Fishery Technology
2011, Vol. 48(2) pp : 133 - 140

Effectiveness of Spices on the Quality and Storage Stability of Freeze-dried Fish Balls

George Ninan[1], A.A. Zynudheen[1], M. Regina[1] and A.C. Joseph[2]
[1] *Central Institute of Fisheries Technology, P.O. Matsyapuri, Cochin - 682 029, India*
[2] *Azheekkakath House, 83/3198, P.O.Vennala, Cochin - 682 028, India*

Five different freeze-dried fish ball samples were prepared from the cooked mince of snapper (*Pristipomoides multidens*) incorporated with spices *viz.*, curry leaf (*Murraya koenigii* S.), mint (*Mentha spicata* L.), turmeric (*Curcuma longa*) and a mixture of ginger (*Zingiber officianale*), garlic (*Allium sativum* L.) and pepper (*Piper nigrum*) with known antioxidant properties. The samples were packed and kept in ambient conditions to assess the antioxidant property of the spices and the storage stability. The evaluation of oxidation indices *viz.*, thiobarbituric acid and free fatty acid values revealed that there was marked protective effect in samples incorporated with spices during storage. Incorporation of spices retarded the rate of oxidation of PUFAs in the samples. A combination of the spices was found to have an added advantage in terms of their synergistic effect against oxidation and in enhancing the taste. There was a gradual decrease in the antioxidant activity as storage time progressed. The control samples had a shorter shelf life when compared to spices incorporated samples.

Key words: Fish balls, freeze drying, spices, quality, shelf life

Fish mince offers enormous potential for development of diverse products such as battered and breaded products which include fish cutlet, fish balls, fish fingers, sausages, dried fish flakes, restructured, dried and formulated products (Venugopal, 2001). Fish ball from surimi is a product popular in South East Asian countries. In India, it is prepared as a deep fried coated product from the raw fish mince and consumed as a snack.

Freeze-drying is a promising area in the value addition of fishery products. However, rancidity is a problem encountered in freeze-dried products. The chances of oxidation are comparatively higher in fishery products due to the high content of polyunsaturated fatty acids. Though synthetic antioxidants were used to prevent oxidation in food products, due to their health implications, it has been replaced by natural antioxidants. The interest in natural antioxidants continues to grow because they are presumed to be safe as they occur in foods and have been used for centuries (Frankel, 1996). Furthermore, evidence is accumulating that natural antioxidants in foods may have clear benefits due to their anticarcinogenic effects and inhibition of biologically harmful oxidation reactions in the body (Simic & Karel, 1980).

The objective of this study is to assess the effect of incorporation of spices on the quality and shelf life of freeze dried coated fish balls during storage at ambient temperature. The spices selected for the study were curry leaf (*Murraya koenigii* S.), mint (*Mentha spicata* L.), turmeric (*Curcuma longa*), ginger (*Zingiber officianale*), garlic (*Allium sativum* L.) and pepper (*Piper nigrum*). The selection of these spices as natural antioxidants was based on the reports cited on their antioxidant potential (Rao *et al.*, 2005; Kanatt *et al.*, 2005; Craig, 1999; Anon, 2006 a,b; Yang *et al.*, 1993, Agbor *et al.*, 2006; Sharma, 1976).

* Corresponding author; e-mail: george66jiji@rediffmail.com

Materials and Methods

Fresh, post rigor snapper (*Pristipomoides multidens*) locally called "velameen" with average weight of 516 ± 20 g and length of 35 ± 5 cm was procured from a landing centre near Cochin and brought to the laboratory in iced condition (fish: ice = 1:1). It was washed, dressed, filleted and minced using the mincing unit of the Hobart Mixer (Model # A 120, M/s Hobart Engineering Co. Ltd, UK). Food grade quality ingredients and refined sunflower oil were used for the preparation of fish balls. Analytical Grade chemicals were used for conducting the experiments. The batter mix and bread crumbs for coating the fish balls were prepared as described by Abbas *et al.* (2009).

