CAUSE OF YELLOW DISCOLOURATION IN ICED AND FROZEN CUTTLEFISH FILLETS AND ITS CONTROL

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THESIS
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2001

To God

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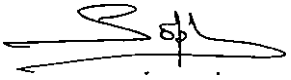

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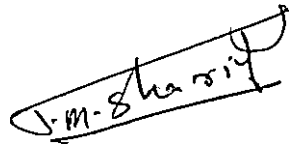

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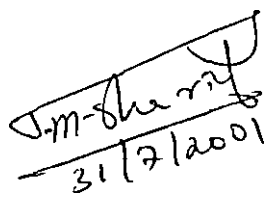

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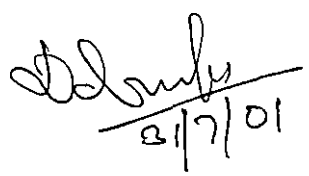

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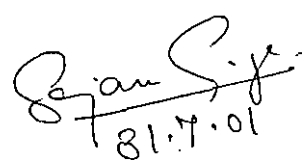

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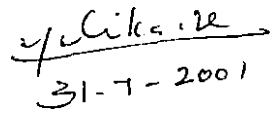

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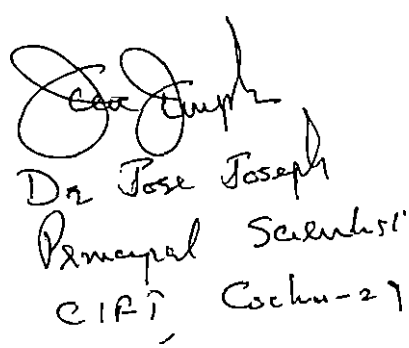

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Sophia Margaret Joseph

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INTRODUCTION

1. INTRODUCTION

Cephalopods are exclusively marine molluscs and there are about 660 species in the world oceans, which are diverse in form, size and nature (Voss, 1977; Worms, 1983). Of these, less than a hundred species are of commercial importance. In Indian seas there are about 80 species of cephalopods of commercial and scientific importance (Oommen, 1997; Sarvesan, 1974). Cuttlefishes, squids and octopus are the three major groups of cephalopods that belong to the highly evolved class of the Phylum Mollusca namely, Cephalopoda, animals with feet around head. Voss (1973) estimated the cephalopod potential from the continental shelf and slope as 8 to 12 million tonnes and opined that in oceanic waters the Oegospid squids' potential could be 8 to 60 times that of the neritic shelf resources.

In recent years, the cephalopods have gained great importance owing to the increasing demand, next to shrimp, in the export trade. Cephalopods were fished from the seas around India from very early times and at present, constitute one of the important exploited marine fishery resources of our country. The cephalopod landings of India were low, less than 1400 t until 1972, and have been gradually increasing only from 1973 onwards with the commencement of export of frozen cephalopod products to several countries. This was a transition, because, for long these have been discarded and then began to be considered a quality resource. The present production has crossed the 50,000 t mark. These are obtained as by-catch in good quantities in trawl fishing, and in addition, a small portion is taken in many types of indigenous gear. About three-fourth of the present catch is landed on the west coast, with the three maritime states- Kerala, Maharastra and Gujarat-

accounting for the lion's share. At present no special gear is exclusively used for the capture of cephalopods, except for a type of hand - jig (anchor hook), is employed for obtaining cuttlefish at Vizhinjam, the Japanese hand - jig for squid in the Palk Bay area and the spear for the octopus fishing in Minicoy.

Cephalopods are neritic, demersal organisms occurring from the coastline to about 110 m depth. But they are more abundant in the upper 40 m depth particularly during breeding season when they migrate shoreward and aggregate in shallow waters. The cuttlefish fishery in India is mainly constituted by two species, viz., *Sepia pharaonis* and *Sepia aculeata*. The less important species are *Sepia elliptica* and *Sepiella inermis*. *Loligo duvaucellii* is the single species that almost constitute the squid fishery of India.

The value of marine products exported from India has substantially increased from Rs.2.46 crores in 1950-'51 to Rs.6443.89 crores in 2000-'01. The beginning of the export of cuttlefish products, other than cuttlebone, was in 1973 with the shipment of 13.8 t of frozen cuttlefish worth Rs.0.2 million to Japan. Since then there was a marked increase in its exports. During April 2000 - Mar 2001, 33677 M.tons of cuttlefish worth Rs.288.99 crores have been exported. This accounted for 7.65%in terms of quantity and 4.48% in terms of value, of the total exports. The quantity of squid exported for the same period was, 37628 M.tons valued at Rs 324.43 crores, which accounted for 8.54%in terms of quantity and 5.03% in terms of value (Anon, 2001).

Almost the entire catch of Indian cephalopods is exported to the overseas market. Japan is the world's largest market and the largest cephalopod producing

nation. Three-fourth of Japan's demand is supplied by domestic catches. Japan was the top buyer of Indian frozen cuttlefish all through the years, but presently the member countries of the European Union, are the major exporters of the cephalopods accounting for, about 53% of the total exports from India in terms of value. Spain alone contributed to 25% (value wise) of the total exports of cephalopods (Shahin and Parameswaran, 2001). Cuttlefish exported to Japan is mainly in the form of fillets, whereas, whole cleaned cuttlefish is exported mainly to European countries such as Spain. Cuttlefish whole, cuttlefish tentacles, cuttlefish fins, cuttlefish roes, baby cuttlefish, etc, are the other varieties exported.

Cephalopod consumption depends to a large degree on traditional habits and consumer tastes. In India, cephalopods were considered as poor man's food for a long time and even today the stigma continues in some parts. In spite of the increased production and great demand as a commodity for export, their inclusion in our diet is still a far cry. This may be due to the conventional food habits and preference for fish and prawns to any other marine products.

On the other hand, consumer acceptability of any product depends upon the quality of the raw product and therefore, the quality of cephalopod raw material and finished products are to be ensured before they are processed and exported. In the case of frozen products, the external appearance, colour, texture, flavour, thickness of mantle and degree of freshness are very important. Quality requirements for export insists that, the raw material must be fresh at the time of freezing, skinned products should be white in colour, not smeared with ink or otherwise discoloured. Cuttlefish with a yellowish or pinkish colour are considered as not fresh. Natural

colour and absence of smell is important. Dressed cuttlefish should be elastic; fillets need to be very clean, very fresh, thick but tender. Appropriate glazing to prevent dehydration is to be done, and the packs should weigh slightly more when frozen, to allow for drip loss during thawing.

The squid and cuttlefish are noted for high yield and lack of bones. Loss of flavour and nutrients as well as hardening of the meat, occur easily unless processing methods are geared to the natural properties of these material. Handling methods, holding time and temperature prior to freezing influence quickly the quality of these fragile raw materials. The change of skin colour within a short period of time after capture is a particular feature of cephalopods. During cold storage also, one problem specific to cuttlefish and squid is discoloration of skin and meat.

Yellow discolouration in cuttlefish fillets is one of the main quality problems encountered in frozen products. No study has been carried out so far in understanding the cause of this problem. The present study is an attempt to find out the effect of immediate freezing and the effect of iced storage and subsequent freezing on the quality of the material and appearance of yellow discolouration. An attempt is also made to find out the effect of, sodium chloride + citric acid dip treatment and the antioxidant, butylated hydroxy anisole, dip treatment on iced and frozen storage characteristics of cuttlefish fillets of *Sepia aculeata.* The result of such a study may enable us to find out the possible cause of yellow discolouration in cuttlefish and suggest a remedial measure for the problem. This in turn will be of valuable help to the industry, since, with the increased production, improved

handling and processing techniques, under strict quality control measures, India has bright prospects in the overseas trade in cephalopod products.

The investigations carried out include: -

1. Effect of treatments: (a) sodium chloride + citric acid, (b) antioxidant BHA (butylated hydroxy anisole), on iced storage characteristics of cuttlefish fillets.

2. Effect of iced storage on the raw material characteristics.

3. Influence of iced storage duration and treatments on the characteristics of the frozen stored material.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. Chemical composition

Investigations of the proximate composition of the meat provide the basic data on the chemical properties of the raw material (Kreuzer, 1984). Japanese scientists, Dr. Juichiro, J. Matsumoto and their colleagues, did fundamental research on chemical components of cephalopods, especially proteins, while E. Tanikawa and his co-workers mainly carried out applied research.

2.1.1. Moisture

Endo *et. al.*, (1962) observed that moisture content in cuttlefish *Sepia esculenta* and squid, *Ommastrephes sloani pacificus,* ranged from 75.8% to 79.6% and 79.0% to 80.0%, respectively. The moisture content of cuttlefish, *Sepia orientalis,* was found to be 80.12 g/100g fresh muscle and of squid, *Loligo vulgaris,* 79.13 g/100g wet weight (Pandit and Magar, 1972). The cuttlefish, *Sepiella inermis,* was reported to have moisture content of 74.7% and squid, *Loligo indica*, 75.05% (Suryanarayanan *et al.*, 1973).

Joseph *et al.*, (1977) reported an approximate moisture content of 83% in squid (*Loligo spp.*). Cuttlefishes, *Sepia pharaonis* and *Sepia esculenta,* were found to have a moisture content of 76.4% and 81.5%, respectively (Suyama and Kobayashi, 1980). They also reported the moisture content of the squids, *Todarodes pacificus,* 76.6% - 78%, *Ommastrephes batrami,* 74.8% - 77.7%, *Nototodarus sloani gouldi,* 78.4%, *N. sloani sloani,* 77.1%, *Illex argentinus,* 78.8% and *Loligo opalescens,* 77%. Borderias, (1982) reported 80.94% moisture content in the mantle

of *Loligo spp.* and 80.99% in the tentacles. Raghunath (1984) has reported a moisture content of 78.33% in the squid, *Loligo duvaucellii.* The cuttlefish, *Sepia aculeate,* was observed to have a moisture content of 76.85% (Joseph and Perigreen, 1988). The moisture contents in *Loligo pealie* and *Illex illecebrosus* sampled during a period of two years were found to range from 81.4% to 84.1% and 78.4% to 86.1%, respectively, (Krzynowek *et al.,* 1989). Selvaraj *et al.,* (1991) reported 82.9% moisture content in squid, *Loligo duvaucelli.*

2.1.2. Protein

Early studies on composition of cephalopod muscle proteins showed that, the main classes of proteins, as well as, composition and content of amino acids, were comparable with those of fish and mammals. Those studies however, gave evidence of the existence of some differences in the properties of squid proteins and that of the muscles of vertebrates (Takagi, 1950; Yoshimura and Kubo, 1952; Okada and Tada, 1954).

The crude protein content on dry weight basis was found to be 80.21% and 81.5%, respectively in, *Sepia orientalis* and *Loligo vulgaris* (Pandit and Magar, 1972). *Sepiella inermis* and *Loligo indica* were reported to have 81.49% and 83.49% protein content on dry weight basis. Sastry and Srikar (1985) reported 3.13g total nitrogen content/100g in *Sepia aculeata.* The true protein content was found to be 15.8%, water soluble protein content to be, 19.04% and salt soluble protein content to be, 80.36%. A total nitrogen content of 3.45%, salt soluble nitrogen content of 85.1% and non protein nitrogen content of 24.61% (of total nitrogen) were observed by Joseph and Perigreen (1988) in *Sepia aculeata.*

Migita and Matsumota (1954) found 55.5% of water extractable protein in squid compared to 27.5% in carps and 22.3% in horse mackerel. It was also observed that, the amount of water soluble proteins of squid muscles increased with the increase of the ratio of extracting water to the weight of muscle, while those of flat fish and horse mackerel remained constant with the increasing water/muscle ratio. It was suggested that myosin and actomyosin might be present in the water extracts of the squid muscle. The actomyosin showed functional characteristics as a contractile protein of the muscle. In some properties, however, it deviates from actomyosin of rabbit and carp, and predominant of this is that, the actomyosin is readily extracted from the fresh muscle with water. Hence, the author suggested to give the actomyosin the name, "M-actomyosin" since, this property was also known from muscles of other molluscs (Matsumoto, 1957 and 1957 a).

The actomyosin from the squid, *Todarodes pacificus*, was found to have considerably different properties compared to those of rabbit and carp actomyosin. It showed distinct properties such as solubility in water, lability in dissolved state, different value of electrophoretic mobility, different electrophoretic behaviour, etc. M-actomyosin occupies more than 60% of the total proteins of the squid muscle and is considered to be the dominant contractile protein of the squid muscle (Matsumoto, 1959).

Kimura *et al.*, (1969) studied the collagen from, *Todarodes pacificus* and *Octopus vulgaris* together with collagen from other invertebrates. Its shrinkage temperature was found to be 49 ^{0}C for both squid and octopus. The thermal stability of the collagen of cephalopods was found to correspond to that of warm water fishes.

It was rich in hydroxylysine, acid amino acids, amide nitrogen and carbohydrates. When compared with vertebrate collagen, 3- hydroxy proline and 4- hydroxy proline was found to be present in squid collagen.

The collagen of *T. Pacificus* and *Octopus vulgaris* had higher sugar content than vertebrate collagen. The total sugar in the collagen was found to be 3.96% in squid and 2.89% in octopus. This was lower than that of abalone collagen (4.18%) and blue crab (12.45%). Glucose and galactose were found to be the main sugar components in the collagen (Kimura, 1972).

In squid (*Loligo spp.*) an approximate protein content of 15-16% was reported by Joseph *et al.*, (1977). Tsuchiya *et al.*, (1977) found the properties of actin from squid to be similar to that from vertebrate muscles, except for its behaviour on extraction. Tsuchiya *et al.*, (1978) studied the structure of squid myosin. It was found to have a fragile structure, less resistant to trypsin, and was more quickly digested than fish myosin. Thus, it was thought that the fragile structure of the squid myosin might be in accordance with the short life cycle of this squid, *Todarodes pacificus*, which is one year. Taneka (1982) found that the presence of actin or sucrose during heat treatment (35^0C) effectively prevented thermal denaturation of myosin.

In the squid, *Loligo duvaucellii*, Selvaraj *et al.*, (1991) reported total nitrogen content of 2.8% and salt soluble nitrogen of 0.6%. The squid, *Loligo duvaucellii,* was reported to have 3.11% total nitrogen, 1.41% water soluble nitrogen and 0.72% non protein nitrogen (Raghunath, 1984). A seasonal variation in protein content was observed by Krzynowek *et al.*, (1989), in *Loligo pealei* and *Illex illecebrosus.*

2.1.3. Lipids.

The lipids in the body and in the diet constitute a concentrated form of energy for metabolism and storage purposes and may also serve important non-caloric metabolic functions (George and Berger, 1966).

A study by Jangaard and Ackman (1965), showed that the fatty acid composition of the mature squid (*Illex illecebrosus*) resembled mainly that of the fish preyed upon. The lipid of the flesh was found to consist mainly of phospholipids, and those of the lecithin and cephaline types resembled those found in teleostean fish in fatty acid composition.

Pandit and Magar (1972) reported a lipid content of 3.9% for *Sepia orientalis* and 4% for *Loligo vulgaris* on dry weight basis. The cephalopods, *Sepiella inermis* and *Loligo indica,* were reported to have a lipid content of 5.56% and 5.4%, respectively, on dry weight basis (Suryanarayanan *et al.*, 1973). An approximate fat content of less than 1% was found in squid (*Loligo spp.*) by Joseph *et al.*, (1977). Nash *et al.*, (1978) analysed the lipid content of *Illex illecebrosus*. He found the total lipid content to be 1.5% in mantle, and 97% of this was found to be phospholipids and 4% to be cholesterol. Triglycerides, free fatty acids and sterol esters were detected only in traces, but liver contained triglycerides as the major component.

The lipid content in the skin free mantle of *Ommastrephes bartrami*, *Todarodes pacificus*, *Nototodarus sloani sloani* and *Illex illecebrosus* were estimated to be 0.2%, 0.3%, 0.1% and 0.1%, respectively, by Hayashi and Takagi, (1979). They also studied the changes in lipid content and fatty acid composition of minced squid meat during processing into dried seasoned products. They found that, the

content of polyenoic acids decreased during processing. It was suggested that, the compounded carbonyl compounds, produced by joint action of light, oxygen and heat during processing might be one factor responsible for browning reaction appearing during storage of dried, seasoned squid products.

Joseph and Perigreen, (1988) observed a fat content of 0.83% in *Sepia aculeate*, on wet weight basis. Krzynowek *et al.*, (1989) studied the seasonal variation in body composition of *Loligo pealei* and *Illex illecebrosus*. Fat contents ranging from 1.07% to 1.84% and 1% to 1.85% on wet weight basis were reported in the two species respectively, over a period of two years.

2.1.4. Minerals

The ash content of a particular animal indicates the amount of inorganic constituents present in the tissue. The trace element, copper (Cu), is essential for the normal functioning of man's metabolism where it is needed for the fixation of iron into haemoglobin. Hence, in diets insufficient in iron, (Fe), the deficiency of Cu may cause anaemia. Copper from seafood is completely absorbed and fully utilized by the human beings. Zinc (Zn) activates insulin and is a cofactor for various enzymes. According to Kuhnau, (1962) the consumption of trace elements rich-marine invertebrates is of particular value in every state of malnutrition especially when there is a lack of animal protein and an excess of starchy food, which are deficient in trace element.

