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Storage Behaviour of Washed Mince from Pink Perch (*Nemipterus japonicus*) at –30°C

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The proximate composition of Pink perch (*Nemipterus japonicus*) used in the study was recorded as protein, 16.22%; fat, 1.3% and ash , 1.7%. The yield of this fish after dressing ie., beheading and evisceration was 76%. The minced meat has a solubility of 91% and no TMA was recorded. Other physico-chemical properties also reflected its prime quality. The minced meat was frozen stored at –30°C. During storage up to 120 days ,slight pH increased up to 6%, a-amino nitrogen to 51% and TVB-N to 80%. However there was no significant change ($P>0.05$) in the FFA and PV during the storage period. Functional properties like solubility, viscosity, Ca^{2+} ATPase activity did not vary significantly ($P>0.05$) with storage period indicating the quality of minced meat. The study indicateed that storage of minced meat of Pink perch (*Nemipterus japonicus*) at –30°C does not alter the major protein fractions as indicated by the functional properties and gel forming ability. This was reflected in the over all acceptability of the cooked meat where, appearance and colour of meat did not vary significantly ($P>0.05$) with storage period.

Key words : Pink perch, mince, frozen storage

In recent years fish mince has become increasingly important for the fish food industry, both as an intermediate in surimi production and as the main raw material for breaded fish sticks and formed fish steaks. Undesirable changes in texture as a consequence of long-term storage are a major consideration in grading the quality of frozen seafoods. Loss of protein functionality during frozen storage is due to denaturation of fish protein, mainly myofibrillar protein (Chang & Regenstein, 1997). Storage temperature has been identified as one of the factors that affect lipolysis (Shewfelt, 1981) and loss of protein functionality (Samson & Regenstein, 1986) in frozen stored fish. The purpose of this study was to examine the effect of frozen storage of washed minced meat on functional properties and shelf life. Hence the present investigation reports preparation of mince and frozen storage at –30°C along with changes in physico-chemical parameters, functional properties and organoleptical characteristics.

Materials and Methods:

Pink perch, *Nemipterus japonicus* (80-250g size) procured from the Thoothukudi fishing harbour in iced condition was brought to the

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laboratory for further studies. The fish was beheaded, eviscerated, washed and fed in to a mechanical deboner to obtain mince. Minced meat was washed with chilled water (4°C) for 4 times (1:5 ratio meat to water) to remove water-soluble proteins, enzymes, blood etc. The excess water was removed by squeezing in a muslin cloth. The minced meat with concentrated myofibrillar protein thus obtained was equally divided and packed in laminated low-density polythene bag of 400m thickness. They were frozen using commercial plate freezer and stored in ultra deep freezer at –30°C. The samples were drawn at 30 days interval and analysed.

Proximate composition like moisture, protein, fat and ash was estimated by the AOAC (1995) procedure. Total Volatile Base Nitrogen (TVB-N) and Trimethylamine content (TMA) (Beatty & Gibbons 1937), Free fatty acid (FFA) (Olley and Lovern, 1960), Peroxide value (AOAC, 1995), a-amino nitrogen (Pope & Stevens, 1939) and Ca^{2+} ATPase activity (Noguchi & Matsumoto, 1970), were estimated. Solubility is defined as the amount of total protein that goes into solution under specified conditions (Xiong, 1997). The solubility of protein from meat was determined as follows: protein from meat was extracted using extraction buffer (Phosphate buffer 0.05M; pH 7.5M and 1M NaCl). About 3g of meat with 25ml of extraction buffer was homogenised. The homogenate was then centrifuged for 10min at 4000 rpm. Two ml of the supernatant was kept for digestion and nitrogen content was estimated using Kjeldahl method. The protein solubility was expressed as percentage of protein extracted to total protein. Viscosity was estimated with digital viscometer (Brook field, U.S.A). pH of the sample with Digital pH meter (335, Systronics,India). Expressible water, folding test and expressible water content of frozen minced meat was determined by the method of Okada (1963). Expressible water content was expressed as percent of meat based on the quantity of water absorbed by filter paper.

Mince was mixed with 2% salt and steam cooked for 10 minutes. Six-member panel carried out the organoleptic evaluation of these samples. The over all quality of meat was ranked using five points hedonic scale (Verma *et al.*, 1995). Correlation (Snedecor & Cochran, 1967) was done with respect to storage period and other parameters.

