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Effect of Iced Storage Duration and Treatments on the Frozen Storage Characteristics of Cuttlefish Fillets

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Effect of iced storage duration and treatments with 2% (w/v) salt + 0.2% (w/v) citric acid solution and with 0.01% (w/v) butylated hydroxy anisole (BHA) solution, each for 10 min was studied in frozen stored cuttlefish fillets. The salt + citric acid treatment was found to be superior in retaining the texture, physical appearance and overall quality of the frozen fillets. Yellow discolouration was observed on the control samples (4-d iced) after eight weeks of frozen storage. A Maillard type reaction is postulated as the possible cause of yellow discolouration in frozen stored cuttlefish fillets.

Key words: Frozen storage, cuttlefish fillets, dip treatment

Freezing and frozen storage prolong the shelf life of seafood by retarding enzymatic and microbial degradation (Selvaraj *et al.*, 1991). Several studies have been reported on the storage characteristics of iced and frozen stored squid and cuttlefish (Joseph *et al.*, 1985; Dhananjaya *et al.*, 1987; Joseph and Perigreen, 1988; Selvaraj *et al.*, 1991; Anon, 1994 a, b; Hisa *et al.*, 1999). The present investigation was undertaken to study the effect of immediate freezing, and the effect of iced storage for different periods and subsequent freezing on the quality of frozen-stored cuttlefish (*Sepia aculeate*-Orbigny) fillets. An attempt was also made to find out the effect of sodium chloride + citric acid dip treatment and the antioxidant BHA dip treatment on the frozen storage characteristics of the fillets.

Materials and Methods

Cuttlefish *Sepia aculeata* (140-150 g size) procured from the local fishing harbour was used in the study. Whole cuttlefish without any bruises or ink sac rupture was selected. The dressed mantles were divided into three lots. One lot was given a 10 min dip treatment in a solution containing 2% (w/v) sodium chloride + 0.2 % (w/v) citric acid. The second lot was given a 10 min dip treatment in 0.01% (w/v) BHA solution. Tween 80, an emulsifier was used for preparing the antioxidant solution. The third lot was kept as control without any chemical treatment. All the three lots were iced in 1:1 ratio in plastic boxes. A temperature of 2 $\pm 1^{\circ}$ C was maintained throughout the period of study. A portion of the iced samples was drawn on the zero day, second day and fourth day of iced storage and subjected to freezing. The mantles were rolled into tubes and frozen at – 40 °C in a blast freezer, which were then dipped in glaze water at around 0°C and sealed in 150 gauge polyethylene pouches and packed in 3-ply corrugated master carton. This was then stored at -18 $\pm 1^{\circ}$C. These samples were drawn after 1w, 4w and 8w of frozen storage and analysed for biochemical and sensory characteristics.

Quality characteristics like alpha amino nitrogen (Pope and Stevens, 1939), non-protein nitrogen (NPN) (AOAC, 1975), total volatile base nitrogen (TVBN) and trimethylamine content (TMA) (Conway, 1947), free fatty acid (AOAC, 1998), peroxide value (Connell, 1975) and thiobarbituric acid reactive substances (TBARS) (Buege and Aust, 1978) were determined. The protease

activity (Heriott, 1955) of mantle tissue at different pH values was also determined on the zero day. The sensory evaluation of the raw and the cooked samples was also carried out. The cooked samples were prepared by boiling the mantles in 2% brine for 10 min (Joseph *et al.*, 1985). The organoleptic evaluation of these samples was carried out by a six- member panel. The overall quality of meat was rated on a five point hedonic scale (Selvaraj *et al.*, 1991) from 5 to 1; samples receiving a score upto 2 were considered acceptable. ANOVA (Snedcor and Cochran, 1967) was done with respect to frozen storage period and treatments using Factorial CRD (completely randomised design) for biochemical characteristics. The Quade test (Rangaswamy, 1995) was done for statistical analysis of the organoleptic quality and randomised block design (RBD) for proteolytic activity.