Most trace elements are however, found to be high in the viscera than in the muscle of marine invertebrates. The inorganic substances in the viscera of *Todarodes pacificus* were determined by Takahashi (1959). It was found that, the contents of

Zn, Mn, and Cu were higher in the viscera of the squid than in fish meat and molluscan meat. Their content in viscera was 29.4 mg percentage, 11.7 mg percentage and 33.8 mg percentage respectively, on fresh weight basis. Saito *et al.*, (1960) reported that the edible parts of cephalopods contain all essential minerals and are a fairly good resource for some of them, for example, phosphorus. Magnesium and calcium content were found to be higher in mantle muscle and tentacles of squid, *Todarodes pacificus*. It was found to be 38.1 mg% and 17.6 mg% in mantle and 34.1 mg% and 22.9 mg% in tentacles, respectively (Taguchi *et al.*, 1969). Pandit and Magar (1972) reported 292 mg% phosphorus in *Sepia orientalis* and 271 mg% in *Loligo vulgaris*. The ash content in the two cephalopods was 8.41 g/100g and 7.4 g/100g, respectively, on dry weight basis. The iron content was 0.057 g/100g and 0.074 g/100g, calcium 0.66 g/100g and 0.83 g/100g, sodium 1.87 g/100g and 1.49 g/100g, potassium 2.56 g/100g and 2.02 g/100g, respectively, in the two cephalopods, on dry weight basis.

Suryanarayanan *et al.*, (1973) reported an ash content of 13.42% in *Sepiella inermis* and 12.5% in *Loligo indica* on dry weight basis. The Iron content was 12.88 mg% and 16.64 mg%, phosphorus 138 mg% and 145 mg%, respectively in the two species. Considine and Considine, (1982) reported 173 mg and 119 mg phosphorus, 29 mg and 12 mg calcium, in 100 g meat of raw octopus and raw squid, respectively, and 0.5 mg iron per 100 gm meat was reported in raw squid.

Joseph and Perigreen (1988) reported 4.53% ash content on wet weight basis in cuttlefish, *Sepia aculeata*. In *Loligo pealei* and *Illex illecebrosus* the ash content

was found to range from 0.8% to 2% and 0.3% to 2% respectively over a period of 2 years (Krzynowek *et al.*, 1989).

2.1.5. Heavy metals

High concentrations of heavy metals were found in the hepatopancreas of Mediterranean *Octopus vulgaris* caught off the coast of Monaco. Between 32% and 98% of the total content of Cd, Cu, Fe and Zn were found in the hepatopancreas (Miramund and Guary, 1980). A cadmium content of 0.32 mg/kg and 1 mg lead /kg meat, on wet weight basis was found in the mantle muscle of squid, *Loligo patagonica,* by Falandsysz (1989). In whole squid the corresponding values were 3.2mg/kg and 0.59 mg/kg on wet weight basis.

Heavy metal contents of frozen stored Californian squid *Loligo opalescens* was analysed by Oehlenschlaeger (1991). Maximum values recorded for the squid tube, viscera and whole squid, respectively were (nanogram /gram wet weight): cadmium, 570, 760 and 680; lead, 30, < 0.5 and < 0.51; copper, 26000, 37000 and 31000 and zinc, 11000, 18400 and 14800. In *Sepia officianalis* caught from the French coast of English Channel was reported to show heavy metal (Cd, Ag, Co, Cu, Fe, Pb and Zn) accumulation mainly in the digestive gland, branchial hearts and in kidney (Miramund and Bentely, 1992). A cadmium content of 1.27 ± 0.07 mg/kg wet weight, was reported in cuttlefish and in octopus the concentration was 0.17 ± 0.02 mg/kg wet weight. It was also observed that careful washing reduced Cd content to a high degree and that in most samples examined cadmium was accumulated mainly in the viscera.

2.2. Nutritive Value

2.2.1. Calories

Suryanarayanan *et al.*, (1973) reported a food value of *Sepiella inermis* and *Loligo indica* as 387.99 calories / 100g and 395.03 calories/100 g respectively, on dry weight basis.

2.2.2. Amino acids

The essential amino acids determine primarily the nutritive value (Kreuzer, 1984) of a protein. Cephalopods are found to be excellent sources of protein. The edible portions (constituting, 68.5% and 71.38%, respectively) of *Sepiella inermis* and *Loligo indica* were found to contain most of the essential amino acids (Suryanarayanan *et al.*, 1973).

Certain free amino acids and some water soluble compounds are responsible for the characteristic flavour and taste of the cephalopod meat. Also, these substances are used by the invading bacteria, with the result that ammonia and putritive smell appear and consequently the quality deteriorates. Proline was reported to occur in concentrations sufficiently high to contribute to sweetness in cuttlefish (Amano and Bito, 1951). The characteristic strong odour of squid during boiling was attributed to be due to a sulphur containing amine with piperidine nucleus, which gets easily decomposed when heated with acid or weak alkali (Yaminishi and Matsuzaka, 1955).

Ito, (1957) found the muscle extracts of cephalopods to contain proline, arginine, alnine, serine and glycine as the most prominent amino acids. The muscle

extracts of *Octopus ochellatus,* was found to have all amino acids in very small quantities. Endo *et al.*, (1962) grouped *Loligo chinensis*, *Loligo kensaki*, *Sepioteuthis lessoniana* and *Sepia esculenta* as having superior taste. These were found rich in free amino acids with glycine, proline, and alanine in greatest quantities. A second group including *Todarodes pacificus* and *Thysanoteuthis rhombus* was found to have trimethylamine oxide as the dominant component, with lesser amount of free amino acids, except arginine. Investigations by Endo and Simidu (1963) showed that the muscle extracts of *Todarodes pacificus,* contained smaller amounts of free arginine and higher amounts of octopine than in *Sepioteuthis lessoniana.* During storage of *S. lessoniana* at 5 ^{0}C - 7 ^{0}C, free arginine rapidly decreased while octopine increased in the muscle. This change was explained by the rapid conversion of arginine to octopine in the muscle during storage.

2.2.3. Fatty acids

The most important fatty acids from the point of view of nutrition are the polyunsaturated fatty acids. Using, the means of the percent of the total fatty acids, the ratios of EPA/DHA were reported to be 15.8%/37.1% (Jangaard and Ackman, 1965), for *Illex illecebrosus.* Nair and Gopakumar (1977) reported the ratios of EPA/DHA for *Loligo spp.*, as 11.5%/27.2% of total fatty acids. Exler and Weihrauch (1977) estimated the content of EPA and DHA in 100 g meat to be 0.21 g, and 0.49 g, on wet weight basis, in *Illex illecebrosus.* Gibson (1983) reported the ratios of EPA/DHA for *Loligo spp.* caught off Queens land as 10.3%/31.0% and off Malaysia as 8.3%/31.2%. Gibson *et al.*, (1984) later reported the ratios of EPA/DHA to be 7.9%/32.2% in Malaysian squid, *Loligo spp.*

Krzynowek *et al.*, (1989) studied the cholesterol content and concentrations of eicosopentaenoic acid (EPA) and docosahexaenoic acid (DHA) in two species of squid, *Loligo pealei* and *Illex illecebrosus* over a period of two years, in order to provide nutritional data for dietary planning. The cholesterol content was found to range from 171 mg% to 450 mg% in Loligo spp. and 108 mg% to 336 mg% in Illex on net weight basis. EPA and DHA accounted for almost 50% of the total fatty acids and 99% of the total polyunsaturates. But, since they have low fat content (1 to 2%), they contribute only about 0.5 g EPA plus DHA for every 100 g consumed. Using the means of the percentage of the total fatty acids, the ratios of EPA/DHA were found to be 15.7%/31.6% and 15.1%/37.5% for *Loligo* and *Illex,* respectively. This showed that the EPA content was about half the amount of DHA. The range of EPA and DHA contents were determined by Krzynowek in this study to be 0.09 g/100 g - 0.23 g/100 g and 0.14g/100g - 0.5 g/100 g, respectively, on wet weight basis

2.2.4. Vitamins

Miyake and Hayashi (1961) found that the vitamin B_6 content in squid and cuttlefish flesh was low compared to that of finfish. The cuttlefish, *Sepia esculenta,* and the squid, *Sepisteuthis lessoniana,* were found to have a vitamin B_6 content of 0.7 µg/g and 0.5 µg/g, respectively, on dry weight basis. The squid, *Ommastrephes sloani pacificus,* had a higher value of 4.5 µg/g in mantle compared to tentacles (0.9 µg/g). Braekkan (1962) reported octopus as a fairly good source of vitamin B_6 its content in *O. bimaculatus* was found to be 3.6 µg/g, on dry weight basis.

2.2.5. Biological value of protein

The nutritive value of cephalopods and other marine animals, was investigated in rats (Kreuzer, 1984) . *Octopus vulgaris* had a biological value of 83.5 ± 0.4, squid, 81.7 ± 0.6 and shrimp, 74 ± 1.6. This high BV was comparable with that of fish. Lagunov *et al.*, (1979) recommended squid meat in infant feeding because of its nutritive value and its easy digestibility.

2.3. Discolouration

The ability to adapt the colour of the skin to the colour of the environment is vital to many of the soft-bodied cephalopods, especially those living on the seabed. This is accomplished by the presence of chromatophores in the skin. Cuttlefish and squid have upto three differently coloured pigments in store - black, yellow and red. These pigments are arranged in different layers of the skin. After death, the muscles attached to the chromatophores are no longer controlled by the nervous system. The chromatophores remain expanded and the muscles relax slowly. The process seems to be concluded with the onset of rigor mortis. This, sometimes, creates serious quality problems, in handling, freezing, cold storage and drying (Kreuzer, 1984).

For IQF squid tubes and cuttlefish fillets a white appearance is important. To enhance whiteness, cleaned squid tubes are placed in a bath of iced water containing a teaspoon of citric acid and 3-4 kg common salt/100 litres of water and agitated regularly (ADB/Infofish, 1985).

2.3.1. Black and red discolouration

Discolouration in cephalopods can be for different reasons. Black discolouration in cold stored meat or during thawing, is caused by the pigment flowing out of broken chromatophores. A red discolouration appears when the released pigment makes contact with basic substances; for example, ammonia or other similar substances produced during protein degradation. Tanikawa (1971) mentions that the pigment of the skin of *Todarodes pacificus* is dark brown during rigor mortis.

Ohmori *et al.*, (1975) studied the effect of minerals present in sea water on colour retention in the skin of the squid held in refrigerated sea water (RSW). The best colour retention was achieved by holding the squid in a solution containing 3% NaCl, 0.5% $CaCl_2$ and 0.5% KCl. This may be achieved by the addition of $CaCl_2$ to the RSW. Magnesium ions affected adversely the skin colour retention.

Studies carried out in India have shown that the colour, flavour and texture of squid begin to deteriorate after one day in ice, and after five days in ice, the squid were rated as "very poor". Joseph *et al.*, (1977) concluded that the squid could be kept in ice in prime condition for a maximum period of two days. Squid stored in chilled sea water (CSW) using a squid to ice to seawater ratio of 3:1:1 showed discolouration within 3-4 days (Learson and Ampola, 1977). According to Stroud (1978) ungutted *Loligo forbesi* keep in ice in first class condition upto 8 days, after which the flesh begins to redden, musty odours develop, and finally become inedible in 13-14 days.

According to Botta *et al.*, (1979) freshwater ice did not preserve the reddish brown skin colour very well. Stored for one day in boxes, the skin colour was rated only "fair" and on the third day it was borderline. In refrigerated sea water on one day storage, the colour was "very good" and "fair" on the third day. The sensory quality of subsequently cooked squid mantle of *Illex illecebrosus*, kept quite well. Acceptability ratings indicated a storage life of approximately 8.5 days in freshwater ice and 11 days in refrigerated seawater.

Ke and co-workers iced squid in polyethylene boxes (squid to ice ratio 2:1) and found that the squid lost colour in less than 12 hours and became inedible after 1.5 days (Ke *et al.*, 1979). The loss of skin colour by melting ice was avoided by separating the squid from the ice by a 2mm thick plastic film. It was also found that the squid iced in insulated or non-insulated boxes, without contact with the ice, could be held for at least two days with excellent quality and skin colour, and remained acceptable for more than 3 days. Addition of seawater or brine to each layer of squid was found to give better results. The advantages found were, that the brine evens out the storage temperature and increases the ionic strength, thereby, inhibiting the colour changes. This modified method of non-contact icing resulted in squid that remained for 4 days in excellent condition and 5 days in acceptable condition.

According to Botta *et al.*, (1979), *Illex illecebrosus* caught in the Newfoundland fishery would have to be frozen within 5-6 hr of capture in order to preserve the reddish brown colour of skin that would be accepted by the Japanese. Prolonged immersion in CSW may lead to undesirable changes in skin colour, changes in salt composition of the flesh and leaching out of soluble components,

including protein (Ampola, 1980). He also observed that, squid *Loligo pealei,* frozen at sea immediately after capture, had vivid reddish - purple pigmentation while the colour of the raw squid, which were iced, or chilled before they were frozen, was purplish-grey. The skin chromatophores of the immediately frozen squid were sharply defined and slightly larger than normal. The vivid skin colour remained during frozen storage and even after thawing. This resulted in higher quality scores for appearance for squid, frozen within a short time after capture, compared with those, that were iced or chilled for 1-2 days, and then frozen.

Juanico (1982) states that, it had been observed on Argentinian freezer trawler and factory ships that squid (*Illex argentinus*) had good colour and elastic skin upto 9 h after capture. Between 9 h and 12 h after capture, squid had a whitish colour and an elastic skin, but after 12 h, the squid became rigid. The skin changed to a wine colour and became loose. Fresh natural skin colour is a sign for squid frozen within a short period of time.

Borderias (1982) suggests skinning the squid before freezing. Melanosis or black discolouration and reddening of the skin are also noticed in squid due to failures during handling or due to protein denaturation. Black discolouration in frozen blocks is often observed, mostly appearing during thawing. The cause is that chromatophore cells have been destroyed during freezing or during cold storage due to temperature fluctuation, and when the surface melts the pigment flows out of the broken cells. Black discolouration of whole cuttlefish block is normally caused by broken ink sacs. The ink can be washed off if still fresh. The rate of colour decline

in whole squid was dependant on the method of storage while; the decline in quality was not (Hincks and Stanley, 1985).

2.3.2. Pink/yellow discolouration

Pink or yellow discolouration takes place in squid and cuttlefish due to processing delays (Badonia *et al.*, 1988). Immediate icing, gutting, removal of ink sac, proper handling through washing and storage in ice will reduce discolouration.

Whole squid developed pink discolouration in the mantles by second day in ice. The intensity of the pink colour was reduced on the third day, but the belly portion of the mantles turned pale yellow, accompanied by off odour. The intensity of the yellow discolouration increased and spread on further iced storage with an increasing stale odour. The cooked material had a dull brown appearance. By dressing the squid immediately after capture, the pink and yellow discolouration was avoided, but it could not be kept in direct contact with ice for longer periods because of the rapid leaching of soluble proteins and non-protein components. This resulted in loss of the characteristic sweet taste within two days in ice (Joseph *et al.*, 1985).

In whole squids iced after 5 hrs from time of capture, pink discolouration started appearing on the second day of iced storage itself. Pink discolouration to the extent of 5% was observed in skin on tubes and in whole squids iced after 5 hrs. On the fourth day, 10% discolouration was observed in samples of whole squids iced immediately after catching and in samples of skin on squid tubes, while, 50% disclouration was observed in whole squids iced after 5h (Dhananjaya *et al.*, 1987). Skinning as quickly as possible seemed to be the most appropriate technique to

control pink discolouration in squids. The skinned samples of squid did not produce any pink discolouration even on the fifth day of storage.

Joseph *et al.*, (1988) found that on frozen storage of cuttlefish fillets, the colour of the fillet in the inside portion turned pale yellow by 7 months storage and the intensity of which increased on further storage. Selvaraj *et al.*, (1991) found the control sample of frozen squid mantles (*Loligo duvaucellii*) developing slight pink discolouration at the end of nine months. The 0.5% ascorbic acid treated samples for 10 minutes, showed no discolouration even after 9 months storage.

Whole cuttlefish packed in polystyrene container with a ratio of 1:2::ice: cuttlefish (direct icing) and whole cuttlefish packed in polystyrene container with a ratio of 1:2:4:: water: ice: cuttlefish (ice water slurry) was found to be in acceptable condition only upto the eighth day. The samples showed pink discolouration and poor appearance due to skin rupture and bruises (Anon, 1994b).

Whole octopus subjected to (1) delayed icing for 8 h before packing in a ratio of 1:2:4::ice:water:octopus and (2) delayed icing for 4 hours and stored in a ratio of 1:2::ice:octopus found to develop a slightly foul odour, slight pinkish discolouration, watery texture and slightly broken skin, on the sixth and eighth day of storage, respectively (Anon,1994a).

The yellow discolouration of frozen mongou cuttlefish in Thailand was found to be due to lack of proper sanitation in the fishing boat and inadequate control of temperature between storage and processing, which caused proliferation of the *Pseudomonas spp.* and subsequent yellow discolouration (Hisa *et al.*, 1999).

2.3.3. White discolouration

Composition of the white spots appearing on surface of mantle muscle of European common cuttlefish (*Sepia officianalis*) during frozen storage was analysed by Tanimoto *et al.*, (2000) for determining the cause of this discolouration. Octopine, an amino acid formed by rapid conversion of arginine on storage, was determined as the major component of the spots. Microscopically these spots had a needle -like structure. Total proteins and peptide content was found to be 25.4% of the spots, while water and ash formed 7% and 5.4%, respectively.

2.4. Iced storage - quality changes

Quality has been defined as aggregate of separate factors, each of which has some influence on acceptability (Howgate, 1978). Effective short -term preservation method is of importance for the development of cephalopod fishery.

Iwamoto and Uchiyama (1969) studied the effect of chemical ice (which is composed mainly of methyl cellulose or other high molecular weight substances) on the keeping quality of marine products. He found no significant difference in volatile base nitrogen, trimethylamine nitrogen and nucleotides in the muscle between ordinary ice storage and the chemical ice storage. During ice storage, the chromatophores in squid skin swelled and the black pigment oozed out, but this was not observed in chemical ice storage. The chemical ice compared to ordinary ice produced no drip.