Fish balls were prepared in five combinations *viz.*, Control samples (A), samples containing; curry leaves (B), mint (C), turmeric (D) and samples with standard recipe (E). The ingredients (Table 1) were mixed thoroughly, shaped into balls of uniform size (~10 g) and cooked for 10 min in 1% boiling brine. The cooked fish balls were pre-dusted using dry batter mix, applied with liquid batter and coated with breadcrumbs. The coated fish balls were deep fried in refined sunflower oil at 180-200°C for four min. The fish balls were frozen in an air blast freezer (Model T 10, Castel MAC SpA, Italy) at - 40°C and were freeze dried (CHRIST, Gamma 1-16 LSC, Serial No.12546, GmBH, Osterode am Harz, Germany) for 12 h. Freeze drying was done in two steps *viz.*, main drying for 10 h at a set shelf temperature of 20°C and set pressure of 0.01mbar and subsequent final drying at set shelf temperature of 25°C for 2 h at a condenser temperature of -55°C. The freeze-dried fish balls were immediately packed in pouches made of metallised polyester of 12 μm thickness laminated with low density polyethylene (LDPE) of 60 μm thickness and then stored in air tight steel containers at ambient conditions. The samples were analysed for physico-chemical and sensory parameters.

Crude protein, fat, moisture and crude ash were determined by standard methods described in AOAC (2000). Thiobarbituric Acid value (TBA) was determined by the method of Tarladgis *et al.* (1960). Free Fatty Acid (FFA) was determined using AOAC (1975) method. Lipid content of the comminuted freeze-dried coated fish balls were separated by the method of Folch *et al.* (1957). The fatty acids separated in the form of methyl esters were detected by AOAC (1975) method using gas chromatograph (GC Varian CP3800, Varian Inc. Lake Forest, USA) and compared with standards supplied by SUPELCO, (595, North Harrison Road, Bellefonte, PA 16823-0048, USA)

The colour of the freeze-dried coated fish balls were measured instrumentally with a Hunter Labscan II Mini Scan XE Plus spectrocolorimeter (Hunter Associates laboratory, Inc., Reston, VA). The parameters determined were CIEL* (L* = 0 [black] and L* = 100 [white]), CIEa* (-a* = greenness and +a* = redness), and CIEb* (-b* = blueness and +b* = yellowness). L* measures relative lightness, a* relative redness and b* relative yellowness (Anon, 1986).

Sensory evaluation of the samples was carried out by a group of ten trained panelists. Overall acceptability of the sample was indicated by giving scores on a nine-point hedonic scale (Peryam & Pilgrim, 1957). The limit of acceptability was 4.

Significance of the differences between the mean values was determined by One-way Analysis of Variance (ANOVA), followed by Duncan's test using the statistical package (SAS, 1989).

Results and Discussion

The proximate composition of freshly prepared freeze-dried coated fish balls is given in Table 2. Freeze dried balls had moisture content slightly above the ideal moisture level of 2% for freeze dried products.

Table 1. Ingredients used for the preparation of fish balls (g)

Ingredients	A	B	C	D	E
Fish Mince	1000	1000	1000	1000	1000
Corn Starch	50	50	50	50	50
Salt	8	8	8	8	8
MSG	1	1	1	1	1
Curry leaf	—	20	—	—	—
Mint	—	—	20	—	—
Turmeric	—	—	—	3	—
Ginger	—	—	—	—	20
Garlic	—	—	—	—	20
Pepper	—	—	—	—	1

A: Control sample; **B:** Sample containing curry leaf **C:** Sample containing mint **D:** Sample containing turmeric **E:** Sample as per standard recipe

Table 2. Proximate Composition of freeze dried coated fish balls

	A	B	C	D	E
Moisture (%)	2.38 (0.56)	2.25 (0.50)	2.30(0.46)	2.88(0.41)	2.42 (0.41)
Crude Protein (dwb) %	32.91(1.01)	36.63 (1.22)	34.80 (1.51)	32.83 (0.96)	31.96 (0.98)
Carbohydrate (%)*	42.97 (1.88)	40.59 (1.29)	49.40 (1.73)	44.34 (1.71)	48.06 (1.64)
Ash (dwb) %	2.55 (0.22)	2.16 (0.19)	1.67 (0.18)	2.27 (0.29)	2.34 (0.31)
Crude Fat (dwb) %	19.19 (0.43)	18.37 (0.51)	11.88 (0.29)	17.68 (0.44)	15.22 (0.40)

n=4; Figures in parantheses indicate std.dev.
* Carbohydrate % = (100%) – [(% Protein) + (% Fat) + (% Moisture) + (% Ash)] (Woods & Aurand, 1977)