Results and Discussion

The results of biochemical evaluation of zero day, second day and fourth day frozen cuttlefish fillets are given in Tables 1, 2, 3 & 4. Among the zero day, second day and fourth day frozen samples, the salt + citric acid treated samples gave the highest alpha amino nitrogen content even after 8 w of frozen storage compared to the control and the BHA treated sample. This sample also showed less decrease in NPN content than the control and BHA treated sample. This can be attributed to the increased water holding capacity of the muscle and less leaching of NPN fractions in salt +citric acid treated sample. All the fourth day frozen samples, control and treated, showed the lowest alpha amino nitrogen content during the period of storage. This can be due to the longer storage of the fillets in direct contact with ice as reported earlier by Raghunath (1984) and Joseph *et al.* (1985).

The TVBN and TMA content was found to be increasing steadily in the control and the treated samples (Table 2). The TVBN content was within the limit even for the 4-d iced and frozen samples stored for 8 w. But the control sample was found not

Table 1. Changes in NPN and α-amino nitrogen during frozen storage at -18 ± 1°C

Days in ice	Parameter	Sample	Frozen storage period in weeks		
			1	4	8
0	NPN	Control	0.569 ± 0.015	0.453 ± 0.08	0.310 ± 0.004
	(%)	Salt + citric acid	0.577 ± 0.011	0.457 ± 0.015	0.427 ± 0.001
		BHA	0.567 ± 0.014	0.350 ± 0.008	0.403 ± 0.008
2	NPN	Control	0.391 ± 0.001	0.273 ± 0.011	0.164 ± 0.005
	(%)	Salt + citric acid	0.434 ± 0.004	0.273 ± 0.008	0.192 ± 0.003
		BHA	0.448 ± 0.001	0.235 ± 0.009	0.191 ± 0.004
4	NPN	Control	0.196 ± 0.002	0.132 ± 0.003	0.094 ± 0.005
	(%)	Salt + citric acid	0.222 ± 0.007	0.181 ± 0.007	0.074 ± 0.003
		BHA	0.198 ± 0.001	0.176 ± 0.009	0.0118 ± 0.001
0	α-amino nitrogen	Control	247.518 ± 2.358	240.374 ± 1.475	228.93± 3.435
	(mg/100g)	Salt + citric acid	254.627 ± 2.844	246.89 ± 1.04	236.26 ± 1.104
		BHA	254.369 ± 0.176	246.428 ± 0.873	231.408 ± 0.911
2	α-amino nitrogen	Control	164.384 ± 0.425	109.729 ± 0.333	83.627 ± 0.079
	(mg/100g)	Salt + citric acid	191.022 ± 0.965	163.643 ± 0.821	102.611 ± 2.143
		BHA	179.80 ± 0.16	151.372 ± 2.217	91.865 ± 2.391
4	α-amino nitrogen	Control	79.704 ± 3.623	49.460 ± 0.708	19.487 ± 2.748
	(mg/100g)	Salt + citric acid	127.54 ± 1.204	99.106 ± 0.823	52.494 ± 2.82
		BHA	108.679 ± 3.468	82.37 ± 0.51	38.635 ± 0.057

