Delayed Freezing on the Quality and Shelf-life of Kalawa (*Epinephelus* spp.)

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Kalawa *(Epinephelus* spp.) caught on board FORV Sagar Sampada was frozen in the absolutely fresh condition as well as after keeping for 5 and 10 h at ambient temperature. Evaluation of changes in the quality of these samples during storage at -20°C indicated appreciable difference between the fresh frozen and delayed frozen fish during the initial stages of storage. Fresh frozen and 5 h delayed frozen fish samples had a shelf-life of more than 62 weeks, whereas the 10 h delayed frozen fish had a shelf-life of about 48 weeks.

The freezing and storage characteristics of many of the Indian marine fishes have been studied (Mathen *et al.*, 1966; Jadav & Magar, 1970; Shenoy & Pillai, 1971; Shenoy, 1976; Perigreen & Joseph, 1980; Badonia & Devadasan, 1980; Perigreen *et al.*, 1988; Sankar & Viswanathan Nair, 1988) and the frozen shelf-life of these fishes varied from 10 to 32 weeks depending upon the species, fat content, method of freezing and storage temperature (Perigreen *et al.*, 1985). But no work has been reported on the effect of delayed freezing on the frozen storage behaviour of *Epinephelus* spp., a lean fish of economical importance which is mainly distributed off the west coast of India. This paper summarises the results of the studies made on the storage characteristics of fresh frozen and delayed frozen *Epinephelus* spp. caught on board FORV Sagar Sampada.

Materials and Methods

Kalawa *(Epinephelus* spp.) caught by the research vessel FORV Sagar Sampada during her cruise No. 21 off the West coast of India in September 1986 was used for the study. The fish were washed immediately after catch, packed in polythene bags and divided into three lots. One lot was immediately quick frozen at -40°C in the blast freezer on board the vessel and used as control (sample 1). The other two lots were similarly frozen after keeping at ambient temperature (about 30°C) for 5 and 10 h respectively (samples 2 and 3). All the three lots of frozen samples were stored in the cold storage of the vessel at -20°C. On arriving the shore at Cochin (after 5 weeks) the frozen samples were transferred to the cold storage of the Institute maintained at -20°C and a portion of the fresh frozen fish (sample 1) was analysed for peoximate composition. The samples from all the three lots were drawn at intervals for physical, chemical, bacteriological and organoleptic examination.

Moisture, fat, total nitrogen and non protein nitrogen (NPN) in the muscle of the fish were determined by the methods of AOAC (1975). The salt soluble nitrogen (SSN) was estimated by the method of Dyer *et al.* (1950) and the total volatile basic nitrogen (TVBN) by the microdiffusion method of Conway (1947) from the trichloracetic acid extract of the muscle. The organoleptic characteristics of the fish cooked in 2% sodium chloride for 10 min were determined by a trained taste panel and the scoring was done using a 10 point hedonic scale, 10 being very good, 0 being bad and 4 being just unacceptable.

Table 1. *Proximate composition of Kalawa*

Moisture, %	79.26
Fat, %	0.79
Crude protein, % (TN x 6.25)	18.75
Ash, %	0.62

Table 2. *Changes in biochemical parameters and organoleptic scores of fresh frozen and delayed frozen kalawa during storage at* -20°C

Storage period weeks	Moisture %			NPN mg/100g			SSN % of TN			TVBN mg/100g			Average organoleptic score		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
9	79.3	80.1	80.3	350	440	420	83	65	74	2.8	14	15.4	8.5	7.4	6.4
14	79.1	79.8	79.1	406	350	364	82	77	71	12.6	16.8	16.8	8.2	7.2	6.1
18	79.0	79.7	78.6	314	364	364	70	73	70	8.4	9.8	11.2	7.4	6.3	5.3
24	79.6	80.5	79.9	350	360	370	63	71	68	11.8	16.8	18.8	7.4	6.2	5.3
36	79.3	79.3	79.6	378	392	392	55	56	53	21	21	23.8	7.4	6.2	5.2
48	78.1	78.1	77.9	390	400	402	49	37	41	11.2	20	42	7.3	6.2	4.5
62	78.1	78.1	77.9	390	405	420	46	35	37	16	20	38	6.5	5.2	4

I = fresh frozen; 2 = frozen after 5 h at ambient temperature, 3 = frozen after 10 h at ambient temperature

Table 3. *Changes in bacterial counts of fresh frozen and delayed frozen kalawa during storage at* -20°C