The keeping time in ice of the squid, *Nototodarus sloani gouldi*, caught off the south west coast of Tasmania, was investigated by Young *et al.*, (1973). It was

found that the material remained acceptable for upto eight days. The reliability of the hypoxanthine and TVB methods, for testing the freshness of iced or chilled squid was also studied. It was observed that, the water content of squid increased significantly with time in ice or chilled seawater. The mean moisture content was estimated to be 81% on the third day, but was found to increase to 87% on the 9^{th} day, thus reducing the solids content. Consequently, the dilution of the flesh with water caused a downward trend in the hypoxanthine content. Hence it is suggested that both hypoxanthine and TVB content should always be expressed on dry weight basis otherwise they may mislead as indicators of freshness.

Joseph *et al.*, (1977) found that during iced storage of whole cleaned squid, the total nitrogen and non protein nitrogen content decreased considerably. The alpha amino nitrogen content became very low after two days storage. Based on biochemical and organoleptic changes it was seen that squid meat could not be kept in ice, in prime condition, for more than two days.

Illex illecebrosus and *Loligo pealei*, iced in boxes with a squid to ice ratio of 2:1, had an average total keeping time of 6.3 days (Learson and Ampola, 1977). *Loligo pealei*, caught in the winter months off New Bedford, Massachusetts (USA), in boxes with a squid to ice ratio of 2:1 immediately after capture, had an estimated average keeping time of nine days after which it was considered unmarketable. They also found the *Illex illecebrosus* bulk iced with a squid to ice ratio of approximately 3:1, to be of unmarketable quality after five days.

Icing squid in bulk is not regarded an appropriate method for preserving high quality squid (Kreuzer, 1984). Ampola (1980) is of the opinion that, bulk icing

cannot be considered a method suitable for maintaining squid in high quality on board the fishing vessels, in particular so far as texture and appearance are concerned, although spoilage is retarded, if properly, iced. He also observed the main advantages of storing squid in chilled sea water, as good appearance of the catch, elimination of the labour - intensive practice of icing in boxes or fish holds and the rapid cooling of squid to temperature between - 1.7 ^{0}C and -1.1 ^{0}C.

Slabyj and True (1981) estimated the shelf life of whole squid (*Illex illecebrosus*), chilled in brine upto 5 days at 0.6 ^{0}C. At the Instituto del Frio, Madrid, total volatile bases (TVB) and sensory analysis were found to be the most suitable quality indices for chilled squid (Kreuzer, 1984). Trimethylamine (TMA), total volatile bases (TVB) and free fatty acids (FFA), have been used in Canada to estimate changes in squid quality. TVB and TMA values gave a satisfactory correlation with organoleptic assessments by using the parametric two-way analysis of variance. Woyewoda and Ke (1980) suggested limits for TVB, as 30-45 mgN/100g and for TMA as 3-10 mg N/100g.

Soluble nitrogen in squid (*Loligo duvaucellii*) during storage in crushed ice and water in the ratio of, 1:2:0.2 by weight, was studied by Raghunath, (1984). The total nitrogen (TN) content of the melt water increased from 0.37 to 3.5 mg/ml after eight hours of storage. Non protein nitrogen (NPN) increased from 0.23 to 3.05 mg/ml for the same period. The moisture content of the squid tubes increased from 78.33 to 83.08% where as, the TN decreased from 3.11% to 2.48%, the water soluble nitrogen from 1.41% to 0.72% and the NPN from 0.72% to 0.43%. Organoleptic

quality, particularly the sweet taste, which is linked to the NPN fraction, was affected adversely.

Sastry *et al.*, (1985) adjudged the ice stored cuttlefish meat as good only upto 2 days, fair upto 4 days and acceptable upto 6 days of storage in ice with reference to both texture and overall acceptance. Steady decrease in total nitrogen, non-protein nitrogen and salt soluble proteins were observed during storage. Alteration of sarcoplasmic and myofibrillar proteins were indicated by electrophoretic studies.

Nakamura *et al.*, (1985) states that in the case of squid, K-value may not be a suitable indicator of freshness. Increase in K-value of common squid after catch is much faster than that of fish, because of differences in degradation pathways of adenosine triphosphate (ATP), and its related compounds.

Yamanaka *et al.*, (1987) studied agmatine as a potential index for freshness of common squid (*Todarodes pacificus*) stored at, 0 ^{0}C, 3.5 ^{0}C and 15 ^{0}C. Agmatine, a non volatile amine, reported to be formed by decarboxylation of amino acid arginine by bacterial enzymes with deterioration of squid freshness, was found to be the most useful index for freshness of common squid, since it could be detected even as a small amount (0.15 mg/100g) in the fresh muscle. Its concentration increased with storage time, exceeding 30 mg/100g at the stage of initial decomposition and finally reached the level of about 40mg/100g at the stage of advanced decomposition. Putrescine and pH values were found useful as indicators for the stage of initial decomposition.

Park *et al.*, (1990) studied changes in freshness during iced storage of common European squid (*Loligo vulgaris*). VBN and TMA were found to be useful

as freshness indices, but K-value was not. Maximum storage period, as evaluated by organoleptic and chemical methods, was 10-12 days.

Ohashi *et al.*, (1991) concluded from a study to find out appropriate indicators of freshness of common squid during storage at 0 ^{0}C, 5 ^{0}C and 10 ^{0}C, that VBN, pH and polyamines, except agmatine, were not suitable as freshness indicators. Agmatine increased before initial decomposition, and thus the change occurred earlier than changes in pH and VBN but it did not seem to reflect early freshness. Changes in HX/AMP (Hypoxanthine/Adenosine mono phosphate) ratio were larger than changes in K values, with loss of freshness and this ratio was suggested to be more useful than K value. Concentrations of free amino acids, arginine and ornithine, changed earlier than agmatine, and hence these could essentially be used as early freshness indicators.

Whole octopus samples were subjected to the following storage conditions (A) delayed icing for 8h before packing in a ratio of 1:2:4::water:ice:octopus, (B) immediately killing of the octopus after catching and cooling in chilled seawater and then storing in a ratio of 1:2::water:ice and (C) delayed icing for 4h and storing in a ratio of 1:2::ice:octopus. The samples A and C developed off odour on the sixth and eighth day of storage, respectively, while the sample B did not develop any off odour even on the twelfth day of storage. The TMA content for sample A and sample C exceeded the acceptable limit on the sixth and eighth day, respectively. However, sample B's TMA was found to be within the acceptable limit even on the twelfth day of storage. (Anon, 1994a).

2.5. Frozen storage

Freezing and frozen storage prolong the shelf life of seafood by retarding enzymatic and microbial degradation. But protein denaturation occurs during prolonged storage resulting in moisture loss and textural changes (Selvaraj *et al.*, 1992).

2.5.1. Textural changes

The mantle muscle of squid is circumferential muscle mainly (Otwell and Hamann, 1979a), with a specific toughness that is quite different from that of fin fishes, and even an excellent toughness is maintained after second freezing and thawing process.

The best way to thaw the frozen squid for the production of high quality dried products was investigated by Ke *et al.*, (1979b). Thawing in air at 5 ^{0}C - 10 ^{0}C, or in seawater at 6 ^{0}C -12 ^{0}C, appeared to be the most suitable methods. Thawing in fresh water may induce some quality changes, but was found still acceptable. A higher thawing temperature than 5 ^{0}C-10 ^{0}C, associated with a shorter thawing time, affected overall quality. Quality tests indicated that frozen squid stored at -18 ^{0}C or lower can be kept for one year in good quality for direct consumption or for processing (Ke *et al.*, 1979a).

It was found that approximately 25% of the original protein content (on wet weight basis) was lost by the end of the first minute of cooking at 100^{0}C (Otwell and Hamann, 1979a). The original moisture content of the mantle meat of squid (*Loligo*

pealei) which was greater than 80%, decreased to 75% after cooking at 100 ^{0}C for 5 min and to 71% after 30 min boiling. During the first few minutes of boiling maximum shrinkage was observed (25%), when the collagen of the outer and inner tunics began to gelatinise and the moisture content markedly decreased. Otwell and Hamann (1979b) hence concluded that, to ensure tender - cooked squid meat, the mantle should be cut into longitudinal strips which have less muscle fibre resistance and be boiled for less than 5 min to avoid excessive mantle dehydration. The cooking medium should quickly gelatinise the tunics, which consist of connective tissues, and should prevent excessive moisture loss from the muscle fibres. Hence the cooking medium composition and properties (pH, ion composition, etc.) would also need to be considered.

The squid mantle consists of five distinct layers of tissue: outer lining, outer tunic, muscle fibres, inner tunic and visceral lining. Scanning electron microscopy viewing revealed loss of structural differentiation in the muscle fibres as the only discernible alteration caused by freezing the mantle of squid (*Loligo pealei*) to -29 ^{0}C. Cooking the mantle to 100 ^{0}C caused gross distortions in all mantle tissues (Otwell and Giddings, 1980). According to Guthworth *et al.*, (1982) the study on textural changes in the mantle tissue of *Illex illecebrosus*, showed that the squid should be cooked either very quickly, for example by frying for 2-3 min, or be simmered in a stew or sauce for periods greater than 16 min for optimal texture properties.

With regard to the frozen resistant nature of the squid, Stanley and Hultin (1982) found that frozen northern Atlantic squids, *Loligo pealei* and *Illex*

illecebrosus, were significantly tougher than their fresh materials, which might be caused by protein cross-linking because of high levels of DMA and formaldehyde.

Temperatures lower than -18 ^{0}C would be recommendable in practical conditions since fluctuations in temperature cannot be avoided for longer periods of storage, perhaps exceeding 8 months. Tests have also indicated that the use of frozen squid as raw material does not reduce overall quality of the finished products, provided the time spent on thawing, processing and re-freezing are kept to a minimum to prevent quality losses. The amount of thaw drip increased with the period of storage, due to denaturation of proteins. The amount of drip also increased with decreasing freshness of the squid meat before freezing. The quality of squid meat during cooking is not only influenced by continuous moisture loss but also by continuous leaching out of proteins due to high solubility of squid proteins (Kreuzer, 1984).

Stanley and Smith (1984) also reported that freezing produced a tendency for squid muscle fibres to lose their outer membranes. The freezing and cold storage characteristics, of cuttlefish fillets have been studied by Joseph and Perigreen, 1988. The firm and chewy texture of the cooked fillets changed to rubbery even though the product was slightly sweet at the end of the storage period of 16 months.

Park and Hur (1990) studied the skin stripping, freezing and thawing conditions of common European squid (*Loligo vulgaris*). The most effective method of skin stripping was found to be, immersing the sample at 5 ^{0}C for 10-15 min in fresh water or 5 -10% (w/v) salt solution. Muscle structure of samples

thawed using fresh water after contact freezing was found to be almost the same as that of raw samples.

Ho *et al.*, (1991) had discovered a slight decrease on the extraction of myofibrillar protein and gel forming ability of surimi based products, and nearly no difference in the Ca-ATP ase activity of myobfibril, when the whole squid of Pacific and Argentina squids (*Illex argentinus*) was stored at -20 ^{0}C for a period of 4-6 months.

During the period prior to freezing, when conditions are not always optimum, and during frozen storage, the functional capacity of the muscle proteins declines, rendering the material useless for certain processes such as conversion to gel. Gomez *et al.*, (1996) examined the reasons for the lack of a good gel forming capacity in frozen giant squid (*Dosidicus gigas*). Above 10 ^{0}C, the ultra structure revealed by scanning microscopy showed to be more cellular, particularly in samples mixed with 2.5% NaCl, and the gel strength was higher at this concentration.

Toughness of the mantles of cuttlefish (*Sepia pharaonis*) and squid (*Loligo edulis* and *Illex argentinus*) were found to increase when they were frozen stored at -10 ^{0}C for 0, 0.5, 1, 2 and 4 months. Histological observation of mantle samples showed that the muscle fibres were injured and aggregated while the frozen storage time increased. The formation and growth of ice crystals might have injured the muscle fibre and enhanced the protein aggregation that caused toughening of the mantle. (Yuh and Chau, 1998).

2.5.2. Biochemical changes

Joseph *et al.*, (1977) studied frozen storage changes in squid, *Loligo duavaucellii*. The whole cleaned squid (*Loligo spp.*) was subjected to the following treatment (1) control (2) dip in 10% NaCl for 30 min. and (3) dip in 10% sodium poly phosphate solution for 5 min. The moisture content and total nitrogen was found decreasing steadily in all the three samples. With the increase in duration of frozen storage, there was a sharp decrease in salt soluble nitrogen, which was suggested to be due to denaturation of protein during frozen storage. Among the treated samples, the rate of increase of drip during storage was more in the salt treated than in the polyphosphate treated sample. It was concluded that frozen squid could be stored for a maximum period of 15 weeks at -18 ^{0}C, which can be extended upto 19 weeks by suitable treatment.

Squid mantle muscle proteins readily degraded during storage probably due to proteinase activity, which was a problem in squid myosin preparation (Tsuchiya *et al.*, 1978). L-sodium glutamate, was found effective in preventing freeze denaturation of squid muscle protein by Iguchi *et al.*, (1981). They also found that squid protein *in situ* appears to be more resistant against freeze denaturation than fish protein. On the other hand, squid muscle proteins (*Todarodes pacificus*) are labile when kept *in situ* near the freezing point or at room temperature. *Loligo vulgaris*, stored for 100 days at -20 ^{0}C was found to show good sensory quality. Protein solubility also remained almost constant during the entire storage period; rancidity was also not detected (Borderias, 1982). But there is a high risk of freezer burn on prolonged storage. Minced squid meat has been found to show good keeping quality

when frozen and stored, similar to whole squid (Tanikawa *et al.*, 1953; Borderias, 1982).

Protein solubility, thiobarbituric acid (TBA) values and sensory tests were used as quality indices for frozen squid at the Instituto del Frio, Madrid (Kreuzer, 1984). When using dimethylamine (DMA) as a quality index, erratic results were obtained.

Effect of raw material quality on the shelf life of squid (*Loligo duvaucellii*) mantles, was studied by Joseph *et al.*, (1985). The protein extractability showed a substantial decrease in dressed squid mantles frozen immediately after catch, and stored (at -20 ^{0}C) by 48 weeks storage. The TMA-N also showed an increase by 22 weeks storage, while the change in TMA-N was negligible in samples that were kept in ice for one day and then frozen and stored at -20^{0}C, and in whole squid kept iced for one day and then dressed, frozen and stored. This was reported to be due to formation of dimethylamine in the latter two. The alpha amino nitrogen content was also found to be high in immediately frozen mantles.

Changes in freshness of Japanese common squid (*Todarodes pacificus*) during cold storage was studied by Nakamura *et al.*, (1985). K- Value of sample maintained at 1^{0}C before storing in refrigerator at 2^{0}C, increased rapidly from 18.5% to 39.5% after 1 day and to 53.5% after 2 days. On the other hand, K- Value of sample maintained at 10 - 13^{0}C before storing at 2^{0}C, increased slowly from 45.6% to 55.0% after 3 days. Increase of K- Value of Japanese common squid was reported to be faster than that of common fishes.

Frozen storage of cuttlefish fillets for a period of 16 months resulted in decrease in salt soluble nitrogen of the fillets from 85.1 to 35.36%, the non protein nitrogen from 24.61 to 20.84% of total nitrogen and alpha amino nitrogen from 252 to 140mg/100g. Loss of water holding capacity of the muscle resulted in increased drip loss and lower moisture content. A slow increase in the TVBN values was also noticed which remained almost same at the end of storage period. (Joseph *et al.*, 1988).

Squid mantle muscle is different from fish muscle in its unique structure of obliquely striated muscle (Tsuchiya, 1989). A unique, denaturation profile in squid mantle muscle myofibrils was reported by Konno (1991). Squid myofibrils were found to be 100 times more stable in the presence of calcium ion than in its absence.

Selvaraj *et al.*, (1991) studied the effect of ascorbic acid dip (0.5% w/v for 10 mins.) treatment on frozen storage of squid (*Loligo duvaucellii*). The treated samples were found to be acceptable even after 9 months storage while the control was unacceptable after 6 months. Decrease in NPN, alpha amino nitrogen and moisture contents and increase in peroxide value and TBA value were also found to be less in the treated samples.

The effect of 5% (w/v) trisodium polyphosphate solution treatment for 5 min, on frozen storage of Indian squid, *Loligo duvaucellii*, for a period of 9 months by Selvaraj *et al.*, (1992) showed that the treatment has a noticeable effect in controlling denaturation of squid fillets as evidenced by less thaw drip, less weight loss on cooking, higher salt soluble nitrogen content and soft texture of the cooked sample.

Konno (1993) reported that the rate of autolysis increased in squid with increasing NaCl concentration upto 0.3 M at which the apparent optimum rate was achieved.

2.6. Enzymes

Enzymes are proteins and part of the body cells. They are involved in cell metabolism, in the digestion of food, in *rigor mortis*, in the production of flavour producing and taste giving substances and others. Enzymatic properties and reactions are, therefore of considerable practical interest.

2.6.1 Lactic dehydrogenase activity

Lactic dehydrogenase activity in squid muscle was found to be very low (Shibata and Yoshimura, 1960). Pyruvic acid was markedly accumulated as the end product of glycolysis. This was assumed to be due to differences in the properties of enzymes of the tricarboxylic acid cycle. Octopine, formed from amino acid arginine was suggested to be an important metabolite of anaerobic glycolysis in some molluscs as a substitute for lactate, which is formed in invertebrates (Saito *et al.*, 1982).

2.6.2. Myosin ATPase activity

The activity of ATPase in myosin and actomyosin of the muscles from squid mantles were studied by Tsuchiya *et al.*, (1978). Squid myosin ATPase was found to be very different from the ATPase of myosin from skeletal muscle and in some

specific points, distinct from the myosin ATPase of invertebrates and from non muscular sources, but it seemed most similar to the myosin from scallops.