Table 3 shows the changes in TBA of freeze-dried coated fish balls during storage under ambient conditions. During the first seven days, the control (A) had a comparatively higher TBA value than the other samples. This implies the antioxidant effect of the added spices in these samples. In control, a gradual reduction in the TBA value was noticed till 28 days of storage. Thereafter, the value remained almost steady till 56 days and again increased gradually with storage. The decrease in TBA values till 28 days of storage in the case of control can be attributed to the reasons cited by Melton (1983). Similar results were also demonstrated by Ghirette *et al.* (1997) in a study on the effect of different antioxidants in Milano salami during a storage period of 5 months. Although the TBA values above the range of 0.5 to 1.0 is expected to produce a negative effect during sensory evaluation, the higher TBA values in the present study did not result in any adverse sensory effect. This might be due to masking of rancid taste and odour by the added spices. However, in the case of control, where no spices were added, development of rancidity corresponded with the increase in TBA values in the 70th and 98th days of storage. Reddy *et al.* (1992) reported an increase in TBA up to 98 days of storage and thereafter decrease for frozen fish fingers.

The changes in FFA of freeze-dried coated fish balls during storage under ambient conditions are given in Table 4. The

Table 3. Changes in thiobarbituric acid value (mg malonaldehyde kg^{-1}) in freeze dried fish balls during storage at ambient temperature

Sample	Storage period (Days)							
	0	7	14	28	42	56	70	98
A	2.67^{a} (0.01)	2.02^{b} (0.03)	1.84^{c} (0.03)	1.41^{d} (0.07)	1.48^{d} (0.05)	1.49^{d} (0.07)	1.89^{e} (0.09)	2.30^{f} (0.08)
B	0.92^{a} (0.03)	1.44^{b} (0.01)	1.45^{b} (0.02)	1.47^{bc} (0.06)	1.51^{cd} (0.04)	1.03^{e} (0.02)	1.19^{f} (0.10)	1.12^{g} (0.03)
C	0.89^{a} (0.10)	1.19^{b} (0.08)	0.99^{c} (0.08)	1.39^{d} (0.04)	1.06^{e} (0.02)	1.05^{ef} (0.09)	1.15^{g} (0.01)	1.12^{h} (0.01)
D	1.54^{a} (0.08)	2.04^{b} (0.08)	1.99^{bc} (0.11)	1.62^{d} (0.10)	1.65^{d} (0.09)	1.82^{e} (0.05)	1.27^{f} (0.02)	1.37^{g} (0.03)
E	1.02^{a} (0.09)	1.59^{b} (0.03)	1.46^{c} (0.08)	1.66^{d} (0.05)	1.60^{de} (0.06)	2.02^{e} (0.11)	1.11^{f} (0.02)	1.06^{g} (0.03)

n=4; Figures in parantheses indicate std.dev.
Means within a row with different superscripts are significantly different ($P < 0.05$)

Table 4. Changes in Free fatty acid (mg % oleic acid) in freeze dried fish balls during storage at ambient temperature

Sample	Storage period (Days)							
	0	7	14	28	42	56	70	98
A	0.53^{a} (0.05)	0.58^{a} (0.03)	0.72^{b} (0.03)	1.05^{c} (0.04)	0.49^{ad} (0.02)	0.33^{e} (0.03)	0.39^{f} (0.02)	0.68^{bg} (0.06)
B	0.21^{a} (0.02)	1.60^{b} (0.09)	0.69^{c} (0.05)	0.67^{c} (0.05)	0.44^{d} (0.03)	0.33^{e} (0.04)	0.28^{f} (0.02)	0.67^{c} (0.02)
C	0.19^{a} (0.01)	2.54^{b} (0.09)	0.67^{c} (0.02)	0.62^{d} (0.02)	0.37^{e} (0.04)	0.32^{ef} (0.01)	0.28^{g} (0.01)	0.63^{ch} (0.06)
D	0.20^{a} (0.01)	2.39^{b} (0.06)	0.65^{c} (0.05)	0.27^{d} (0.03)	0.17^{e} (0.02)	0.08^{f} (0.01)	0.06^{fg} (0.02)	0.43^{h} (0.04)
E	0.23^{a} (0.01)	2.82^{b} (0.07)	0.59^{c} (0.04)	0.42^{d} (0.02)	0.38^{e}(0.02)	0.31^{ef} (0.01)	0.29^{fg} (0.02)	0.57^{c}(0.03)

n=4; Figures in parantheses indicate std.dev.
Means within a row with different superscripts are significantly different ($P < 0.05$)

initial FFA content of control was slightly higher than the other samples treated with spices. In the control samples, there was a gradual increase upto 28 days (1.05 mg % oleic acid) followed by a decline and remained stable till 70 days. Except for the first 7 days of storage, the FFA content of the spices incorporated samples was less than that of the control samples. The FFA content was low in all samples since cooking of mince might have deactivated the lipolytic enzymes.