All values are given as average ± SD of three determinations

Table 2. Changes in TVBN and TMAN during frozen storage at –18 ± 1°C

Days in ice	Parameter	Sample	Frozen storage period in weeks		
			1	4	8
0	TVBN (mg/100g)	Control	4.812 ± 0.045	5.464 ± 0.033	8.273 ± 0.024
		Salt + citric acid	4.151 ± 0.046	5.14 ± 0.32	7.832 ± 0.365
		BHA	4.821 ± 0.02	5.538 ± 0.043	8.264 ± 0.032
2	TVBN (mg/100g)	Control	6.164 ± 0.016	6.857 ± 0.021	10.450 ± 0.012
		Salt + citric acid	5.537 ± 0.052	6.475 ± 0.309	9.707 ± 0.059
		BHA	5.787 ± 0.336	7.225 ± 0.369	9.678 ± 0.03
4	TVBN (mg/100g)	Control	10.642 ± 0.226	12.365 ± 0.177	13.574 ± 0.322
		Salt + citric acid	9.017 ± 0.77	11.699 ± 0.097	12.85 ± 0.405
		BHA	9.374 ± 0.379	12.355 ± 0.077	13.108 ± 0.019
0	TMAN (mg/100g)	Control	2.75 ± 0.026	3.415 ± 0.021	4.136 ± 0.012
		Salt + citric acid	2.075 ± 0.023	2.414 ± 0.368	3.404 ± 0.010
		BHA	2.073 ± 0.001	2.768 ± 0.022	4.132 ± 0.016
2	TMAN (mg/100g)	Control	3.757 ± 0.342	4.798 ± 0.671	7.314 ± 0.339
		Salt + citric acid	3.46 ± 0.032	4.772 ± 0.024	5.896 ± 0.383
		BHA	3.407 ± 0.002	4.816 ± 0.017	6.221 ± 0.019
4	TMAN (mg/100g)	Control	5.494 ± 0.06	7.207 ± 0.241	9.050 ± 0.017
		Salt + citric acid	4.158 ± 0.039	6.535 ± 0.29	8.288 ± 0.009
		BHA	4.427 ± 0.416	6.863 ± 0.043	8.968 ± 0.013

All values are given as average ± SD of three determinations

acceptable organoleptically with regard to texture, flavour and appearance. The low value of TVBN in the control even when it was not acceptable may be attributed to its loss by leaching in the thaw drip (Joseph & Perigreen,1988). The statistical analysis also showed the control to be significantly different from the two treatments.

There is an increase in free fatty acids (Table 3) implicating lipolysis during frozen storage of control and treated samples of cuttlefish fillets. This increase is less pronounced in salt + citric acid treated sample showing probably the inhibition of phospholipases either by salt or citric acid or by both. Free fatty acids are formed in fish tissue primarily from phospholipids by the action of phospholipases (Bligh, 1961).

PV increased gradually in all the three samples (Table 3). The BHA treated samples gave the lowest value of PV throughout the storage period. In the fourth day frozen sample, the rise in PV was slow, apparently due to the progressive break down of peroxides taking place whereby, the PV showed not much increase. BHA treated sample gave the lowest mean value showing its antioxidant property. Control gave the highest values for TBARS. The lower values of TBARS in salt + citric acid treated sample compared to the control can be attributed to the antioxidant property of citric acid through its metal chelating property.

The results of sensory evaluation are given in figures 1, 2 and 3. The control and treated samples frozen on the zero day icing were found to remain in acceptable condition even after 8 w of storage. In the case of the second day frozen fillets, the salt + citric acid treated sample remained in acceptable condition with respect to the appearance, texture and flavour even after 8 w of storage. On the other hand, the BHA treated sample and the control were found to remain in fair and acceptable condition only upto 4 w of storage. The change noticed in the BHA treated and in the control samples after 8 w