Table 1. Raw material characteristics of fresh Pink perch meat

	Characteristics	Values
A.	Proximate composition	
i)	Moisture (g / 100g meat)	81.47 ± 0.01
ii)	Protein (g / 100g meat)	16.22 ± 0.01
iii)	Fat (g / 100g meat)	1.3 ± 0.01
iv)	Ash (g / 100g meat)	1.7
B.	Physico-Chemical characteristics	
i)	pH	6.7
ii)	Tri Methylamine Nitrogen (TMA-N) (mg /100g meat)	Nil
iii)	Total Volatile Base Nitrogen (TVB-N) (mg /100g meat)	1.2
iv)	Peroxide Value (PV) (milli equivalent / kg of fat)	1.14 ± 0.02
v)	Free fatty acids (FFA) (% of oleic acid)	0.001
vi)	a-amino nitrogen (g/100g meat)	13.12 ± 0.04
vii)	Viscosity (Cp)	7.0
viii)	ATPase activity (μg pi/mg protein/min)	0.81 ± 0.10
ix)	Extractability in EB (% of total protein)	90.56 ± 0.03

Results and Discussion

In the present study, the average length and weight of pink perch used was 19.9+2.18cm and 111.33+1.15 g respectively. The yield of minced meat from whole fish was 46% and yield after dressing was 76%. The physical and chemical characteristics of fish vary with general

factors such as season, size, maturity and climate. Proximate composition and biochemical characteristics of pink perch mince are presented in Table 1. Proximate values of the present study are in agreement with that reported by previous workers (Reddy *et al.*; 1992; Ninan *et al.*, 2002) TMA was absent. Values of Ca^{++} ATPase activity was with in the range of 0.182 to 1.8mg pi/ mgprotein/min reported by many workers (Numakura *et al.*, 1989 Chan *et al.*, 1995; Rathnakumar & Shamasundar, 1998). High solubility value and viscosity indicated the freshness and conformational status of myofibrillar proteins. Lower values of nitrogen bases, PV and FFA are mainly due to repeated washing of meat at 4°C. Fresh meat had total plate count of $2.7x10^5$ cfu/g, Staphylococci and coliforms were found to be $1.2x10^3$ cfu/g and 1.6 MPN/g respectively Salmonella and Vibrio were absent.

Changes in the biochemical characteristics of pink perch meat during frozen storage in presented in table 2. Increase in pH to a level of 0.4 units at the end of frozen storage was recorded. Vandenverg (1959) stated that the change of pH occurring immediately in freezing was atleast partially the result of increase in the protein concentration in the lipid phase. The increase in pH is probably due to the production of basic volatile compounds. Similarly a-amino nitrogen and TVB-N shows slight increase from 23.12 to 46.67%, 2.8 to 24.92 mg/100g meat respectively. Increase in a-amino nitrogen and TVB-N directly reflects in the pH value. In general fishes with value below 30mg/100g can be considered as suitable for edible purposes (Hall and Ahmed, 1992). Trimethylamine (TMA) was found to be absent throughout the storage period. Similarly Rathinam (1995) also observed the absence of TMA in lizard minced meat stored for 30 days. Reddy *et al.* (1992) found that TMAN which was not detectable during the first two months of storage, increased to 2.1-mg% at the end of 180 days. Peroxide value, which was found to be absent for 45 days registered its presence during 60th day and found to be negligible at the end of study period. Very low level of FFA indicates that the lipid in the mince did not undergo any hydrolysis and PV value also corroborates the same.

The changes in functional properties of pink perch mince during frozen storage are presented in table 3. Solubility of fresh pink perch minced meat was found to be 91.46% and it was reduced to 58.62% at the end of storage period. Solubilisation of myofibrillar protein is prerequisite for many functional properties.

Table 2. Changes in the biochemical characteristics of pink perch mince during frozen storage

Parameter / Days of Storage	pH	a-amino nitrogen (%)	TVB-N (mg/100g meat)	FFA (% of oleic acid)	PV (milli equivalent /kg of meat)
Fresh	6.7	23.13±0.04	2.77±0.05	0.005	Nil
Immediately after freezing	6.6	23.12±0.01	5.0±0.08	0.004	Nil
15	6.7	18.6±0.05	9.08±0.05	0.003	Nil
30	6.8	16.4±0.03	12.39±0.29	0.002	Nil
45	6.8	14.0±0.02	14.42±0.32	0.002	Nil
60	6.9	14.0±0.04	17.61±0.07	0.001	0.6
75	6.9	11.3±0.05	18.54±0.02	0.001	0.9
90	6.9	20.63±0.01	20.49±0.03	0.001	0.9
105	7.0	23.3±0.01	22.45±0.01	0.09	1.2
120	7.1	46.67±0.02	24.92±0.01	0.09	1.4

