pa.s). The viscosity of gel dispersion was adjusted to 1.0 Pa.s.

Individual frozen mince blocks of mackerel (12 Nos.) were coated with protein dispersion by dipping for 10 sec in the

chilled mackerel protein dispersion (400ml). A second set of blocks (12 Nos.) was dipped in chilled distilled water (400ml). Some of the blocks (12 Nos.) were not coated with either water or dispersion, which served as control. Fish mince blocks coated with protein dispersion and water were labeled as protein glazed and water glazed respectively, while uncoated ones represented the unglazed samples. The frozen samples were stored at –17 ± 2°C in a laboratory freezer.

Fresh seer fish steaks (66 Nos.) were coated by dipping in chilled seer fish protein dispersion (2000 ml); the steaks were divided in two troughs containing 1000ml dispersion and 33 Nos. in each for 1 h with periodic changing of sides for uniform absorption of the dispersion. The samples were packaged aerobically in 500 gauge polyethylene pouches and stored under ice (2-4°C) along with uncoated control samples.

In order to understand the influence of combination of glazing and irradiation on chilled storage life of seer fish steaks, a part of the coated steaks were subjected to gamma irradiation. The steaks were held under ice and irradiated by gamma rays at a dose of 1 kGy using a package irradiator (Atomic Energy of Canada Ltd, ^{60}Co source, dose rate 0.043 kGy/min). Uncoated steaks were also irradiated under similar conditions. Uncoated and unirradiated steaks served as controls.

The samples were removed at periodic intervals for quality evaluation. The frozen stored mackerel mince blocks were analyzed for loss of weight due to dehydration, rancidity developement in terms of thiobarbituric acid (TBA) value estimation and for total bacterial counts. The chilled stored seer fish steaks were analyzed for total bacterial counts, total volatile basic nitrogen (TVBN) estimation, TBA value and sensory evaluation by taste panel involving the scientists from the laboratory.

The total bacterial count of each sample was determined by pour plate technique using plate count agar medium (HiMedia Laboratories Limited, Mumbai, India). Chilled stored seer fish steaks were brought to room temperature on the day of experiment by keeping under running tap water. The fish samples (10g) from each treatment were aseptically homogenized for one min in 90 ml sterile saline in a presterilized Sorvall Omnimixer cups. The colony forming units (cfu) were determined by plating 1 ml aliquot of appropriate dilutions in sterile petri plates using the medium. The plates were incubated at room temperature for 48 h and an average of two replicates was taken.

The TBA value was determined following the method of Witte *et al.*, (1970). The sample (5 g) was homogenized in 20 ml of chilled 10% aqueous trichloroacetic acid (TCA) containing 0.5 M ortho-phosphoric acid in a mortar held under ice. The homogenate was filtered through Whatman filter paper no.1.This extract was mixed with equal volume of aqueous solution of TBA reagent (0.05 M in 1.0 M phosphoric acid) and incubated in a boiling water bath for 45 min. After cooling, equal volume of n-butanol was added to the sample to extract the pigment, and the optical density (O.D) of the extract was measured at 532 nm in a UV-VIS Recording Spectrophotometer UV-2500 (Chemito, Japan). The TBA values were expressed as mg malonaldehyde per g sample. Average of three independent estimations of each sample was noted.

The TVBN of each sample was determined by Conway microdiffusion method of Farber and Ferro (1956). A 10% TCA extract was added to the outer well of the Conway unit along with saturated K_2CO_3. The inner well contained 1 ml of TVBN reagent. The TCA extract and the alkaline solution were mixed by gently rotating the Conway unit circularly. After three hours of incubation at room temperature the reagent in the inner well was titrated with 0.002 N H_2SO_4. The TVBN values are expressed as mg N/100 g.