Table 3. Changes in fat during frozen storage at −18 ± 1°C

Days in ice	Parameter	Sample	Frozen storage period in weeks		
			1	4	8
0	FFA (as %oleic acid)	Control	19.116 ± 2.073	26.782 ± 1.218	30.672 ± 0.127
		Salt + citric acid	18.816 ± 0.15	22.555 ± 0.778	31.305 ± 0.194
		BHA	13.248 ± 0.477	15.70 ± 0.30	21.266 ± 0.733
2	FFA (as %oleic acid)	Control	29.614 ± 0.538	42.79 ± 0.28	45.309 ± 0.509
		Salt + citric acid	27.30 ± 0.7	35.70 ± 0.7	38.69 ± 0.509
		BHA	17.242 ± 0.442	23.046 ± 0.646	25.923 ± 0.077
4	FFA (as %oleic acid)	Control	37.755 ± 0.425	29.40 ± 1.4	42.775 ± 0.775
		Salt + citric acid	34.524 ± 0.063	19.133 ± 0.467	22.654 ± 0.254
		BHA	24.384 ± 0.384	16.521 ± 0.521	19.514 ± 0.848
0	PV (milliequivalent/ kg fat)	Control	9.347 ± 0.652	10.717 ± 0.283	13.363 ± 0.636
		Salt + citric acid	6.558 ± 0.108	8.332 ± 0.556	12.916 ± 0.416
		BHA	5.572 ± 0.309	6.333 ± 0.333	11.047 ± 0.381
2	PV (milliequivalent/ kg fat)	Control	11.922 ± 0.384	11.709 ± 0.598	11.454 ± 0.545
		Salt + citric acid	7.50 ± 0.5	10.00 ± 0.0	10.378 ± 1.288
		BHA	5.131 ± 0.131	7.128 ± 0.205	8.90 ± 0.329
4	PV (milliequivalent/ kg fat)	Control	12.666 ± 0.666	14.000 ± 0.0	15.277 ± 0.277
		Salt + citric acid	10.09 ± 0.679	11.555 ± 0.444	11.833 ± 0.167
		BHA	5.633 ± 0.249	6.335 ± 0.621	7.339 ± 0.067
0	TBARS (mg malonaldehyde/ kg meat)	Control	0.906 ±0.005	1.258 ± 0.006	1.816 ± 0.047
		Salt + citric acid	0.889 ± 0.002	0.924 ± 0.005	1.258 ± 0.006
		BHA	0.662 ± 0.004	0.697 ± 0.004	0.892 ± 0.002
2	TBARS (mg malonaldehyde/ kg meat)	Control	0.993 ± 0.004	1.428 ± 0.046	2.771 ± 0.078
		Salt + citric acid	0.864 ± 0.003	0.910 ± 0.002	1.006 ±0.003
		BHA	0.756 ± 0.002	0.789 ± 0.003	0.807 ± 0.004
4	TBARS (mg malonaldehyde/ kg meat)	Control	2.446 ± 0.004	2.815 ± 0.006	3.296 ± 0.031
		Salt + citric acid	1.69 ± 0.007	1.893 ± 0.012	2.519 ± 0.028
		BHA	1.565 ± 0.006	1.751 ± 0.10	2.336 ± 0.008

All values are given as average ± SD of three determinations

were, loss in firmness of texture, which became soft in raw condition and rubbery when cooked, loss of the sweet taste and fading of the white fillets to a dull white colour. The control samples also showed desiccation seen as white patches, after 8 w.

After the eighth week of storage, the control sample showed yellow discolouration on the surface of the mantle. The texture, flavour and appearance also reached the borderline of acceptability. Desiccation of the samples was also observed. Compared to the salt + citric acid treated sample, the BHA treated sample, after 8 w, gave a score below '3' for all the attributes even though, it did not reach the borderline of acceptability. Desiccation was also noticed in the BHA treated samples after 8 w storage. The salt + citric acid treated sample remained in fair and acceptable condition with respect to

Table 4. Protease activity of different regions of cuttlefish mantle at different pH values on zero day (expressed as µg Tyrosine liberated/ml/h).