Magnesium ions are assumed to play an important role in the contraction of the obliquely striated mantle muscle of squid (Mori *et al.*, 1980). This was because squid actomyosin ATPase was activated with Mg^{++} in high ionic strength media in contrast to the ATPase of skeletal muscle actomyosin.

ATPase activity of myosin ATPase from squid, *Todarodes pacificus*, mantle muscle was studied by Yoshitomi and Konno (1982), under various conditions. The properties were found to be similar to those of rabbit myosin in many respects, but unique in few others such as the KCl dependency of the Mg-ATP ase activity. This was also confirmed by Kimura *et al.*, (1980).

The ATPase activity of squid myosin was found to be reduced with Mg^{++} ion to some extent, but not so definitely as it is in the case of vertebrate skeletal muscles. At 35^0C, the activity of the ATPase of actomyosin was found to decrease (Tanaeka, 1982).

2.6.3. Proteinase activity

Rodger *et al.*, (1984) studied the effect of alkaline protease activity on some properties of comminuted squid (*Loligo forbesi*) . A highly active protease, capable of autolysing the muscle such that its rheological properties were changed was detected. The whole squid muscle incubated under the same conditions as the

comminuted, did not result in any marked proteolysis. It was concluded that the enzyme system is either compartmentalized and released on comminution, or that the disruption of muscle structure effectively increased the availability of substrate or necessary co-factors.

Hurtado *et al.*, (1999) characterized the proteinase activity of octopus arm muscle. Extremely high autolytic activity was observed in octopus arm muscle, which was 40-500 fold higher than those of various other fish species. The proteinase exhibited optimum activity at pH 2.5 and 40 ^{0}C, although it contained a sulph-hydril group in the active site. It was concluded that due to its high affinity for myosin, the enzyme activity should be controlled during processing of octopus to ensure the functionality of myosin.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Procurement of raw materials

The cuttlefish, *Sepia aculeata,* caught by trawlers were procured from Sakthikulangara fishing harbour. Fresh material, without any bruises or broken ink sac, each weighing 140-150 g size, was used for the study. After collection from the harbour, the specimens were immediately transported to the laboratory in iced condition.

3.2 Dressing to cuttlefish fillets

In the laboratory the specimens were washed in ice cold water. Then, the dark (dorsal) side of the animal was split by using a knife to about the middle of the back, the shell (cuttle bone) was taken out, and the viscera, head and tentacles were pulled off. Care was taken not to break the ink sac. The mantle was cleaned from the remaining viscera. Then, cuts were made on both sides of the fins, which were then torn off. A cut along the neck of cuttle fish allowed the removal of the skin by tearing it towards the tail end (Kruezer, 1984). The whole operation was carried out under running water.

3.3 Iced storage

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 and 0.2% (w/v) citric acid solution. The second lot was given a 10 minutes dip treatment in 0.01% (w/v) butylated hydroxy anisole (BHA) solution. The third lot was kept as control

without any chemical treatment. All the three lots were kept well iced with 1:1 icing in plastic boxes. The temperature was maintained at 2 ± 1 ^{0}C throughout the period of study. The ice was replenished as and when required. Samples were drawn on zero, second, fourth and sixth day of storage in ice and analysed for alpha amino nitrogen, non-protein nitrogen, total volatile base nitrogen, trimethylamine nitrogen, thiobarbituric acid reactive substances, free fatty acids and peroxide value. The chemical composition and proteolytic activity of the mantle was estimated on the zero day. The sensory evaluation of the raw and cooked samples was also carried out.

3.4 Freezing and storage

From the treated samples and the control subjected to iced storage, a portion of them, as required was taken and frozen on zero day, second day and fourth day of iced storage. The mantles were rolled into tubes, packed individually in 150 gauge polyethylene pouches and frozen at –40 ^{0}C in a blast freezer. They were then dipped in glaze water around 0 ^{0}C, then sealed in the polyethylene pouches, packed in 3-ply corrugated master carton and stored at -18 ^{0}C ± 1 ^{0}C.

3.5 Biochemical composition of the mantle muscle

The material was subjected to biochemical analysis for the estimation of moisture, crude protein, lipid content and ash on the zero day, according to standard procedures.

3.5.1. Moisture

A known weight of the sample was taken in a pre-weighed, tared crucible and was dried to a constant weight at 105 ^{0}C in hot air oven. The percentage moisture was then calculated from the loss of weight of the meat after drying.

3.5.2. Crude protein.

It was estimated by Microkjeldahl's method (AOAC, 1984). The sample is digested with concentrated sulphuric acid in presence of the catalyst $CuSO_4$/Hg/Hgo, so that the protein N_2 is converted to ammonium sulphate. The ammonium sulphate formed is distilled with alkali and the ammonia evolved is absorbed in boric acid containing Tachirho's indicator. The ammonia absorbed is then titrated against N/70 H_2SO_4. From the titre value the percentage of total nitrogen in the sample is calculated. Since proteins on an average contains 16% N_2, the crude protein content of the sample is obtained by multiplying the percentage of total nitrogen by 6.25.

. One gram meat was digested in Kjel Plus (KPS020) equipment after adding one spatula of digestion mixture and 10 ml of Conc.H_2So4. When the digestion was completed about 25 ml of distilled water was carefully added into the sample, along the side of the Kjeldahl's flask. The flask was swirled to dissipate off the heat evolved. When the solution attained room temperature, it was quantitatively transferred into a 50ml standard flask. The Kjeldahl's flask was washed two or three times with small volumes of distilled water and was collected in the standard flask. The solution was then made upto 50 ml using distilled water and mixed thoroughly well. Five ml of the made up solution was used for distillation in Kjel Plus (Distil-M)

equipment. The ammonia fixed by boric acid as ammonium borate was titrated against N/70 H_2SO_4 back to the original pink colour.

3.5.3. Lipid

The method adopted for lipid estimation was according to Radin (1981). About 1g of minced meat was taken in a mortar and homogenised with 18 ml of extraction solvent (hexane:isopropanol::3:2v/v). It was then filtered into a pre-weighed beaker. The residue was washed two or three times with minimum volume of solvent mixture. The solvent was evaporated off on boiling water bath, then cooled to room temperature in a desiccator and weighed. From the difference in weight the percentage of lipid was calculated.

3.5.4. Ash

This was determined by the method of AOAC (1984). The ash content was determined by igniting the pre-weighed sample at 550 ^{0}C in a muffle furnace until free of carbon, allowed to cool and weighed to find the percentage ash.

3.6 Determination of alpha amino nitrogen

The method adopted was that of Pope and Stevens (1939). The method is based on the formation of a soluble copper complex through the reaction between amino acid and excess copper in the form of copper phosphate. The amount of copper taken into solution by amino acid is determined by iodometry.

About 10 g meat was homogenised in a mortar with 20 ml 10% TCA. The protein gets precipitated out. This was filtered and the filtrate was collected in a 50ml

standard flask. The precipitate was washed 3 times more with 5 ml each of TCA solution, collecting the filtrate in the standard flask. It was then made upto 50 ml using the TCA solution. 25 ml of this TCA extract was used for estimation of alpha amino nitrogen.

25 ml of the extract was taken in another 50 ml standard flask. Two drops of thymolphthalein indicator was added to this and neutralized with 10N NaOH till a faint blue colour was obtained. This was then made upto 50 ml using cupric phosphate suspension. It was mixed and allowed to stand for 30 min, filtered and 10 ml of the filtrate was taken in a conical flask. 2 g KI and 1 ml glacial acetic acid was added to this. It was then titrated against 0.01N thiosulphate (containing 0.1%sodium borate) solution using starch as the indicator. The content of alpha amino nitrogen was calculated from the relation, 1 ml of 0.01N thio is equivalent to 0.28 mg of alpha amino nitrogen.

3.7. Determination of non protein nitrogen (NPN)

The NPN content was determined by the method described in A.O.A.C. (1975). The tissue is extracted with 10% TCA to precipitate out the protein. The extract contains non-protein nitrogenous compounds, the nitrogen content of which is then determined by Microkjeldahl's method.

10 ml of the 10% TCA extract prepared was transferred into a Kjeldahl's flask. A pinch of digestion mixture and 10 ml of conc. H_2SO_4 was added to this. It was then digested in the Kjel Plus equipment, cooled, and made up to 50 ml. 5 ml of this solution was used for distillation in Kjel Plus equipment.

3.8. Determination of total volatile base nitrogen (TVBN)

TVBN was determined by Conway's microdiffusion method (Conway, 1947) as a test for spoilage. The tissue is extracted in the TCA solution to obtain the NPN compounds. The extract is treated with saturated solution of sodium or potassium carbonate solution to liberate the volatile bases in the outer chamber of the Conway's microdiffusion apparatus. The liberated volatile bases absorbed in H_2SO_4 present in the inner compartment. The absorbed bases titrated against standard acid.

1 ml of the 10% TCA extract was taken in the outer chamber of the Conway's unit. 1 ml of saturated sodium carbonate solution was also added to the outer chamber. 1ml of H_2SO_4 was taken in the inner chamber. The unit was closed immediately. The solution in the outer chamber was mixed by slow rotation of the apparatus. It was then incubated at room temperature overnight. The amount of unreacted acid in the inner chamber was determined by titration against 0.02N NaOH, using Tachirho's indicator. A blank was also run using 1ml of 10% TCA in the outer chamber.

3.9 Determination of trimethylamine content (TMA).

TMA was also determined by the Conway's microdiffusion method (Conway, 1947). On addition of alkali to TCA extract, TMA is liberated while, all the other amines (primary and secondary) and ammonia are held back by adding formaldehyde. The TMA is absorbed in standard acid and can be estimated by titration.

The 10% TCA extract prepared was used for determination of TMA. The procedure done was similar to the determination of TVBN except that, 1 ml of formaldehyde is added into the outer chamber of the Conway's micro diffusion unit along with the TCA extract and saturated sodium carbonate solution

3.10. Determination of free fatty acid

Method adopted was according to AOAC (1998). The free fatty acid present in the fat is titrated against standard alkali using phenolphthalein as indicator.

The fat extract prepared by method of Radin (1981), was used. A known volume of the extract was taken in a clean weighed beaker. The solvent was evaporated off on a water bath and weight of fat determined. Then, 10 ml of the made up extract was transferred into a conical flask. The solvent was evaporated off and to this fat, 10 ml of neutralised alcohol was added. This was then titrated against 0.01N NaOH using phenolphthalein as indicator to the appearance of the first permanent pink colour that, persist for 30 seconds. Percentage free fatty acid was calculated as oleic acid.

3.11. Determination of Peroxide Value (PV)

The method of Connell (1975) was adopted for peroxide value determination. Hydroperoxides oxidise KI in acid medium and liberates iodine. The iodine liberated can be measured by titration against standard sodium thiosulphate solution using starch as the indicator.

10 ml of the lipid extract prepared by method of Radin (1981), was taken in a conical flask. The solvent was evaporated off. To this 20 ml of glacial acetic acid

and a pinch of KI was added. The flask was closed and the reagents were mixed carefully and incubated for 30 min under dark at room temperature. Then, the flask was taken and sides washed with distilled water. A few ml. of starch solution was then added and titrated immediately against N/500 thiosulphate solution till the blue colour disappeared.

3.12. Determination of Thiobarbituric acid reactive substances.

Method adopted was that of Buege and Aust (1978). Malonaldehyde formed from the breakdown of polyunsaturated fatty acids, serves as a convenient index for determining the extent of the peroxidation reaction. Malonaldehyde has been identified as a product of lipid peroxidation that reacts with thiobarbituric acid to give a red colour species absorbing at 531 nm.

One gram meat was weighed accurately into a homogeniser. 3ml of distilled water was added and homogenised. It was then transferred to a centrifuge tube, added 0.15 ml 7.25% BHA and 6 ml of TCA-TBA-HCl solution. The mixture was vortexed and then incubated in boiling water bath to develop colour. It was then cooled in water for 10 min, centrifuged and absorbance of the resulting supernatent determined at 531 nm, against blank containing 3 ml distilled water and 6 ml TCA-TBA-HCl solution.

3.13. Determination of protease activity.

The method of Heriott (1955) was used. The tyrosine liberated due to the proteolytic activity is determined directly at 280 nm. The total enzyme activity is expressed as, mg of tyrosine liberated in 1 h/g of wet tissue (enzyme units /gm tissue).

Muscle tissue was collected from the anterior, posterior, lateral region and from middle region of the mantle for determining the activity level of protease. One gram muscle from each region was homogenised with 6 ml cold distilled water. 2 ml of each of the homogeneate was added to 4 ml of pre-warmed buffers at 40 ^{0}C and incubated for 1 h at pH 3,4,7 and 8. The reaction was terminated with trichloroacetic acid (5% final concentration) and the supernatent after centrifugation were estimated for the released tyrosine at 280 nm directly.

3.14. Sensory evaluation.

The thawed and cooked samples were subjected to sensory evaluation. The cooked samples were prepared by boiling the mantles in 2% brine for 10 min (Joseph *et. al.*, 1985). The organoleptic qualities were assessed for both raw and cooked samples, by a six-member panel on the basis of colour, appearance, texture and flavour (for cooked sample), using a five-point hedonic scale (Selvaraj *et. al.*, 1991). Sensory evaluation sheet format is given as Appendix-I.

3.15. Statistical analysis

The whole experiment was planned using factorial completely randomized design, taking the duration of iced storage as one factor and the three treatments as

another factor for iced storage study. For frozen storage study, the duration of frozen storage was taken as the third factor (Snedcor and Cochran, 1967). Each treatment combination was replicated 2 times.

The statistical analysis of the organoleptic evaluation was done using the Quade test (Rangaswamy, 1995). The proteolytic activity of the mantle muscle was analysed using randomized block design taking, the different regions of mantle as blocks and the pH as treatments.

RESULTS

4. RESULTS

4.1. Biochemical composition

Results of the biochemical analysis of cuttlefish fillets carried out on the zero day are presented in Table 1.

4.2. Biochemical evaluation of ice stored cuttlefish fillets.

The biochemical evaluation was carried out for the determination of alpha amino nitrogen, non protein nitrogen (NPN), total volatile base nitrogen (TVBN), trimethylamine nitrogen (TMAN), free fatty acids (FFA), and peroxide value (PV) and thiobarbituric acid reactive substances (TBARS). Samples were analysed on the zero day, second day, fourth day and sixth day of storage in ice.

4.2.1 Alpha amino nitrogen

In the case of control, the alpha amino nitrogen content was 249.385 ± 4.658 mg/100g meat on the initial day. By the sixth day of storage, it decreased to 27.131 ± 0.089 mg/100g

The sample treated with salt + citric acid, had an initial content of 252.6 ± 0.792 mg/100g which, by the end of storage period decreased to 38.723 ± 0.072 mg/100g.

In the case of BHA treated sample, the initial content was 255.239 ± 0.240 mg/100g. By the sixth day, this decreased to 32.586 ± 0.338 mg/100g

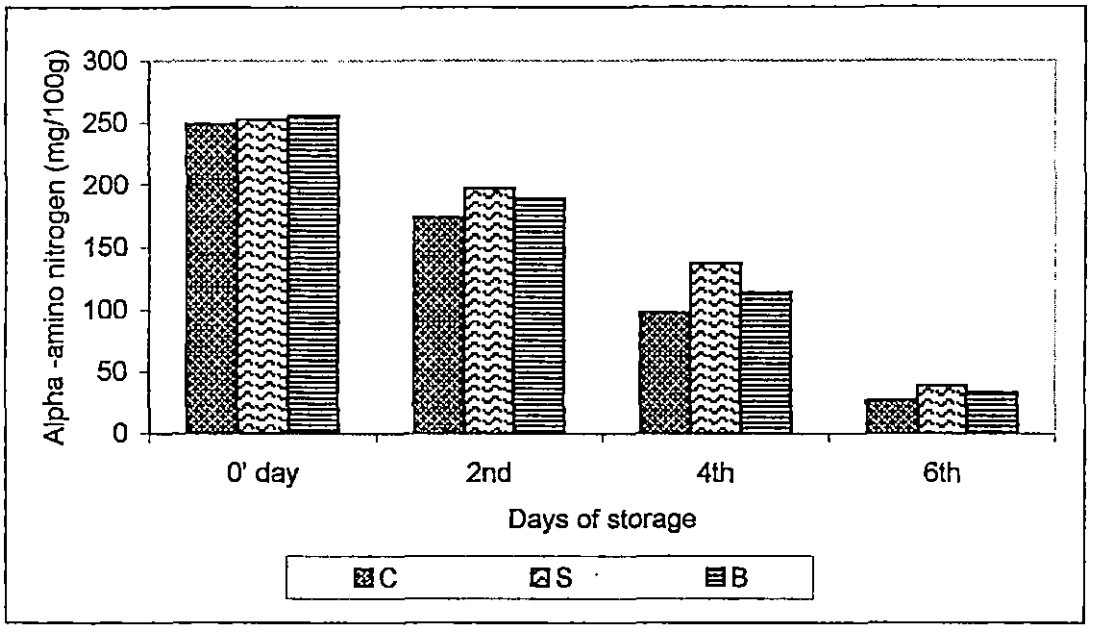
Table 1. Biochemical composition of cuttlefish fillets.

Parameters / Treatments	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Control	77.797 ± 0.525	19.429 ± 0.076	0.816 ± 0.001	0.968 ± 0.008
Treatment I	78.245 ± 0.245	19.608 ± 0.495	0.8165 ± 0.0005	0.960 ± 0.029
Treatment II	77.548 ± 0.681	19.59 ± 0.406	0.8215± 0.0005	0.994 ± 0.026

Treatment I – Salt + citric acid treated

Treatment II – BHA treated

Fig. 1. Variation of alpha amino nitrogen in cuttlefish fillets during iced storage.