Protein and moisture of the fish were determined according to AOAC (1990). The protein content of the sample was determined by Kjeldahl's method using a Kjel Plus digestion and distillation units (Pelican instruments, Chennai). A pre-weighed sample was digested with concentrated H_2SO_4 and a pinch of digestion mixture (consisting of K_2SO_4, $CuSO_4.5H_2O$ and Na_2SeO_3 in the ratio of 50:10:1) at 350-400°C and steam distilled after making the solution alkaline with 40% NaOH. The liberated ammonia was collected in a receiving flask containing 20 ml of 4% boric acid and indicator (consisting of mixture of methyl red and bromocresol green). The amount of ammonia in boric acid was titrated back with 0.05 N H_2SO_4. Protein content was determined by multiplying the nitrogen value by 6.25.

The lipid content was determined by the procedure of Bligh and Dyer (1951). For the extraction of lipid, a pre-weighed sample (10g) was homogenised with mixture of chloroform, methanol and water in the ratio of 1:2:0.8 in a food processor (Singer) for 1 min. Chloroform and water were then added to the homogenate in succession to shift ratio of the extractant to 2:2:1.8. Total lipid content was determined gravimetrically.

A panel of six scientists from the department conducted sensory evaluation of the samples during the storage period. The odor score of the fish was assessed on a 10-point hedonic scale as follows: 10, characteristic of fresh odor; 9, marginal loss of fresh odor; 8, light loss of fresh odor; 7, definite loss of fresh odor; 6, almost no fresh odor; 5, most detectable and rancid odor; 4-2, increasing off odor; and 1, putrid odor (Bilinski, 1983). Fish that scored between 10-5 were considered acceptable and those scoring less than 5 were considered as spoiled.

Results

Table 1. shows the proximate composition of mackerel and seer fish muscle used in the experiments. As the table shows, mackerel used was a medium fatty fish, with a lipid content of 7.5% while seer fish was a lean fish.

Table 1. Proximate composition of Indian mackerel and Seer fish meat

Fish	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
Mackerel	77.19	14.21	7.51	1.33
Seer fish	82.07	15.87	3.42	0.68

Fig.1 depicts dehydration losses during frozen storage of mackerel mince. In the initial stages, in mackerel muscle mince moisture was retained in all samples, losing only 9% weight after 40 days of storage. With further storage there was subsequent decline in weight of the frozen mackerel samples. After 80 days of storage unglazed and water glazed samples showed 35% reduction in weight, while protein glazed samples had better moisture retention, with a loss in weight of only 17%.

As shown in Fig. 2, prolonged frozen storage resulted in oxidation of lipids in the mince. Thus, after 80 days of frozen storage,

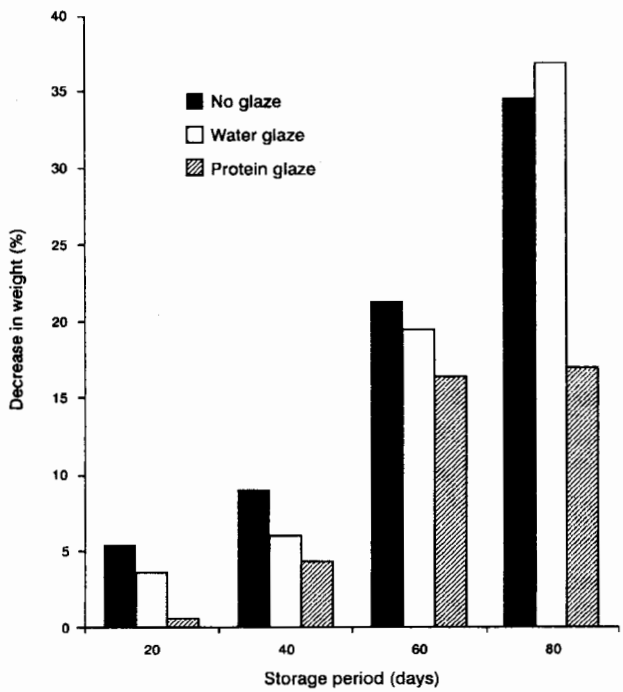

Fig. 1. Influence of glazing treatment on loss in weight of frozen mackerel mince blocks.