Storage period weeks	Total bacterial count/g			Faecal streptococci/g			Total presumptive coliforms/g		
	1	2	3	1	2	3	1	2	3
9	2.68×10^4	5.34×10^4	7.23×10^5	7.5×10^2	4.8×10^2	3.78×10^4	2.8×10^2	5.9×10^2	3.5×10^3
14	2.70×10^4	9.80×10^3	9.30×10^4	Nil	Nil	1.18×10^4	35	—	4.8×10^2
18	1.58×10^4	1.76×10^4	2.07×10^5	Nil	Nil	—	1.6×10^2	2.2×10^2	8.3×10^2
24	2.5×10^4	4.5×10^4	7.0×10^4	Nil	Nil	1.3×10	Nil	40	3.2×10^2
36	1.45×10^3	5.52×10^3	2.37×10^4	Nil	Nil	2.9×10^2	Nil	Nil	97
48	3.73×10^3	5.86×10^3	3.75×10^4	Nil	Nil	Nil	Nil	Nil	Nil
62	1.44×10^3	1.47×10^3	2.5×10^3	Nil	Nil	Nil	Nil	Nil	Nil

E. coli and coagulase positive staphylococci were absent in all the samples
I = fresh frozen; 2 = frozen after 5 h at ambient temperature; 3 = frozen after 10 h at ambient temperature

Total aerobic bacterial plate count (TPC) of the muscle was determined using tryptone glucose beef extract agar (TGA). The plates were incubated at 28 ± 2°C (RT) for 48h and counts taken. Total coliforms were determined using desoxycholate lactose agar and *Escherichia coli* by standard method (FDA, 1973). Faecal streptcocci was estimated using KF agar and coagulase positive staphylococci using Baird Parker Agar (Difco, 1971).

Results and Discussion

Epinephelus used for the study had an average weight 700 g and length 40 cm. The proximate composition of the flesh of the fish is given in Table 1. The flesh was white in colour and the taste of the cooked meat was good.

The changes in moisture, non protein nitrogen, salt soluble nitrogen, total volatile basic nitrogen and organoleptic score of fresh frozen sample (1) and delayed frozen samples (2 and 3) are given in Table 2. There was no significant difference in moisture content among the samples and it indicates a slight decrease in all cases during storage. NPN values of samples 2 and 3 were higher than that of sample 1 at the beginning probably due to the effect of autolysis and bacterial action in the muscle of delayed frozen samples. The values did not indicate a definite pattern of change during storage due to the elimination of some of the non protein constituents along with thaw drip. SSN value was maximum in sample 1 and minimum in sample 2 at the initial stage which may be due to the effect of rigor mortis on the extractability of proteins. Nikkila & Linka (1954) have shown that the amount of protein that can be extracted from Baltic herring *(Clupea herengus)* falls off as much as 50% when the fish enters rigor. A slight increase of SSN values in sample 3 compared to sample 2 at the initial stage and in sample 2 upto certain period of storage may be due to resolution of rigor before freezing in sample 3 and during thawing in sample 2. SSN values of all the three samples indicated a reduction of about 45–50% after 62 weeks storage. TVBN values of the samples clearly indicated the extent of spoilage. The value was minimum in sample 1 and maximum in sample 3 through out the period of storage. Fluctuations in values during storage are presumably due to the elimination of dissolved volatile constituents through drip.

The organoleptic scores also clearly indicated the extent of spoilage, the score was maximum for sample 1 and minimum for sample 3. Though a decrease in score was noticed in all samples during storage it remained more or less constant from 18 weeks to 48 weeks of storage. Samples 1 and 2 were in acceptable condition even after 62 weeks of storage whereas sample 3 was only in just acceptable condition after 48 weeks storage.

Table 3 gives the changes in the total bacterial counts, faecal streptococci and total presumptive coliforms in fresh frozen and delayed frozen kalawa during storage. *Escherichia coli* and coagulase positive staphylococci were absent in all samples throughout the period of storage. The total bacterial count was least in fresh frozen kalawa (sample 1) and highest in sample frozen after 10 h at ambient temperature (sample 3). During storage, there was a steady decrease in the bacterial counts of the three samples. The faecal streptococci and coliform counts were the least in sample 1 and highest in sample 3, and they steadily decreased during storage. Whereas the faecal streptococci completely disappeared in sample 1 and 2 after 14 weeks of storage, it persisted upto 36 weeks in sample 3. In the case of coliforms, they were completely destroyed after 18 weeks of frozen storage in sample 1, after 24 weeks in sample 2 and after 36 weeks in sample 3.

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