Where, C-control; S- salt+citric acid treated; B-BHA treated

Table 2. ANOVA for alpha amino nitrogen in cuttlefish fillets during iced storage.

Source of variation	DF	S.S.	M.S.S	F-ratio
Between treatments	2	1745.061	872.53	84.15*
Between days	3	160028.723	53342.907	
Interaction	6	574.231	95.705	
Error	12	124.421	10.368	
Total	23			F(5%)=4.75

Where, D.F- Degrees of freedom; SS-Sum of squares; MSS-Mean sum of squares

* Significant at 5% level.

Pairwise comparison of treatments. (t_{12}) at 5% level = 2.179

Control [a]

Salt + Citric acid treated [b]

BHA treated [c]

Treatments with different superscripts show significant variation.

The trend is shown in Fig.1. The result of statistical analysis is provided in Table 2. The statistical analysis showed a significant difference, at 5% level of significance, among the control and the two treatments.

4.2.2. Non protein nitrogen (NPN)

The percentage NPN content, in the case of control was 0.618 ± 0.0045%,on the zero day, which decreased to 0.373 ± 0.001 % on the sixth day.

In the case of salt + citric acid treated sample the NPN content was 0.610 ± 0.014 % on the zero day and on the sixth day, this decreased to 0.444 ± 0.011%.

The BHA treated sample, showed a decrease from 0.599 ± 0.0015 % on zero day to 0.392 ± 0.006% on the sixth day.

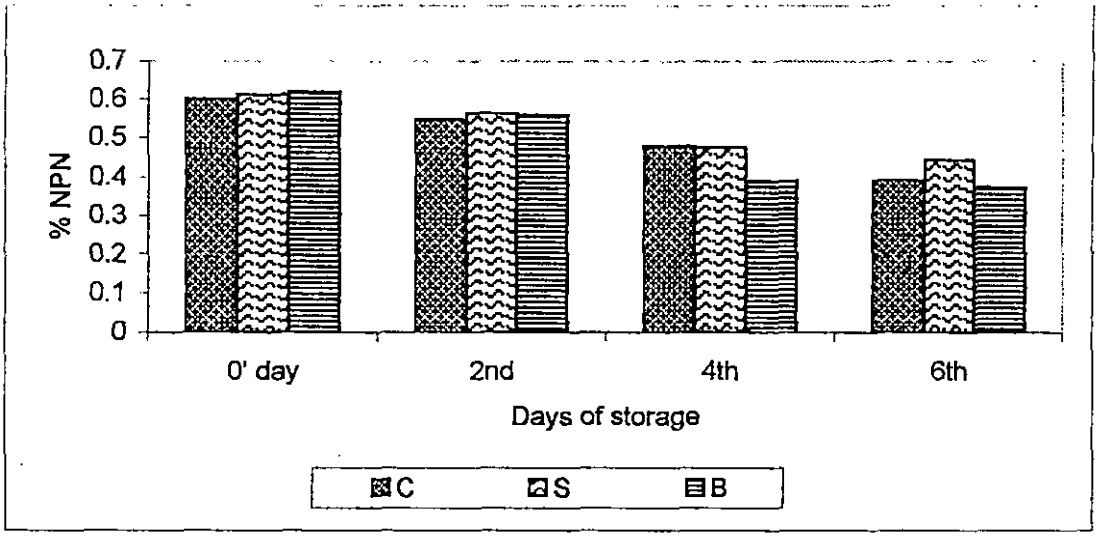
The trend is shown in Fig.2. The statistical analysis showed significant difference among the control and the two treatments. The result of ANOVA is given in Table 3.

4.2.3. Total volatile base nitrogen (TVBN).

In all the samples, the TVBN content increased steadily, from an initial value of 3.388 ± 0.063 mg% to 16.619 ± 0.394 mg% in the control, 3.395 ± 0.026 mg% to 14.867 ± 0.373 mg% in the salt + citric acid treated sample, and 3.814 ± 0.343 mg% to 15.271 ± 0.181 mg% in the BHA treated sample by the end of the sixth day.

The trend is shown in Fig.3. The result of statistical analysis is given in Table 4. The statistical analysis showed significant difference between the salt + citric acid

Fig.2. Variation of NPN in cuttlefish fillets during iced storage



Where, C-control; S- salt+citric acid treated; B-BHA treated

Table 3. ANOVA for NPN in cuttlefish fillets during iced storage.

Source of variation	DF	SS	MSS	F-ratio
Between treatments	2	0.00612	0.00306	24.48*
Between days	3	0.1623	0.0541	
Treatment × Days	6	0.00958	0.00159	
Error	12	0.0015		
Total	23	0.1		F (5%)= 4.75

Where, D.F- Degrees of freedom; SS-Sum of squares; MSS-Mean sum of squares

* Significant at 5% level.

Pairwise comparison of treatments. (t_{12}) at 5% level = 2.179

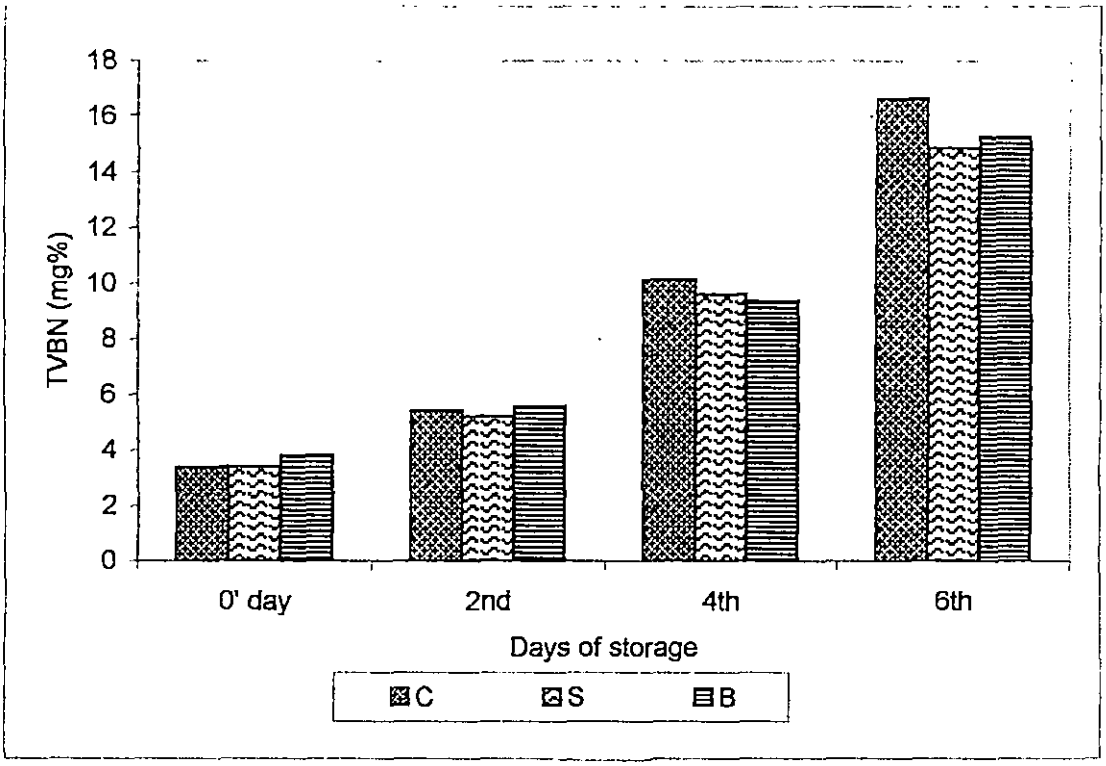
Control [a]

Salt + Citric acid treated [b]

BHA treated [c]

Treatments with different superscripts show significant variation.

Fig. 3. Variation of TVBN in cuttlefish fillets during iced storage.



Where, C-control; S- salt+citric acid treated; B-BHA treated

Table 4. ANOVA for TVBN in cuttlefish fillets during iced storage.

Source of variation	DF	SS	MSS	F-ratio
Between treatments	2	1.5785	0.789	5.635*
Between days	3	515.562	171.854	
Treatments × Days	6	2.777	0.4628	
Error	12	1.6855	0.14	
Total	23	521.603		F(5%)=4.75

Where, D.F- Degrees of freedom; SS-Sum of squares; MSS-Mean sum of squares

* Significant at 5% level.

Pairwise comparison of treatments. (t_{12}) at 5% level = 2.179

Control [a]

Salt + Citric acid treated [b]

BHA treated [a]

Treatments with different superscripts show significant variation.

treated sample and the control. There was no significant difference between the control and the BHA treated sample.

4.2.4. Trimethylamine (TMA)

In the case of the control, salt + citric acid treated and the BHA treated samples, the TMA content increased from a zero day value of, 1.355 ± 0.025 mg%, 1.357 ± 0.010 mg% and 1.386 ± 0.0015 mg%, respectively, to 9.495 ± 0.0315 mg%, 8.644 ± 0.362 mg % and 8.825 ± 0.0915 mg %, respectively, by the end of the sixth day.

The trend is shown in Fig.4. The result of statistical analysis is given in Table 5. The statistical analysis showed significant difference among the control and the two treatments.

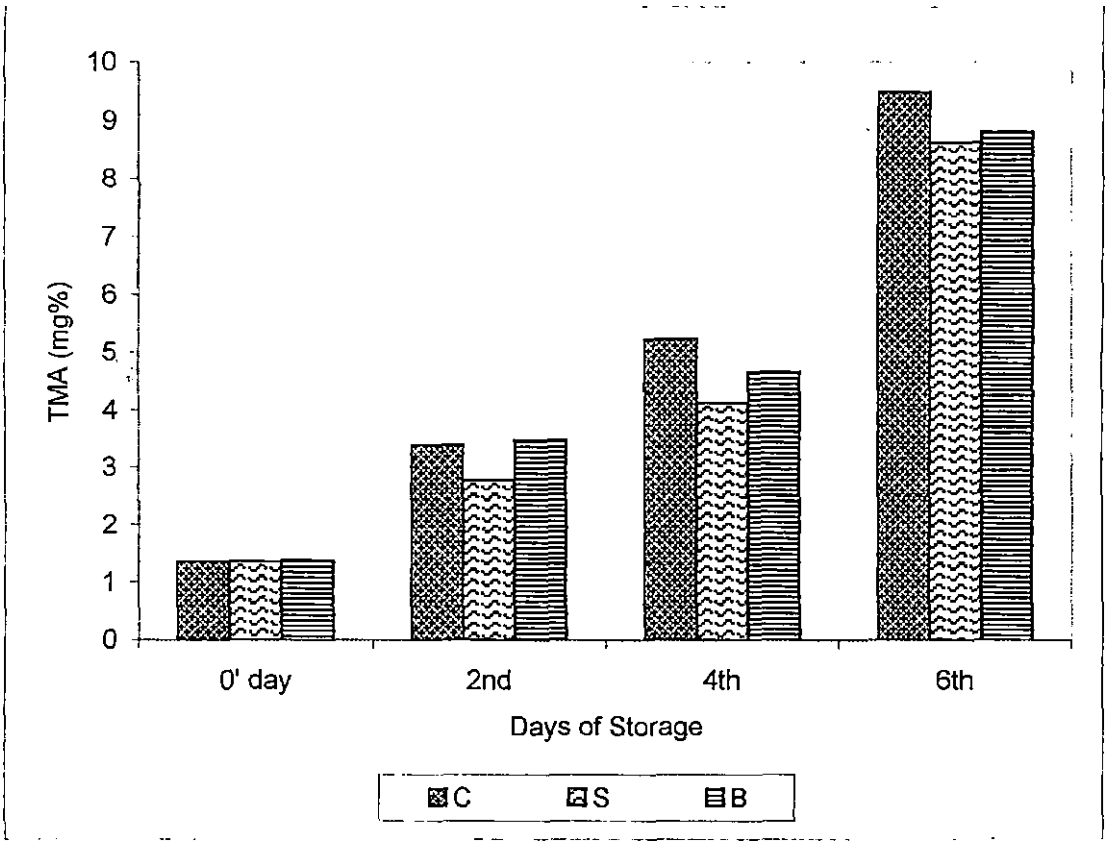
4.2.5. Free fatty acids (FFA)

The FFA content in the control was found to be 19.833 ± 1.167 %, on the zero day. This increased to 42.097 ± 1.174 % on the fourth day, and then showed a slight decrease to 35.807 ± 0.807 %, on the sixth day.

In the case of the salt + citric acid treated sample also, a similar trend was observed. The percentage FFA increased from an initial content of 19.692 ± 0.308 to 31.32 ± 0.987 on the fourth day, and then showed a lower value of 28.875 ± 0.875, on the sixth day.

In the case of the BHA treated sample, the percentage FFA content on the zero day was very low, 4.486 ± 0.179 %, which increased sharply after the fourth day

Fig. 4. Variation of TMA in cuttlefish fillets during iced storage



Where, C-control; S- salt+citric acid treated; B-BHA treated

Table 5. ANOVA for TMA in cuttlefish fillets during iced storage.

Source of variation	D.F	SS	MSS	F-ratio
Between treatments	2	1.681	0.8405	16.676*
Between days	3	189.759	63.253	
Treatments × days	6	0.971	0.1618	
Error	12	0.605	0.0504	
Total	23	193.016		F(5%) = 4.75

Where, D.F- Degrees of freedom; SS-Sum of squares; MSS-Mean sum of squares

* Significant at 5% level.

Pairwise comparison of treatments. (t_{12}) at 5% level = 2.179

Control [a]

Salt + Citric acid treated [b]

BHA treated [c]

Treatments with different superscripts show significant variation.

to 25.173 ± 0.673, and by the sixth day the percentage FFA content reached 36.974 ± 0.359.

The trend is shown in Fig.5. The result of ANOVA is given is Table 6. The statistical analysis showed significant difference among the control and the two treatments.

4.2.6. Peroxide value

The control sample showed a PV of 7.499 ± 0.83 milliequivalent /kg fat on the zero day, which increased to 11.805 ± 0.694 on the second day, and then showed a slight increase to 12.132 ± 0.368 milliequivalent /kg fat on the sixth day.

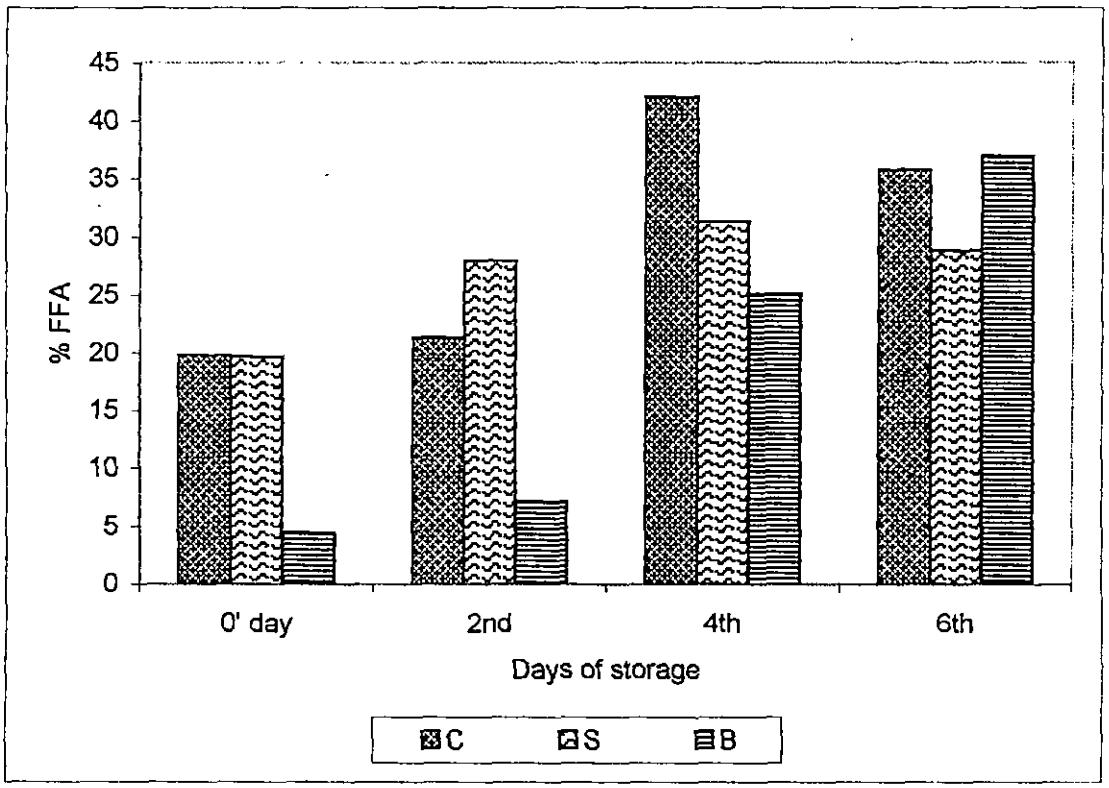
The salt + citric acid treated sample gave a PV of 5.931 ± 0.221 on the zero day, 7.856 ± 0.714 on the second day, and by the sixth day it gave a value of 9.999 ± 0.909 milliequivalent /kg fat. The BHA treated sample showed a PV of 4.8 ± 0.2 on the zero day, which increased steadily to 8.012 ± 0.32 by the sixth day of iced storage.

The trend is shown in Fig.6. The result of statistical analysis is given in Table 7. The statistical analysis showed significant difference between the control and the two treatments.

4.2.7. Thiobarbituric acid reactive substances (TBARS)

The TBARS content showed a steady increase in all the samples. The initial content of TBARS was 0.9005 ± 0.0025 mg malonaldehyde/kg meat, in the control. This increased to 2.458 ± 0.016 mg malonaldehyde/kg meat, by the sixth day.