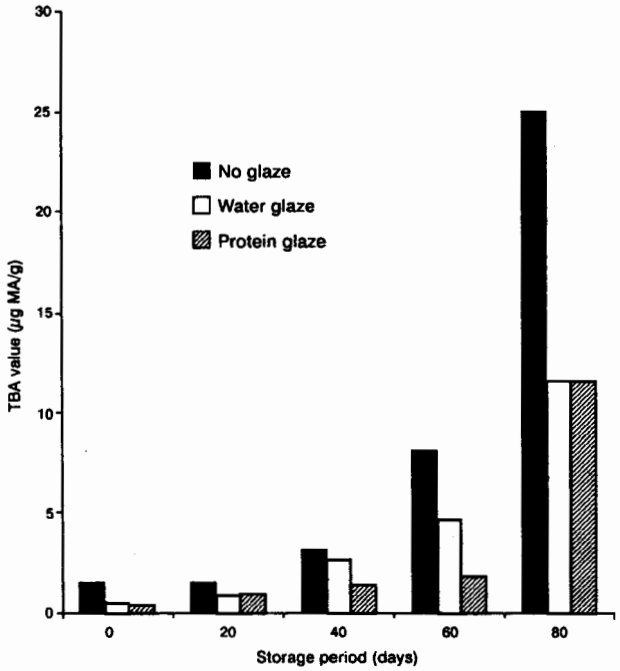

Fig. 2. Influence of glazing treatment on TBA value of frozen mackerel mince blocks.

unglazed samples had a TBA value as high as 25mg MA/g while both water and protein glazed samples had TBA values of about 11mg MA/g. The initial bacterial counts of unglazed, water and protein glazed mince block samples were $4.9x10^5$, $9.3x10^4$ and $1.16x10^5$ per g, respectively. Bacterial count did not change significantly in all the samples after frozen storage for a period up to 80 days (Figure not given).

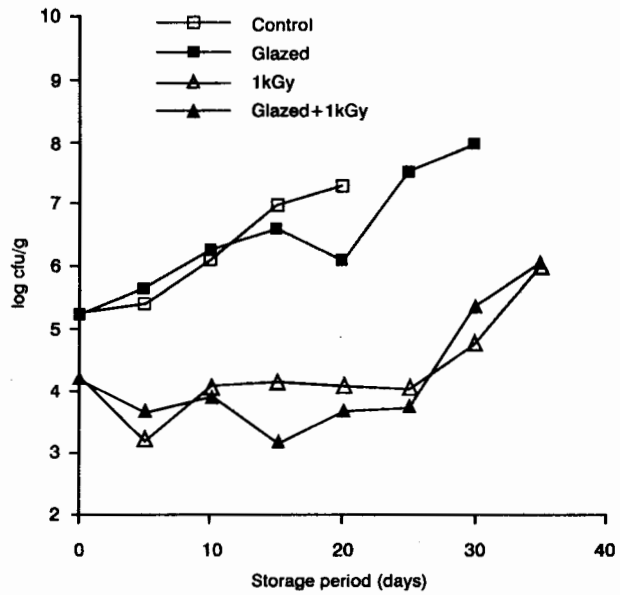

Fig. 3. Effect of glazing and irradiation on aerobic plate counts of seer fish steaks during ice storage.

Fig. 3 shows the microbial profiles of seer fish steaks during chilled storage. The untreated fish had an initial microbial count of $1.6x10^5$ cfu/g. The microbial count increased to $1.9x10^7$ cfu/g within 20 days of storage in the case of untreated steaks. Irradiation alone at 1 kGy decreased the initial count to $1.7x10^4$ cfu/g. After storage for 35 days the microbial count reached to $9.5x10^5$ cfu per g in irradiated samples. The initial microbial load of glazed fish was $1.7x10^5$ which increases to $3.4x10^7$ cfu/g after 25 days of storage on ice. Irradiation reduced the initial load of glazed fish to $1.5x10^4$ cfu/g, the number decreased by one log cycle during ice storage after 5 days and remained constant at this level during ice storage for 20 days of storage. Subsequently the value increased to $1.2x10^6$ cfu/g after 35 days.