Table 3. Changes in the functional properties of pink perch mince during frozen storage

Parameter / Days of Storage	Solubility (% of Protein to total Protein)	Viscosity (Cp)	ATPase activity (µg pi/protein mg protein/min.)
Fresh	91.46±0.10	7.0	0.68±0.15
Immediately after freezing	57.29±0.07	7.0	0.28±0.30
15 days	57.25±0.06	6.7	0.33±0.25
30 days	60.72±0.40	6.7	0.37±0.30
45 days	59.88±0.02	6.7	0.45±0.30
60 days	59.78±0.03	6.7	0.44±0.08
75 days	59.07±0.20	6.3	0.35±0.09
90 days	59.07±0.40	6.7	0.36±0.10
105 days	58.02±0.40	7.0	0.32±0.40
120 days	52.62±0.07	6.7	0.33±0.03

Freezing reduces the extractable protein to the level of 37%. Subsequent storage at -30°C does not show any variation till the end of 120 days. The fresh meat has the viscosity of 7.0 Cp. A slight decrease in viscosity was noticed in the present study. The decrease in viscosity could be due to alterations in protein with a subsequent formation of small size aggregates, which corresponds to protein-protein interactions (Hermansson, 1979). The formation of aggregates increased in size with increase in frozen stored period giving rise to a greater loss of extractable protein and drastic decrease in viscosity (Borderias *et al.*, 1985). The decrease in viscosity with increase in frozen storage period of ray muscle and hake actomyosin have been reported (Pastoriza *et al.*, 1994, Montecchia *et al.*, 1997). Ca^{2+} ATPase activity showed a steep fall due to freezing process and during subsequent storage period a reduction up to 52% is observed. From the initial value of 0.68 mg pi /mg protein/min, Ca^{++} ATPase activity reduced to 0.33 mg pi /mg rotein/min at the end of 120 days. Both solubility and Ca^{2+} ATPase activity indicates that no significant ($P>0.05$) alteration in the myofibrillar protein has occurred. It is suggested that during freezing and frozen storage induced association-dissociation reaction did not takes place, which normally cause alteration in the major protein fractions. The increase in ATPase enzyme activity during the initial phase of frozen storage period was attributed to modification of a natural barrier between enzymes and substrates are activated (Partman, 1977).

To find out the gel forming capacity , raw meat was minced with salt, stuffed in the casing and cooked. After cooling the meat was cut in to thin slice and folding test was conducted and changes in gel forming ability of pink perch mince during frozen storage are presented in Table 4. The meat has excellent (AA) gel forming capacity till the end of 120 days. Kurokawa (1979) recorded grade AA of Kamaboko

Table 4. Changes in Gel forming ability of pink perch mince during frozen storage

Parameter / Days of Storage	Expressible water(%)	Folding test
Fresh	24.89 (±0.27)	AA
Immediately after freezing	26.62 (±0.26)	AA
15	25.65 (±0.17)	AA
30	25.82 (±0.19)	AA
45	26.55 (±0.31)	AA
60	26.89 (±0.26)	AA
75	27.10 (±0.28)	AA
90	26.92 (±0.19)	AA
105	27.43 (±0.16)	AA
120	27.82 (±0.23)	A

prepared from the frozen stored lizard meat. Nozaki *et al.* (1986) has also observed the similar results in the meat of lizardfish. Noguchi *et al.* (1975) observed that fish meat of horse mackerel retain grade AA till subsequent storage. They also found good correlation between organoleptic characteristic and folding test. The water holding capacity of after cooking as examined by expressible water (Table 4) shows marginal loss in retaining the water (2.9%). Many workers observed that with a reduction in gel strength increase in the expressible water of gel (Noguchi & Matsumoto, 1970). In the present study expressible liquid in mince did not increase in greater extent. This indicates that the protein did not under go any change during storage. Increase in expressible water is corroborates with decrease in meat quality was reported by many workers (Noguchi *et al.*, 1975;Samson & Regenstein, 1986).