Fig. 5. Variation of FFA in cuttlefish fillets during iced storage



Where, C-control; S- salt+citric acid treated; B-BHA treated

Table 6. ANOVA for FFA in cuttlefish fillets during iced storage

Source of variation	D.F.	S.S	MSS	F-ratio
Between treatments	2	555.364	277.682	275.751*
Between Days	3	1709.962	569.987	
Treatment x Days	6	576.298	96.049	
Error	12	12.095	1.007	
Total	23	2853.719		F(5%)=4.75

Where, D.F- Degrees of freedom; SS-Sum of squares; MSS-Mean sum of squares

* Significant at 5% level.

Pairwise comparison of treatments. (t_{12}) at 5% level = 2.179

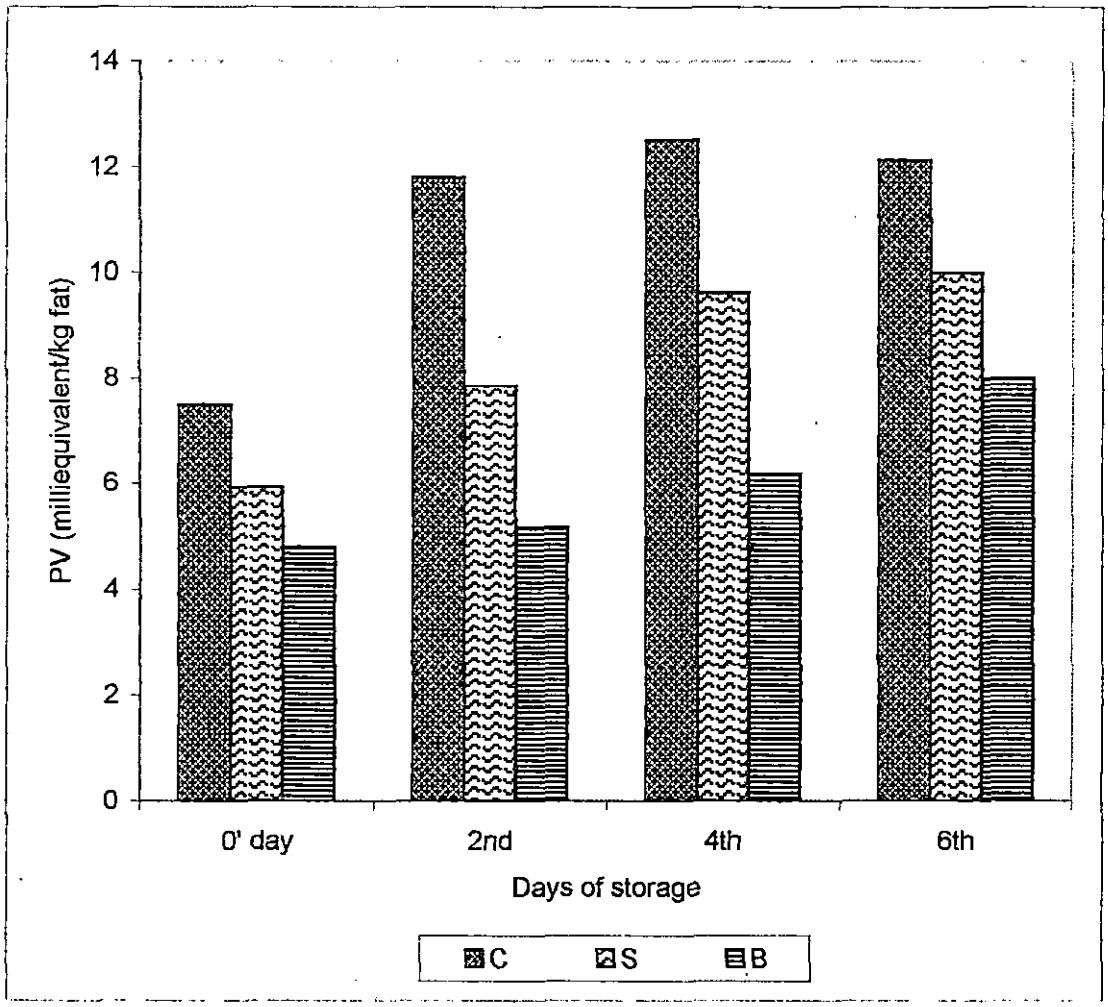
Control [a]

Salt + Citric acid treated [b]

BHA treated [c]

Treatments with different superscripts show significant variation.

Fig. 6. Varation of PV in cuttlefish fillets during iced storage.



Where, C-control; S- salt+citric acid treated; B-BHA treated

Table 7. ANOVA for PV in cuttlefish fillets during iced storage.

Source	D.F.	S.S	MSS	F-ratio
Between treatment	2	97.895	48.947	959.745*
Between days	3	55.221	18.407	
Treatment × days	6	11.011	1.835	
Error	12	6.128	0.51	
Total	23	170.255		F(5%) = 4.75

Where, D.F- Degrees of freedom; SS-Sum of squares; MSS-Mean sum of squares

* Significant at 5% level.

Pairwise comparison of treatments. (t_{12}) at 5% level = 2.179

Control [a]

Salt + Citric acid treated [b]

BHA treated [c]

Treatments with different superscripts show significant variation.

In the case of the salt + citric acid treated sample, the initial content of 0.858 ± 0.01 mg malonaldehyde/kg increased to 2.255 ± 0.162 mg malonaldehyde/kg on the sixth day. In the case of the BHA treated sample, the TBARS content increased from a lower value of 0.6495 ± 0.0015 to 2.0895 ± 0.0035 mg malonaldehyde/kg on the sixth day.

The trend is shown in Fig.7. The result of statistical analysis is given in Table 8. The statistical analysis showed significant difference among the control and the two treatments.

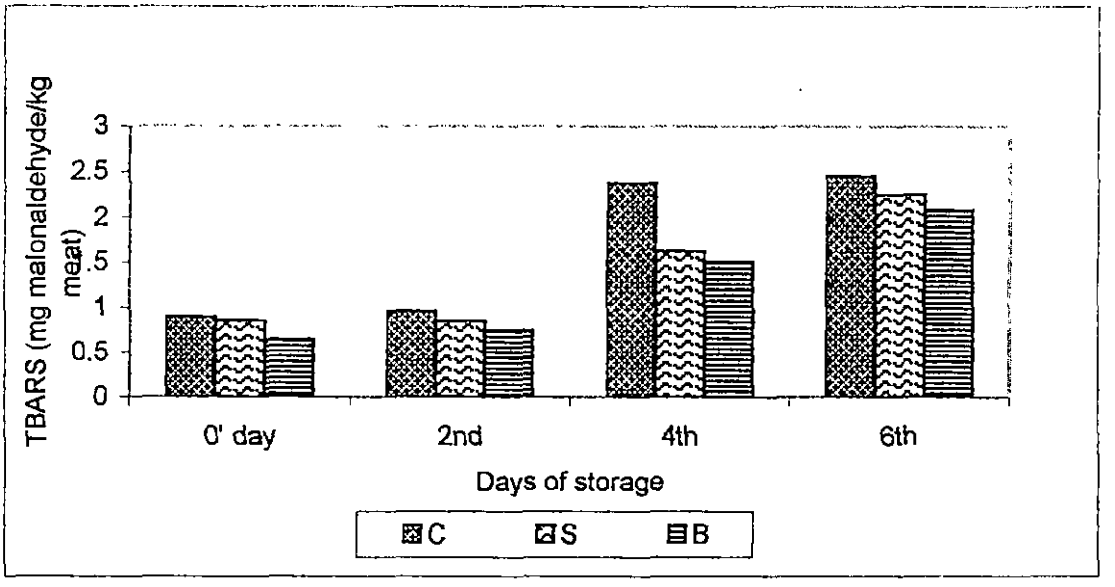
4.3 Biochemical changes in frozen stored cuttlefish fillets

The cuttlefish fillets subjected to different durations of iced storage, viz. zero day, two days and four days, were frozen and stored at -18 ± 1^0c. These samples were drawn after one week, four weeks and eight weeks of frozen storage, and the biochemical evaluation was carried out for all the characteristics similar to that in ice stored fillets.

4.3.1. Alpha amino nitrogen.

In the case of the zero day frozen samples, the decrease in alpha amino nitrogen content even after the eighth week of storage was less, compared to the second day and the fourth day frozen samples. In the case of the control, the content of alpha amino nitrogen, in the zero day frozen sample was 247.518 ± 2.358 mg/100g after one week, and after the eighth week it was reduced to 228.93 ± 3.43 mg/100g, whereas, in the salt + citric treated and BHA treated samples, the content

Fig. 7. Variation of TBARS in cuttlefish fillets during iced storage



Where, C-control; S- salt+citric acid treated; B-BHA treated

Table 8. ANOVA for TBARS in cuttlefish fillets during iced storage.

Source of variation	D.F	SS	MSS	F-ratio
Between treatments	2	0.730775	0.3653875	3463.38*
Between days	3	10.08587	3.361956	
Treatment × Days	6	0.435399	0.072566	
Error	12	0.001266	0.0001055	
Total	23	11.25331		F(5%) = 4.75

Where, D.F- Degrees of freedom; SS-Sum of squares; MSS-Mean sum of squares

* Significant at 5% level.

Pairwise comparison of treatments. (t_{12}) at 5% level = 2.179

Control [a]

Salt + Citric acid treated [b]

BHA treated [c]

Treatments with different superscripts show significant variation.

decreased from 254.627 ± 2.844 and 254.369 ± 0.176 mg/100g to 236.26 ± 1.104 and 231.408 ± 0.911 mg/100g, respectively, after the eighth week.

In the case of the second day frozen samples, the control, salt + citric treated and BHA treated samples showed an alpha amino nitrogen content of 164.385 ± 0.425, 191.022 ± 0.965 and 179.8 ± 0.160 mg/100g, respectively, after one week of storage which decreased to 83.627 ± 0.079,102.611 ± 2.143 and 91.865 ± 2.391 mg/100g, respectively, after the eighth week.

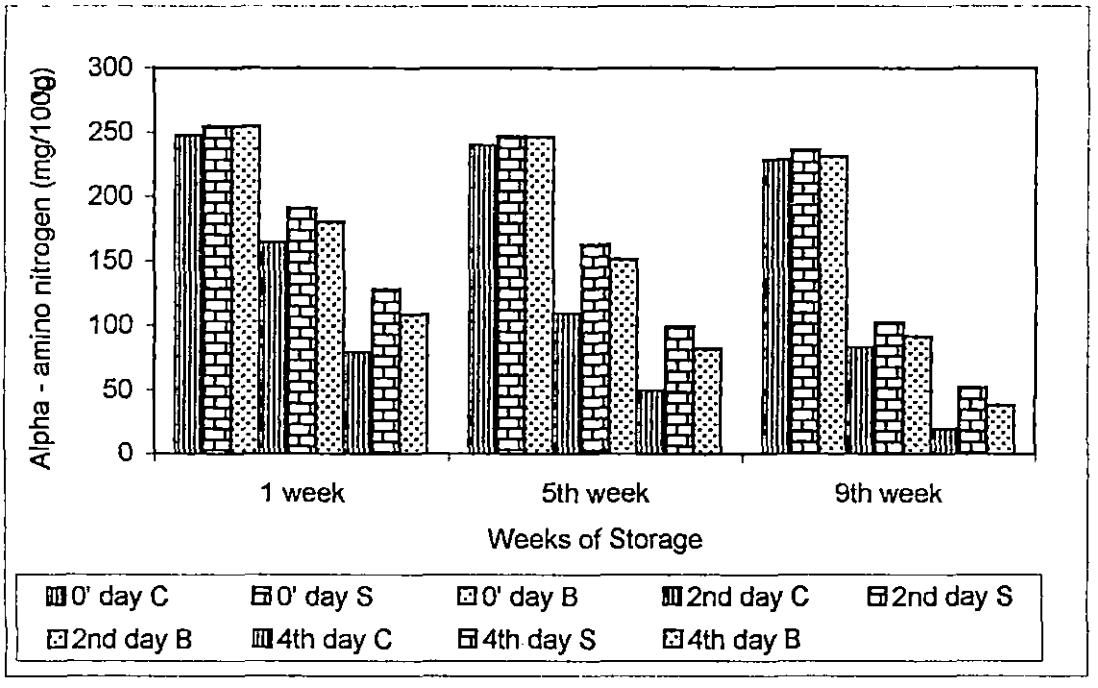
The fourth day frozen samples showed the lowest content of alpha amino nitrogen after the eighth week of storage. After one week, the samples showed an alpha amino nitrogen content of 79.704 ± 3.623 mg/100g in control, 127.54 ± 1.204 mg/100g in salt + citric acid treated and 108.679 ± 3.468 mg/100g in BHA treated sample. The content of alpha amino nitrogen decreased to 19.487 ± 2.748 mg/100g, 52.494 ± 2.82 mg/100g and 38.635 ± 0.057 mg/100g, respectively, in control, salt + citric acid treated and BHA treated sample, respectively, after the eighth week of storage.

The trend is shown in Fig.8. The result of statistical analysis is given in Table. 9. The statistical analysis showed significant difference among the control and the two treatments.

4.3.2. Non protein nitrogen (NPN)

The percentage NPN content was found to be very low in the samples frozen on the fourth day. In the case of the zero day frozen samples, the control showed a percentage NPN content of 0.569 ± 0.015 after one week of storage, and

Fig. 8. Variation of alpha amino nitrogen in frozen stored cuttlefish fillets.



Where, C-control; S- salt+citric acid treated; B-BHA treated

Table 9. ANOVA for Alpha amino nitrogen in frozen stored cuttlefish fillets.

Source of variation	D.F.	S.S	MSS	F-ratio
Between treatments	2	7213.613	3606.8	243.67*
Between Days	2	264771.673	132385.83	
Between Weeks	2	30582.055	15291.02	
Treatment × Days	4	2145.301	536.32	
Treatment × Weeks	4	583.99	145.99	
Days × Weeks	4	7003.46	1750.86	
Treatment ×Days × Weeks	8	265.052	33.13	
Error	27	399.657	14.802	
Total	53	312964.801		F(5%) =3.35

Where D.F, Degree of freedom; SS, sum of squares ; MSS, mean sum of squares.

*significant at 5% level.

Pairwise comparison of treatments. t_{27} at 5% level = 2.052.

Control [a]

Salt + citric acid treated [b]

BHA treated [c]

Treatments with different superscripts show significant variation.

0.310 ± 0.004 after the eighth week. The salt + citric acid treated and the BHA treated samples showed a percentage NPN content of 0.577 ± 0.011 and 0.567 ± 0.014, after one week. After the eighth week, the percentage NPN content reached 0.4275 ± 0.0015 and 0.403 ± 0.008, respectively.

In the case of the second day frozen samples, the control, salt + citric acid treated and BHA treated samples showed a percentage NPN content of 0.391 ± 0.001, 0.434 ± 0.0045 and 0.448 ± 0.0015, respectively, after the first week and 0.164 ± 0.005, 0.192 ± 0.0035 and 0.1915 ± 0.0045 %, respectively, after eight weeks of storage.

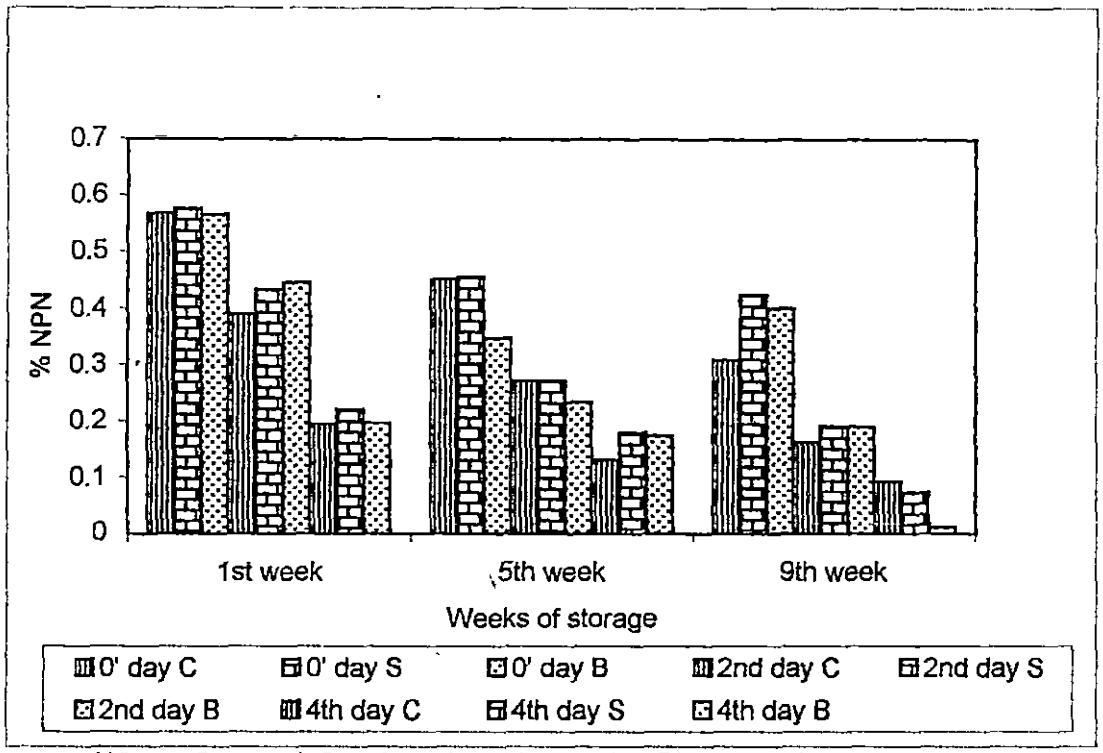
The fourth day frozen samples showed a percentage NPN content of 0.196 ± 0.002, 0.222 ± 0.007 and 0.198 ± 0.001 after one week, in the control, salt + citric acid treated and BHA treated sample. After the eighth week, the percentage NPN content was 0.094 ± 0.005, 0.0749 ± 0.0038 and 0.0118 ± 0.001, respectively.