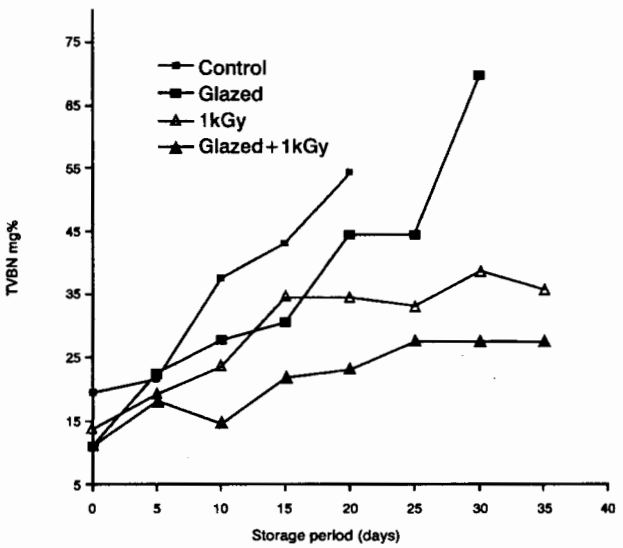

Fig. 4. Effect of glazing and irradiation on TVBN values of seer fish steaks during ice storage.

Fig. 4 depicts the data on total volatile basic nitrogen formation during storage of seer fish fillets under ice. Initial value of TVBN in treated and untreated fish was as low as 11mg N%, the value reached to 54 mg N% after 20 days of storage in untreated samples. Irradiation suppressed TVBN formation and gave value of 36 mg N% after 35 days. Surface glazing and irradiation of seer fish reduced the formation of TVBN and a value of 28 mg N% was reached after 25

days of storage, which did not increase further after 35 days of storage.

Fig. 5 displays rancidity measurement of samples stored under ice. The TBA values were within a value of 2mg MA/g in all the samples immediately after treatment. During storage, the values tended to increase in all the samples, giving a value of 11mg MA/g after 25 days of storage in the case of irradiated samples. Higher levels of values were also observed in fish treated by glazing and irradiation. Nevertheless, all the values were below 13mg MA/g.

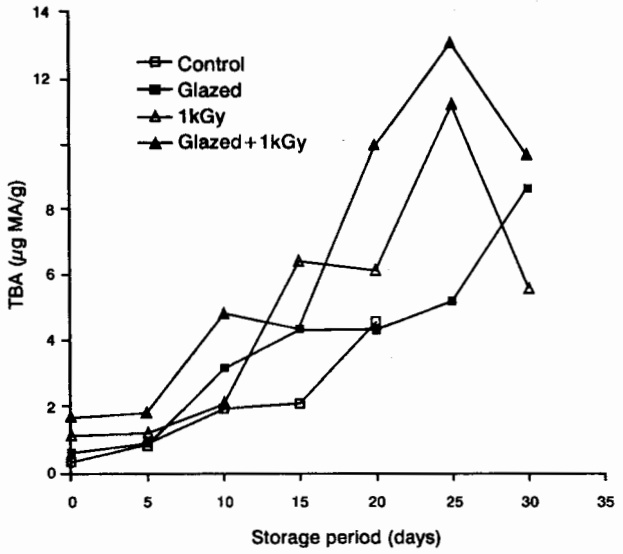

Fig. 5. Effect of glazing and irradiation on TBA values of seer fish steaks during ice storage.