The important fatty acids identified in fish balls were Palmitic (C_{16}), Palmitoleic ($C_{16:1}$), Stearic (C_{18}), Oleic ($C_{18:1}$), Linoleic ($C_{18:2}$), Linolenic ($C_{18:3}$) and Arachidonic ($C_{20:4}$) acids (Table 5). Although the content of polyunsaturated fatty acids *viz.*, $C_{18:3}$, $C_{20:5}$ and $C_{22:6}$ are expected to be high in fish lipids, their percentage was very less in the fish balls. This could be due to frying of fish balls in sunflower oil causing dilution of the fish lipids. There was a marked decrease in the $C_{18:1}$ and $C_{18:2}$ during storage of the freeze-dried coated fish balls (Table 5).

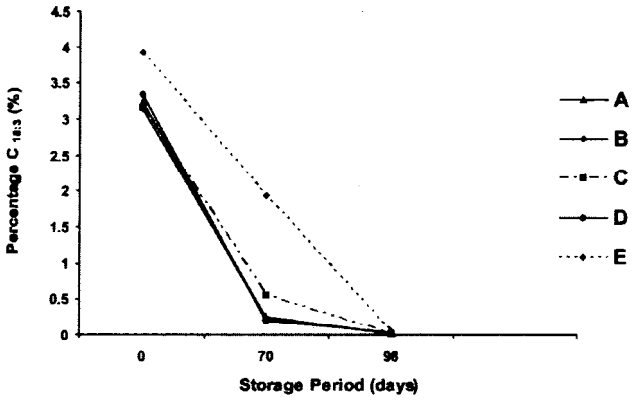
However, when PUFA found in the fish lipids of the samples alone was taken into account (Fig. 1-3), a marked difference could be observed between the control and spices incorporated samples indicating the protective effect of the added spices. It is reported that 82% of the fatty acid content of sunflower oil is monounsaturated fatty acids (Anon, 2003).The highest concentration of $C_{18:3}$ was observed in E (Fig. 1) while control and other samples had almost same

Table 5. Changes in fatty acid composition in freeze dried fish balls during storage at ambient temperature

DAYS	A			B			C			D			E		
	Storage period (Days)														
	0	70	98	0	70	98	0	70	98	0	70	98	0	70	98
C_{16}	9.67	10.36	12.21	4.25	9.59	10.04	2.26	7.81	10.47	10.33	10.18	13.22	11.98	5.17	8.74
$C_{16:1}$	1.62	0.63	0.55	1.01	0.44	0.34	0.36	0.30	0.24	1.07	1.01	0.97	0.61	0.47	0.11
C_{18}	3.15	5.06	7.79	2.95	4.58	7.72	0.73	6.19	12.31	0.39	2.43	3.68	0.84	9.21	12.66
$C_{18:1}$	30.33	27.91	27.53	41.38	40.11	30.15	13.44	8.33	2.97	43.82	40.25	8.69	31.45	23.67	17.46
$C_{18:2}$	38.06	37.17	33.42	41.47	28.21	22.26	76.95	5.18	5.05	70.99	23.19	11.57	42.62	17.15	16.88
$C_{20:4}$	0.58	0.09	0.03	0.43	0.19	0.11	1.61	0.32	0.30	0.25	0.22	0.20	0.28	0.65	0.62