The trend is shown in Fig.9. The result of statistical analysis is given in Table 10. The statistical analysis showed significant difference between the control and the salt + citric acid treated sample. There was no significant difference between the control and the BHA treated sample.

4.3.3. Total volatile base nitrogen (TVBN)

The TVBN content in the zero day frozen samples, was 4.812 ± 0.045 mg% in the control, 4.151 ± 0.046 mg% in the salt + citric acid treated and 4.821 ± 0.02 mg% in the BHA treated sample, after one week of storage. After the eighth week,

Fig. 9. Variation of NPN in frozen stored cuttlefish fillets



Where, C-control; S- salt+citric acid treated; B-BHA treated

Table 10 : ANOVA for NPN in frozen stored cuttlefish fillets.

Source of variation	DF	S.S.	MSS	F-ratio
Between treatments	2	0.0111	0.00555	50*
Between days	2	0.8908	0.4454	
Between weeks	2	0.3401	0.17005	
Treatment × Days	4	0.0025	0.000625	
Treatment × Weeks	4	0.0058	0.00145	
Days × Weeks	4	0.0301	0.00752	
Treatment × Days × weeks	8	0.03	0.00375	
Error	27	0.003	0.000111	
Total	53	1.3134		F(5%) 3.35

Where D.F, Degree of freedom; SS, sum of squares ; MSS, mean sum of squares.

*significant at 5% level.

Pairwise comparison of treatments. t_{27} at 5% level = 2.052.

Control [a]

Salt + citric acid treated [b]

BHA treated [a]

Treatments with different superscripts show significant variation.

the TVBN increased to 8.273 ± 0.024, 7.832 ± 0.365 and 8.264 ± 0.032 mg%, respectively.

In the case of the second day frozen samples, the control, salt + citric acid treated and BHA treated samples showed a TVBN content of 6.164 ± 0.016, 5.587 ± 0.052 and 5.787 ± 0.336 mg%, respectively, after one week. After eight weeks, the TVBN content increased to 10.45 ± 0.012, 9.707 ± 0.059 and 9.678 ± 0.03 mg% respectively.

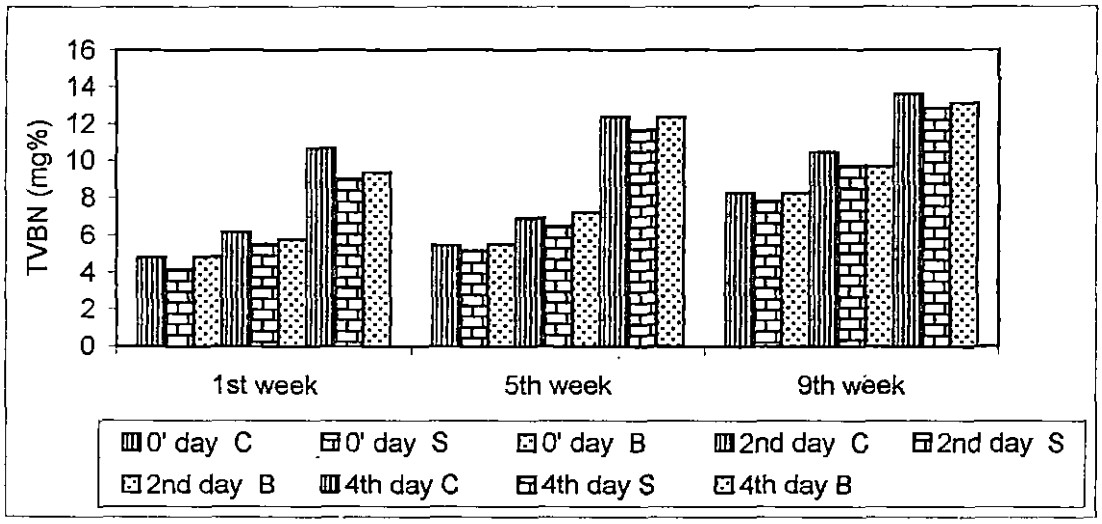
The fourth day frozen samples showed a higher content of TVBN after one week of storage. The TVBN content in the control, salt + citric acid treated and BHA treated sample was found to be 10.642 ± 0.226, 9.017 ± 0.077 and 9.374 ± 0.399 mg%, respectively, after one week. After the eighth week the TVBN content reached 13.574 ± 0.322, 12.85 ± 0.405 and 13.108 ± 0.019 mg%, respectively.

The trend is shown in Fig. 10. The result of statistical analysis is given in Table 11. The statistical analysis showed significant difference among the control and the two treatments.

4.3.4. Trimethylamine (TMA)

The zero day frozen sample, showed less increase in TMA content compared to second day and fourth day frozen sample during the period of storage. The zero day samples showed a TMA content of 2.75 ± 0.026, 2.075 ± 0.023 and 2.0735 ± 0.0015 mg%, respectively in the control, salt + citric acid treated and BHA treated sample, after one week of storage. After the eighth week, the TMA content showed a

Fig. 10. Variation of TVBN in frozen stored cuttlefish fillets



Where, C-control; S- salt+citric acid treated; B-BHA treated

Table 11 ANOVA for TVBN in frozen stored cuttlefish fillets.

Source of variation	DF	S.S.	MSS	F-ratio
Between treatments	2	4.324	2.162	17.023*
Between days	2	305.984	152.992	
Between weeks	2	126.453	63.226	
Treatment × Days	4	0.71	0.1775	
Treatment × Weeks	4	0.953	0.2382	
Days × Weeks	4	8.607	2.151	
Treatment × Days × weeks	8	0.801	0.1	
Error	27	3.43	0.127	
Total	53	451.262		F(5%) 3.35

Where, D.F- Degrees of freedom; SS- sum of squares ; MSS- mean sum of squares.

*significant at 5% level.

Pairwise comparison of treatments. t_{27} at 5% level = 2.052.

Control [a]

Salt + citric acid treated [b]

BHA treated [c]

Treatments with different superscripts show significant variation.

steady increase to 4.136 ± 0.012, 3.404 ± 0.010 and 4.132 ± 0.016 mg%, respectively.

In the case of the second day frozen samples, the control, salt ± citric acid treated and BHA treated samples showed a TMA content of 3.757 ± 0.342, 3.460 ± 0.032 and 3.407 ± 0.0025 mg%, respectively, after one week. After the eighth week, the TMA content was 7.314 ± 0.339, 5.896 ± 0.383 and 6.221 ± 0.019 mg%, respectively.

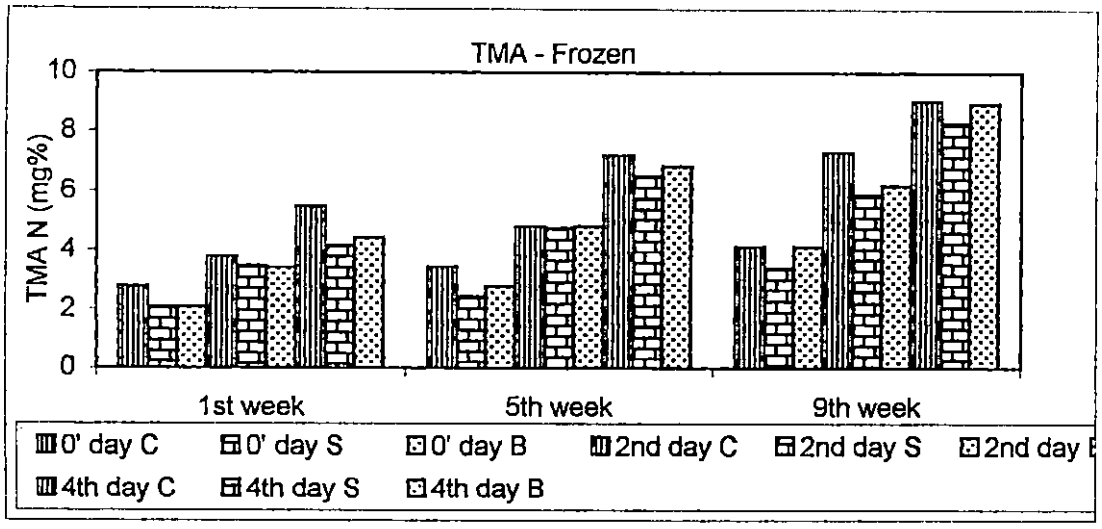
After one week of storage, the fourth day frozen samples showed a TMA content of 5.494 ± 0.060 mg% in control, 4.158 ± 0.039 mg% in salt + citric acid treated and 4.427 ± 0.416 mg% in BHA treated sample. After the eighth week, the TMA content was 9.05 ± 0.017, 8.288 ± 0.009 and 8.968 ± 0.013 mg%, respectively, in the control, salt + citric acid treated and BHA treated sample.

The trend is shown in Fig. 11. The result of statistical analysis is given in Table 12. The statistical analysis showed significant difference between the control and the two treatments. The salt + citric acid treated and BHA treated samples showed no significant difference.

4.3.5. Free fatty acids (FFA)

In the zero day frozen control sample, the FFA content showed an increase from 19.116 ± 2.073% in the first week to 30.672 ± 0.127% after the eighth week. The salt + citric acid treated and the BHA treated samples showed a percentage FFA content of 18.8165 ± 0.15 and 13.248 ± 0.477, respectively, after one week which increased to 31.305 ± 0.194 and 21.266 ± 0.733%, respectively.

Fig. 11. Variation of TMA in frozen stored cuttlefish fillets



Where, C-control; S- salt+citric acid treated; B-BHA treated

Table 12. ANOVA for TMA in cuttlefish fillets during frozen storage

Source of variation	DF	S.S.	MSS	F-ratio
Between treatments	2	5.411	2.705	12.57*
Between days	2	127.133	63.566	
Between weeks	2	74.148	37.074	
Treatment × Days	4	0.24	0.06	
Treatment × Weeks	4	0.588	0.147	
Days × Weeks	4	9.621	2.405	
Treatment × Days × weeks	8	1.613	0.201	
Error	27	2.583	0.2152	
Total	53	221.337		F(5%) 3.35

Where D.F- Degree of freedom; SS, sum of squares ; MSS, mean sum of squares.

*significant at 5% level.

Pairwise comparison of treatments

Control [a]

Salt + citric acid treated [b]

BHA treated [b]

Treatments with different superscripts show significant variation.

The second day frozen samples showed a percentage FFA content of 29.614 ± 0.538, 27.3±0.7 and 17.242 ± 0.442, respectively, in the control, salt + citric acid treated and BHA treated samples after one week. After the eighth week, the percentage FFA showed an increase to 45.309 ± 0.509, 38.690±0.509 and 25.923 ± 0.77, respectively.

The fourth day samples showed a percentage FFA of 37.755 ± 0.425 in the control, 34.524 ± 0.063 in the salt + citric acid treated, and 24.384 ± 0.384 in the BHA treated sample after one week. This decreased to 29.4 ± 1.4, 19.133 ± 0.467 and 16.521 ± 0.521%, respectively, on the second week and then, showed an increase to 42.775 ± 0.775, 22.654 ± 0.254 and 19.514 ± 0.848%, respectively, after the eighth week.

The trend is shown in Fig.12. The result of statistical analysis is given in Table 13. The analysis showed significant difference among the control, salt + citric acid treated and BHA treated samples.

4.3.6. Peroxide value (PV)

The zero day frozen sample showed a PV of 9.347 ± 0.652, 6.558 ± 0.108 and 5.572 ± 0.309 milliequivalent /kg fat, respectively, after one week in the control, salt citric acid treated and BHA treated sample. After the eighth week the PV was found to be 13.363 ± 0.636, 12.916 ± 0.416 and 11.047 ± 0.381 milliequivalent/kg fat, respectively.

The second day frozen samples showed an increase of PV from 7.5 ± 0.5 and 5.1315 millequivalent/kg fat, respectively, in the salt ± citric acid treated and BHA

Fig. 12. Variation of FFA in frozen stored cuttlefish fillets

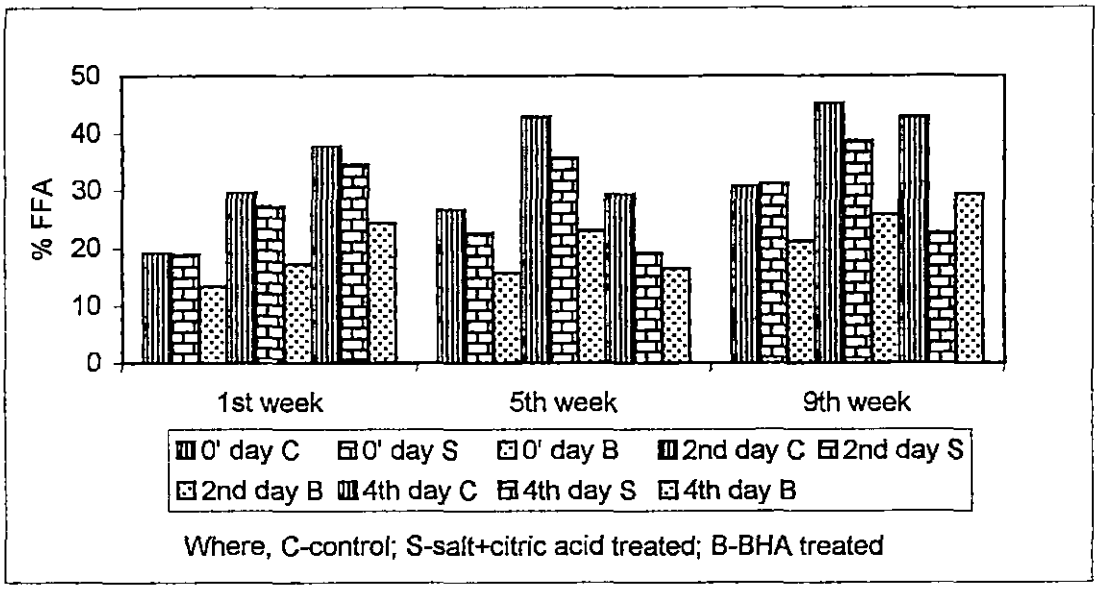


Table 13. ANOVA for FFA in frozen stored cuttlefish fillets.

Source of variation	DF	S.S.	MSS	F-ratio
Between treatments	2	1817.8	908.9	879.013*
Between days	2	827.215	413.607	
Between weeks	2	400.119	200.059	
Treatment × Days	4	230.292	57.573	
Treatment × Weeks	4	99.29	24.822	
Days × Weeks	4	751.646	187.911	
Treatment × Days × weeks	8	126.746	15.843	
Error	27	27942	1.034	
Total	53	1809.749		

Where, D.F- Degree of freedom; SS- sum of squares ; MSS- mean sum of squares.

*significant at 5% level.

Pairwise comparison of treatments

Control [a]

Salt + citric acid treated [b]

BHA treated [c]

Treatments with different superscripts show significant variation.

treated after one week, to 10.378 ± 1.288 and 8.9 ± 0.329milliequivalent/kg fat, respectively, after the eighth week. The control sample showed a slight decrease from 11.922 ± 0.384 to 11.454 ± 0.343 milliequivalent/kg fat after the eighth week

The fourth day frozen samples showed a PV of 12.666 ± 0.666, 10.09 ± 0.679 and 5.633 ± 0.249 milliequivalent/kg fat, respectively, after one week in the control, salt + citric acid treated and BHA treated samples. After the eighth week, the PV was found to be 15.277 ± 0.277, 11.833 ± 0.1167 and 7.339 ± 0.067 milliequivalent/kg fat, respectively.

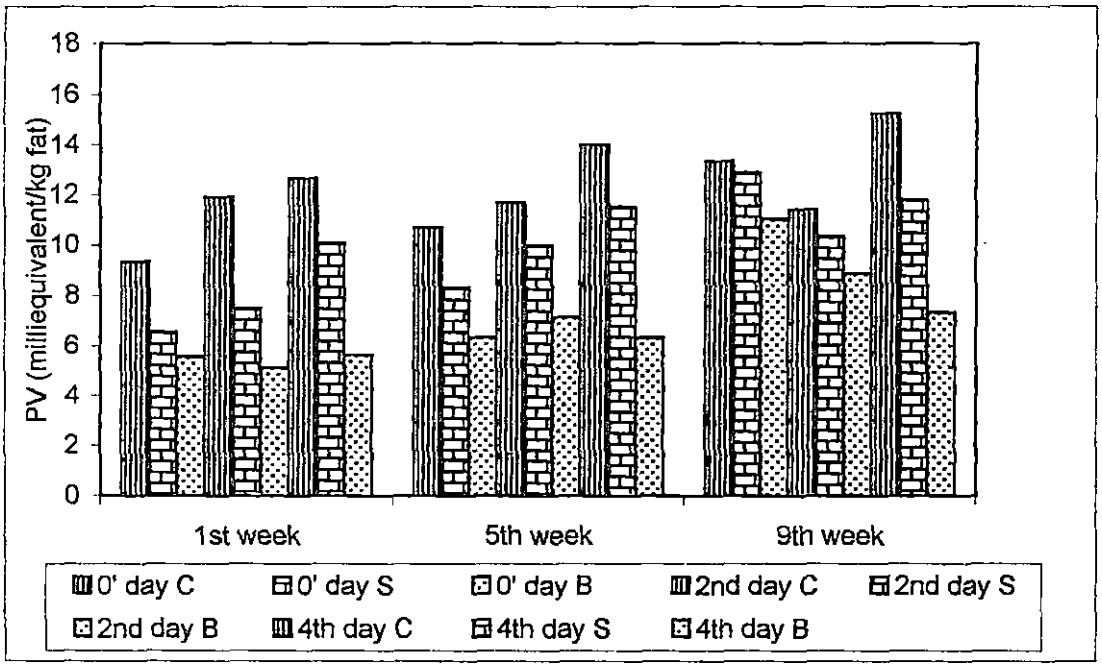
The trend is shown in Fig.13. The result of statistical analysis is given in Table 14. The analysis showed significant difference among the control, salt +citric acid treated and BHA treated samples.