Sensory evaluation of the seer fish steaks during chilled storage indicated enhanced acceptability of the products as a result of treatment. Glazed and irradiated seer fish steaks had acceptability up to 30 days as compared with about 25 days for irradiated and 20 days for glazed samples, while untreated steaks had lowest acceptability of 15 days (Table 2).

Discussion

The serious disadvantages of prolonged storage of frozen fishery products, particularly fatty fish items, are lipid oxidation and dehydration. Fish mince is a major item of commerce intended for preparation of surimi and seafood analogues. Since mincing results in exposure of the meat to air and resulting oxidation, lipid oxidation and associated flavour changes are major quality problems that hamper fish mince industry (Jeon *et al.*, 2002). Furthermore, weight loss is another major problem with respect to several high value fish fillets, which are traded under frozen conditions (Balachandran, 2001).

Table 2. Sensory score of seer fish steaks on storage

Storage period (days)	Control	Glazed	1kGy	Glazed+ 1kGy
0	8.0±0.9	7.5±1.2	7.0±0.5	7.0±1
10	5.5±1	6.0±0.89	6.5±1.0	6.5±0.8
15	5.0±0.5	5.5±0.31	6.0±0.55	6.5±0.58
20	-	5.0±0.41	5.5±0.9	6.2±0.48
25	-	-	5.5±0.41	6.0±0.71
30	-	-	4.5±0.4	5.5±0.51
35	-	-	-	5.0±0.77

- indicates samples not taken for analysis.

The present results suggest advantages of protein glazing in quality retention of frozen fishery products. Conventionally, water is used for glazing of frozen stored fishery items (Aitken, 1976). The treatment has limitation in controlling moisture loss and rancidity development during prolonged storage, particularly in the case of fatty fish like mackerel where development of rancidity is a major problem. Protein glazing significantly prevented the lipid oxidation. Both water and protein glazed samples showed less rancidity as indicated by TBA values compared to control samples on storage. The results suggest potential advantages in using coating of protein dispersion, prepared from the meat of the same fish as glaze for high value fishery products, as compared with conventional water glazing. Till now preparation of water stable dispersion of fish structural proteins has not been developed. This has been made possible by making use of gel forming capacity of fish muscle under mild acidic conditions. A number of products could be developed

such as spray dried protein powders and biofilms making use of the thermostable nature of the proteins in the dispersion (Venugopal, 1997). The present study indicates potential of protein dispersion from the same fish as a glaze for enhancement of keeping quality of chilled or frozen fishery products.

Use of antimicrobials is recommended in packaging films for inhibition of surface microorganisms (Suppakul *et al.*, 2003). Coating of dispersion could also be supplemented by incorporation of small amounts of anti-microbials and antioxidants. Thus, we have observed that the antimicrobial effect of dispersion could be enhanced by incorporation of nisin. The nisin supplemented seer fish dispersions prevented growth of *Staphylococcus aureus* in fish as shown by inoculated pack studies (unpublished data).

The results also suggest that dispersion coating can enhance the refrigerated shelf life of fresh fish. A low level of gamma irradiation can have additional advantage in combination with dispersion coating on the storage life of seer fish steaks. It is likely that these treatments exert a synergistic effect in controlling microbial proliferation and associated spoilage of the fish steaks, which could be operative through the 'Hurdle concept' (Leistner, 1992). The dispersion treatment could be advantageously used for shelf life extension of other fish items also under chilled conditions. Influence of protein glazing in combination with gamma radiation at a dose of 1 kGy on shelf life extension in ice of seer fish steaks was assessed in terms of different quality parameters. The bacterial load of the irradiated sample was reduced by one log cycle. The combination of protein coat with irradiation not only reduced the bacterial load by one log cycle but also kept it low during the entire storage period. The TVBN values were observed to be twice in the untreated samples as compared to coated and irradiated samples at the end of their respective storage life. The results are also indicative of additional advantage of dispersion coating in quality retention of fresh fish, as proved by the sensory evaluation apart from its benefits observed in the case of frozen fish products.

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