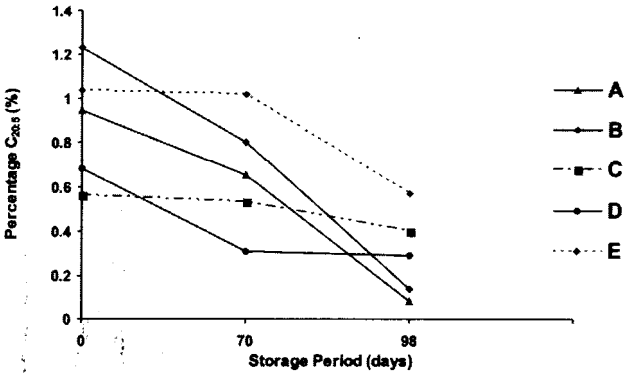
A: Control sample; **B:** Sample containing curry leaf **C:** Sample containing mint **D:** Sample containing turmeric; **E:** Sample as per standard recipe

concentration of the said fatty acid. After 70 days of storage, about 49% of $C_{18:3}$ was retained in sample E. At the end of the storage period, the content of $C_{18:3}$ was reduced to negligible quantity in all the samples. Initial levels of $C_{20:5}$ and $C_{22:6}$ were maximum in sample B (Fig 2 & 3). These fatty acids were also reduced to insignificant levels by the end of storage period in all the samples. However, the reduction was greater in the case of control samples. The antioxidant activity of spices *viz.*, turmeric, curry leaf, mint leaf, ginger and garlic was reported (Subramanian *et al.*, 1994; Sharma, 1976; Rao *et al.*, 2005; Kanatt *et al.*, 2005; Anon, 2006 b; Sallam, 2004). Though these spices were proven for their antioxidant activity, it was observed in the present study that there was a gradual reduction in the percentage of unsaturated fatty acids during storage. This indicated the reduction in antioxidant activity of the spices on storage.



A: Control sample; B: Sample containing curry leaf C: Sample containing mint D: Sample containing turmeric; E: Sample as per standard receipe

Fig. 1. Freeze dried coated fish balls - Changes in $C_{18:3}$ fatty acid during storage under ambient conditions



A: Control sample; B: Sample containing curry leaf C: Sample containing mint D: Sample containing turmeric; E: Sample as per standard receipe

Fig. 2. Freeze dried coated fish balls - Changes in $C_{20:5}$ fatty acid during storage under ambient conditions

Table 6 shows the changes in colour parameters CIE L*, CIE a* and CIE b* values of freeze-dried coated fish balls during storage under ambient conditions. In all, the sample lightness (L*) increased significantly ($p < 0.05$) with storage time. At the end of the storage period, spices incorporated

Table 6. Changes in colour in freeze dried fish balls during storage at ambient temperature

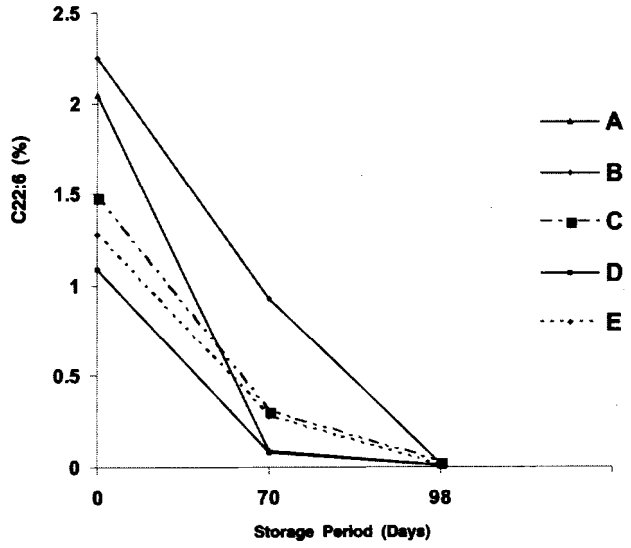
Days	A			B			C			D			E		
	Storage period (Days)														
	L *	a*	b*	L *	a*	b*	L *	a*	b*	L *	a*	b*	L *	a*	b*
0	58.68^a	8.88^a	26.84^a	55.17^a	6.66^a	23.66^a	57.56^a	6.24^a	23.56^a	57.82^a	8.06^a	30.03^a	54.78^a	8.39^a	21.25^a
	(0.12)	(0.04)	(0.15)	(0.24)	(0.02)	(0.11)	(0.16)	(0.06)	(0.03)	(0.09)	(0.06)	(0.11)	(0.15)	(0.07)	(0.20)
70	58.89^b	8.09b	26.14^b	57.49^b	6.09^b	23.95^b	61.29^b	5.45^b	23.79^b	59.65^b	7.94^b	29.44^b	55.45^b	7.07^b	23.48^b
	(0.10)	(0.05)	(0.22)	(0.35)	(0.14)	(0.13)	(0.29)	(0.03)	(0.11)	(0.17)	(0.03)	(0.21)	(0.23)	(0.11)	(0.46)
98	59.41^c	7.87c	25.34^c	61.51^c	5.87^c	22.42^c	62.15^c	5.35^c	24.9^c	62.29^c	6.39^c	31.43^c	62.98^c	6.45^c	27.63^c
	(0.11)	(0.03)	(0.39)	(0.33)	(0.15)	(0.09)	(0.22)	(0.07)	(0.09)	(0.23)	(0.06)	(0.30)	(0.30)	(0.12)	(0.31)