Region of mantle pH	Anterior	Middle region	Lateral side	Posterior region
3	102.844	121.637	138.706	163.27
4	144.396	137.413	145.172	182.068
7	202.5	206.982	224.137	224.396
8	111.379	139.741	147.758	181.81

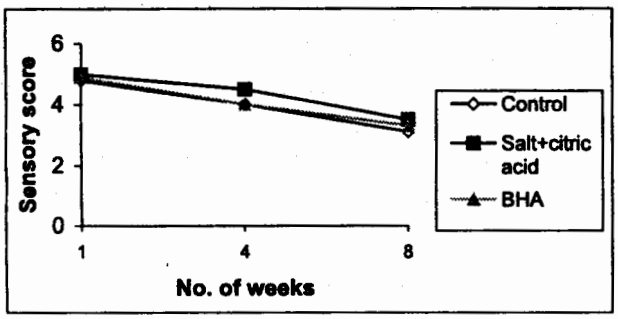

Fig. 1. Changes in overall organoleptic acceptability of zero day frozen raw cuttlefish fillets during storage.

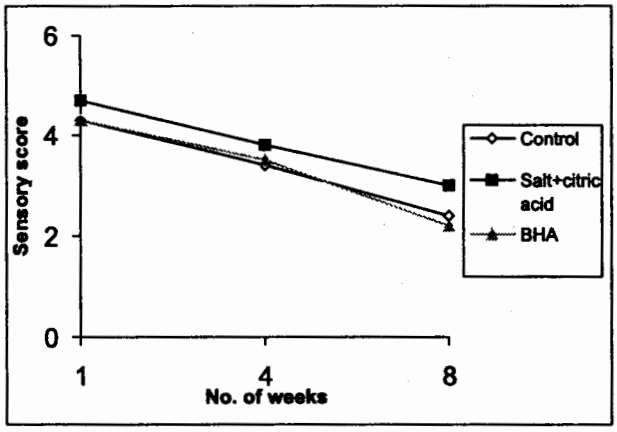

Fig. 2. Changes in overall organoleptic acceptability of second day frozen raw cuttlefish fillets during storage.

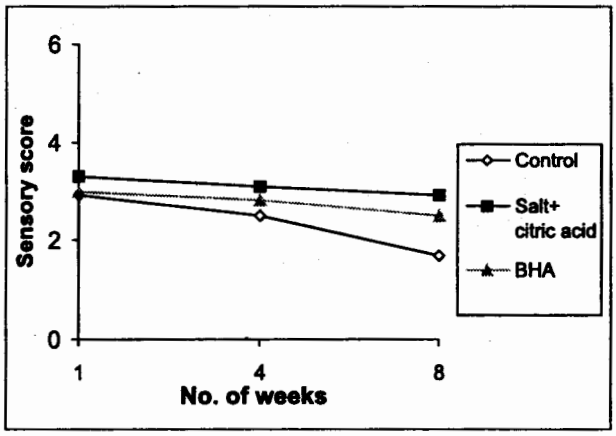

Fig. 3. Changes in overall organoleptic acceptability of fourth day frozen raw cuttlefish fillets during storage.

texture and colour even after 8 w. No desiccation was noticed in this sample. Statistical analysis also showed the salt + citric acid treated sample to be significantly different from the control with respect to flavour, texture, colour and appearance.

Hence the study shows that the salt + citric acid treatment is a better treatment than BHA treatment. The former treatment prevents protein degradation as well as lipid autoxidation, supporting the Olcott's hypothesis that the yellow discolouration is due to a Maillard or an aldehyde amine type reaction (Schultz, *et al.*, 1962). Surface desiccation during frozen storage may also play a pivotal role in the yellow discolouration reaction. So it may be postulated that a delay in the processing of cuttlefish leads to the formation of amino compounds by bacterial/endogenous proteolysis, and carbonyl compounds by lipid autoxidation. Surface desiccation occurring during frozen storage may accelerate a Maillard type reaction between the amino and carbonyl compounds leading to yellow discolouration.

The proteolytic activity of the cuttlefish fillets evaluated on the initial day was found to be maximum in the lateral and posterior region of the mantle as compared to the anterior and middle region (Table 4). The region where yellow discolouration was observed in the fourth day frozen untreated sample after eight weeks of storage was near the lateral side. Hence, a correlation between the region of appearance of yellow discolouration and region of maximum proteolytic activity cannot be ruled out.

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