Table 5 shows the changes in organoleptic evaluation of cooked minced meat during frozen storage. The organoleptic quality of the pink perch meat was ranked using 5-point hedonic scale and the parameter such as appearance,

colour, odour, texture was found to be excellent. The above qualities are found to be good till the end of storage period. Low temperature storage has an advantageous impact in retarding the deterioration process of fish and fish products. It retains their freshness for longer time owing to biochemical and microbial activity. Lower the storage temperature longer is the shelf life. The storage life of frozen stored product is very much dependent on the quality of raw materials, method and duration of freezing and storage condition. However there is significant correlation ($P<0.01$) in the overall acceptability of mince in relation to frozen storage period.

Table 5. Organoleptic evaluation of cooked minced meat during frozen storage

Parameter / Days of Storage	Appearance	Colour	Odour	Texture	Over all acceptability
Fresh	4.83±0.37	4.75±0.45	4.83±0.37	4.92 ± 0.18	5.0 ± 0.00
Immediately after freezing	4.83±0.37	4.70±0.25	4.81±0.19	4.83 ± 0.37	4.97 ± 0.05
15	4.83±0.37	4.70±0.25	4.81±0.19	4.83±0.37	4.97±0.05
30	4.83±0.37	4.70±0.25	4.83±0.37	4.75±0.45	4.83±0.04
45	4.83±0.37	4.65±0.42	4.33±0.24	4.70±0.25	4.75±0.25
60	4.49±0.20	4.68±0.42	4.33±0.24	4.67±0.37	4.78±0.06
75	4.50±0.23	4.58±0.42	4.25±0.25	4.25±0.25	4.65±0.40
90	4.48±0.37	4.55±0.60	3.92±0.19	4.25±0.25	4.5±0.06
105	4.50±0.06	4.77±0.30	3.83±0.25	3.83±0.23	4.48±0.08
120	4.50±0.08	4.70±0.25	3.67±0.37	3.83±0.25	4.40±0.12

Changes in total plate count, *Staphylococci, E.coli, Salmonella, Vibrio* during storage period is presented in Table 6. *Salmonella* and *Vibrio cholera* was absent through out the storage period. The present study revealed a gradual reduction in the total plate count, *Staphylococcus,* and *E.coli* count during frozen storage. Decrease in microbial load may be due to the exposure to low temperature for long duration. This is in agreement with reports of Nickelson *et al.* (1980) and Siddaiah *et al.* (2001) for frozen mince of black drum, sandfront tilapia, pink perch and common carp. Licciardello & Hill (1978) reported that there was no significant correlation between aerobic plate count and indicator organisms .

The present study indicates storage at –30°C does not affect the functional properties and gel forming ability of the pinkperch meat. This is reflected in the over all acceptability of the cooked meat. Appearance and colour of meat did not vary significantly ($P>0.05$) with storage period. It is also inferred that minced meat of pink perch fish, water washed at 4°C and frozen stored at –30°C retained its physico-chemical, functional and organoleptic properties without any significant change.

Table 6. Microbiological properties of minced meat during frozen storage

Storage Days	TPC (cfu/g)	*Staphylococci* (cfu/g)	*E.coli* (MPN/g)	*Salmonella*	*Vibrio cholera*
Raw	2.1×10^5	2.5×10^3	1.4	Nil	Nil
30	1.9×10^5	1.8×10^3	1.4	Nil	Nil
60	2.1×10^5	2.7×10^3	1.4	Nil	Nil
90	1.8×10^4	2.4×10^3	1.1	Nil	Nil
120	1.43×10^4	2.1×10^3	1.1	Nil	Nil

Table 7. Correlation Coefficient between storage Days and bio-chemical, functional, gel forming, and organoleptic characteristics

Parameter	Correlation
Bio-chemical characteristics of pink perch mince during frozen storage	
Storage days Vs pH	0.96513 *
Storage days Vs α -amino nitrogen	0.41140 N.S
Storage days Vs TVB-N	0.980534 *
Storage days Vs FFA	0.691577 **
Functional properties of pink perch mince during frozen storage	
Storage days Vs Solubility	-0.503648 N.S
Storage days Vs Viscosity	-0.309695 N.S
Storage days Vs ATPase activity	-0.374402 N.S
Solubility Vs Viscosity	0.372085 N.S
Solubility Vs ATPase activity	0.921157 *
Storage days Vs expressible water	0.856548 *
Organoleptic evaluation of cooked minced meat during frozen storage	
Storage days Vs Appearance	-0.875022 N.S
Storage days Vs Colour	-0.252355 N.S
Storage days Vs Odour	-0.971347*
Storage days Vs Texture	-0.949621*
Storage days Vs Over all acceptability	-0.984342*

N.S – Not Significant ; *- P< 0.01 ; ** -P<0.05

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