A: Control sample; **B:** Sample containing curry leaf **C:** Sample containing mint **D:** Sample containing turmeric; **E:** Sample as per standard recipe

n=4; Figures in parantheses indicate std.dev.

Means within a column with different superscripts are significantly different ($P < 0.05$)

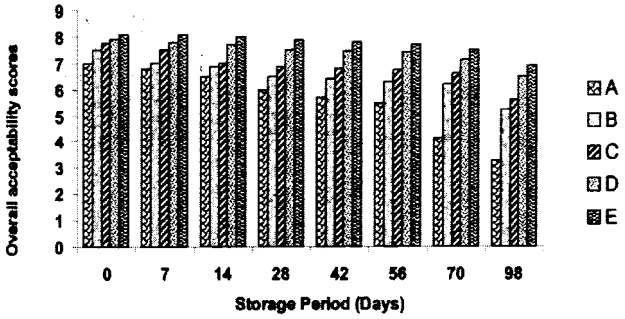
samples had high L* values compared to the control (A). This could be due to the presence of antioxidants in the spices. Similar observations were made in meat products where antioxidants in spices retarded the formation of metmyoglobin and thereby preventing decrease in the lightness values (Genot *et al.*, 1991; López *et al.*, 2005).



A: Control sample; B: Sample containing curry leaf C: Sample containing mint D: Sample containing turmeric; E: Sample as per standard recipe

Fig. 3. Freeze dried coated fish balls - changes in $c_{22:6}$ fatty acid during storage under ambient conditions

The overall acceptability scores were obtained by pooling the scores for each attribute *viz.*, appearance, colour, odour, taste and texture of the samples which are presented in Fig. 4. Sample A (control) reached the overall acceptability score of 3.25 by 98 days which was below the acceptable limit of 4. In the case of the other samples, the scores were above 5 even after this storage period. Hence, all the samples except control were organoleptically good. The scores of samples B to E ranged from 6.9 to 5.25 by 98 days and the overall acceptability was in the order sample B < C < D < E.



A: Control sample; B: Sample containing curry leaf C: Sample containing mint D: Sample containing turmeric; E: Sample as per standard recipe

Fig. 4. Freeze dried coated fish balls -changes in overall acceptability during storage under ambient conditions

From the five sensory characteristics evaluated, only three characteristics *viz.*, texture, taste and odour provided useful information regarding the changes due to treatment and storage. Differences in texture were observed between the samples as a result of the slow and gradual uptake of moisture by the product during the storage. The increase in moisture content during the storage period was in the range of 2-7%. This was sufficient enough to make the product less crispy which was prominent in the case of sample A (control).

A noticeable difference could be observed between the control and the spice treated samples in the case of taste and odour characteristics during the storage period. The decline in overall acceptability of sample as a result of this difference in taste and odour was more prominent during 70-98 days. This corresponded well with the TBA values of sample A, during this period. Compared to other samples, samples D and E had greater overall acceptability. The added spices, ginger and garlic imparted a good flavour and odour to the product thereby masking any off-flavour. From the overall acceptability point of view, sample E was more acceptable throughout the storage study. Since the samples under study were deep fried ones, there was no marked difference in appearance and colour during the storage period.

The present study on the effect of spices on the quality and storage stability of freeze-dried coated fish balls establishes the protective effect of the antioxidants in the spices. The spice incorporated freeze-dried coated fish balls had a shelf life of more than 98 days when compared to control samples (70 days). Also, a combination of the spices was found to have an added advantage in terms of their synergistic effect against oxidation and in enhancing the taste. Even though the added spices delayed oxidation, their antioxidant activity reduced during storage.

The authors are thankful to the Director, Central Institute of Fisheries Technology, Cochin for according permission to publish the paper. The technical assistance rendered by Ms. K.B. Beena, Technical Officer, Fish Processing Division of the Institute is gratefully acknowledged.

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