4.3.7. Thiobarbituric acid reactive substances (TBARS)

The TBARS showed a steady increase in the frozen stored samples. After one week of storage, the zero day frozen samples showed a TBARS content of 0.906 ± 0.0055, 0.889 ± 0.002 and 0.662 ± 0.004 mg malonaldehyde/kg meat, in the control, salt + citric acid treated and BHA treated sample, respectively. After the eighth week, the TBARS increased to, 1.8165 ± 0.0475, 1.258 ± 0.006 and 0.8925 ± 0.0025 mg malonaldehyde/kg meat, repectively, in the control, salt + citric acid treated and BHA treated sample.

The second day frozen sample showed a TBARS content of 0.993 ± 0.004, 0.8645 ± 0.0035 and 0.756 ± 0.0025 mg malonaldehyde/kg meat respectively in control, salt + citric acid treated and BHA treated sample after one week.

Fig. 13. Variation of PV in frozen stored cuttlefish fillets



Where, C-control; S-salt+citric acid treated; B-BHA treated

Table 14. ANOVA for PV in frozen stored cuttlefish fillets.

Source of variation	DF	S.S.	MSS	F-ratio
Between treatments	2	256.575	123.287	264.33*
Between days	2	16.566	8.283	
Between weeks	2	88.485	44.248	
Treatment × Days	4	29.161	7.29	
Treatment × Weeks	4	6.299	1.574	
Days × Weeks	4	28.129	7.032	
Treatment × Days × weeks	8	9.495	1.186	
Error	27	12.593	0.4664	
Total	53	437.303		

Where, D.F- Degree of freedom; SS- sum of squares ; MSS- mean sum of squares.

*significant at 5% level.

Pairwise comparison of treatments

Control [a]

Salt + citric acid treated [b]

BHA treated [c]

Treatments with different superscripts show significant variation.

After the eighth week, the TBARS content increased to 2.771 ± 0.078, 1.006 ± 0.0035 and 0.807 ± 0.004 mg malonaldehyde/kg meat respectively.

The fourth day frozen sample showed an increase of TBARS content from 2.446 ± 0.004, 1.6905 ± 0.0075 and 1.5655 ± 0.0065 mg malonaldehyde /kg meat after one week to 3.296 ± 0.031, 2.519 ± 0.0285 and 2.336 ± 0.008 mg malonaldehyde/kg meat, respectively, after eight weeks in the control, salt + citric acid treated and BHA treated samples.

The trend is shown in Fig. 14. The result of statistical analysis is given in Table 15. The statistical analysis showed, significant difference among the control, salt + citric acid treated and BHA treated sample.

4.4 Sensory evaluation of ice stored cuttlefish fillets

The sensory evaluation was carried out for both raw and cooked samples

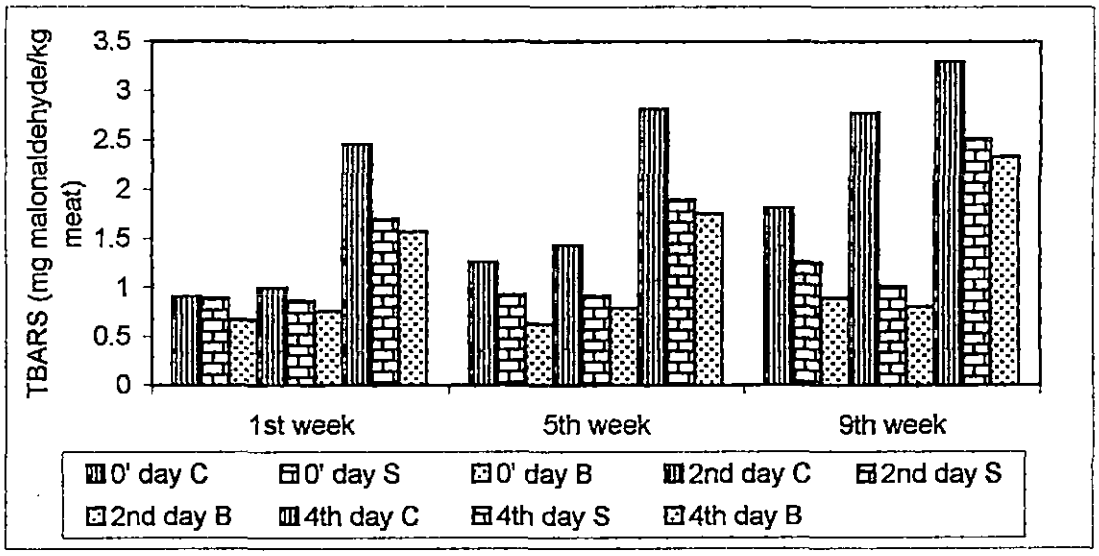
4.4.1. Sensory evaluation of raw cuttlefish fillets

Sensory attributes like appearance, colour and texture were evaluated according to the score given in the score sheet.

4.4.1.1. Appearance of raw cuttlefish fillets.

On the initial day, the appearance score was 5.0 in all the three samples, control, salt + citric acid treated and BHA treated. During the period of storage, a steady decline in the score was shown and by the sixth day the scores were found to be 2.55 ±0.05, 3.1±0.1 and 2.6 ± 0.1, in the control, salt + citric acid treated and BHA treated.

Fig.14. Variation of TBARS in frozen stroed cuttlefish fillets



Where, C-control; S- salt+citric acid treated; B-BHA treated

Table 15. ANOVA for TBARS in frozen stored cuttlefish fillets.

Source of variation	DF	S.S.	MSS	F-ratio
Between treatments	2	6.83	3.415	2845.8*
Between days	2	16.46	8.23	
Between weeks	2	4.142	2.071	
Treatment × Days	4	0.56	0.14	
Treatment × Weeks	4	0.151	0.037	
Days × Weeks	4	1.255	0.313	
Treatment × Days × weeks	8	1.09	0.136	
Error	27	0.033	0.0012	
Total	53	30.521		

Where, D.F- Degree of freedom; SS- sum of squares ; MSS- mean sum of squares.

*significant at 5% level.

Pairwise comparison of treatments

Control [a]

Salt + citric acid treated [b]

BHA treated [c]

Treatments with different superscripts show significant variation.

These variations are shown in Fig. 15. The statistical analysis showed significant difference between the control and salt + citric acid treated samples.

4.4.1.2 Colour of raw cuttlefish fillets

The white colour of the fillets changed to dull white in the control and in the BHA treated sample as the storage progressed. The sensory score on the initial day for control, salt + citric acid treated and BHA treated samples was 5.0, and on the final day it was reduced to 2.55 ± 0.05, 3.05 ± 0.05 and 2.65 ± 0.05.

These variations are represented in Fig.16. The statistical analysis showed the control and the salt + citric acid treated samples to be significantly different.

4.4.1.3. Texture of raw cuttlefish fillets.

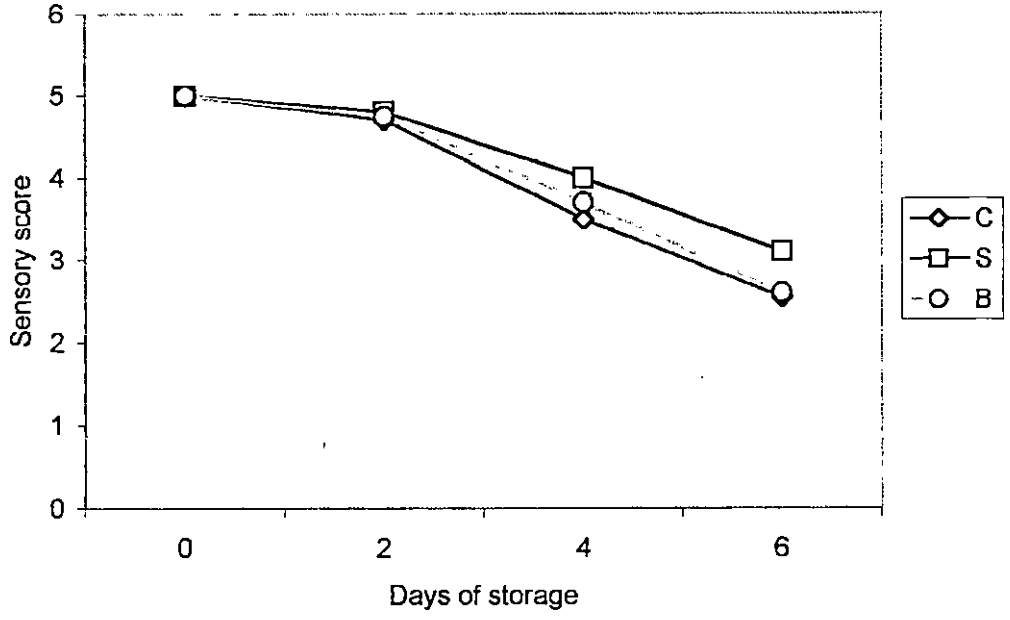
The salt + citric acid treated samples maintained the firmness of texture compared to the control and the BHA treated samples. By the sixth day, the control and the BHA treated samples became soft and on cooking became rubbery and difficult to chew. On the initial day, the texture score was 5.0 in all the three, i.e. control, salt + citric acid treated and BHA treated samples. On the sixth day of storage it was reduced to 2.0, 2.7 ± 0.1 and 2.0 respectively.

These variations are shown in Fig.17. The statistical analysis showed the control and salt + citric acid treated samples to be significantly different.

4.4.2. Sensory evaluation of cooked cuttlefish fillets

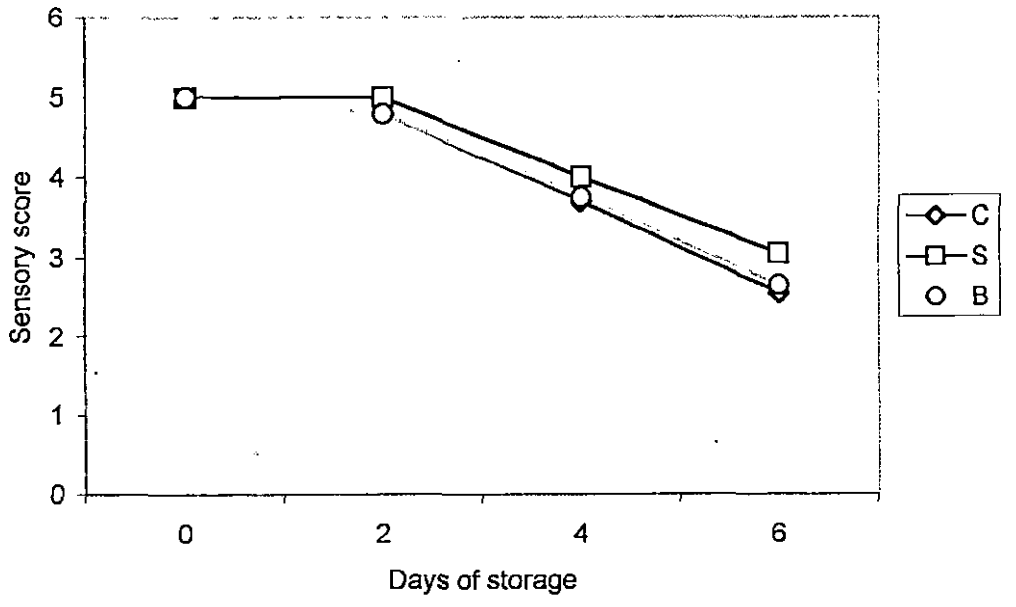
The samples were cooked in 2% boiling brine for 10 min and the sensory evaluation was carried out for appearance, colour, texture and flavour.

Fig. 15. Variation of appearance of raw cuttlefish fillets during iced storage



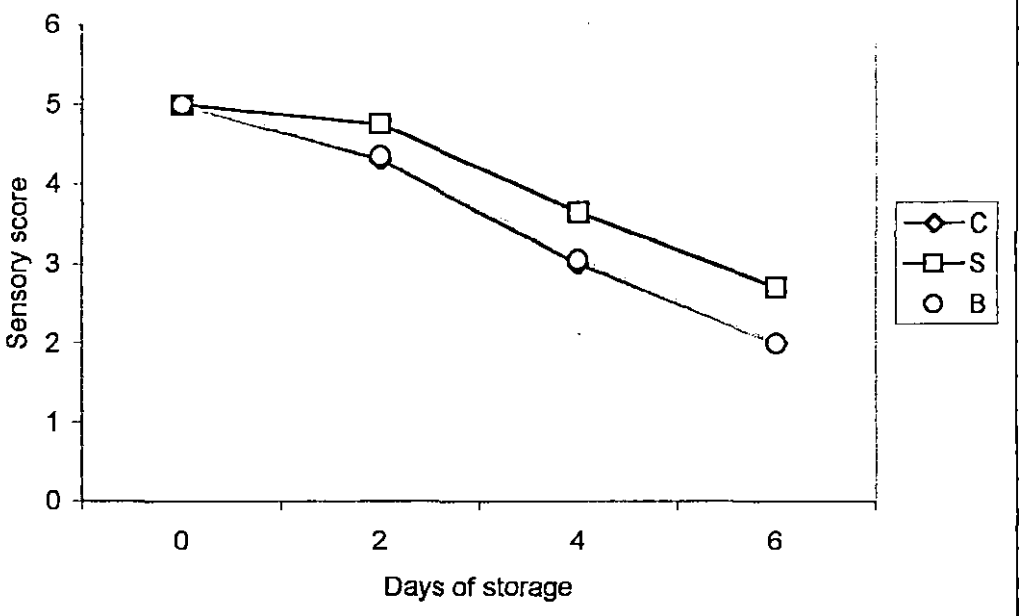
Where, C - Control, S - Salt + citric acid treated, B - BHA treated

Fig. 16. Variation of colour of raw cuttlefish fillets during iced storage



Where, C - Control, S - Salt + citric acid treated, B - BHA treated

Fig. 17. Variation of texture of raw cuttlefish fillets during iced storage



Where, C - Control, S - Salt + citric acid treated, B - BHA treated

4.4.2.1. Appearance of cooked cuttlefish fillets.

The score for colour was 5.0 in all the three samples, on the initial day. On the sixth day of storage, it was reduced to 2.4, 3.0 and 2.5 in control, salt + citric acid treated and BHA treated samples. The variations are shown in Fig. 18. The statistical analysis showed no significant difference between the three samples.

4.4.2.2. Colour of cooked cuttlefish fillets.

On the initial day the score for colour was 5.0 in case of control, salt + citric acid treated and BHA treated samples. On the final day of storage it was reduced to 2.5, 3.0 and 2.55 ± 0.05. These variations are shown in Fig. 19. The statistical analysis showed no significant difference among the three treatments.

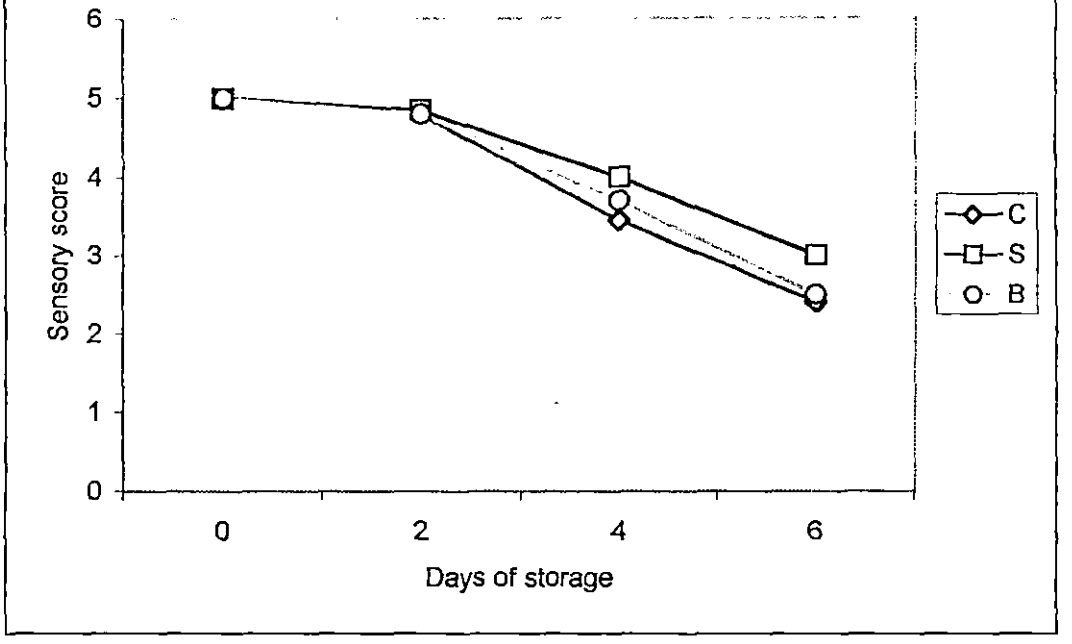
4.4.2.3. Texture of cooked cuttlefish fillets.

The sensory score for texture was found to be 5.0 in all the three samples on the zero day where as on the sixth day of storage it decreased to 2.0, 2.6 and 2.1. These variations are shown in Fig. 20. The statistical analysis showed significant difference between the control and the salt + citric acid treated sample.

4.4.2.4 Flavour of cooked cuttlefish fillets.

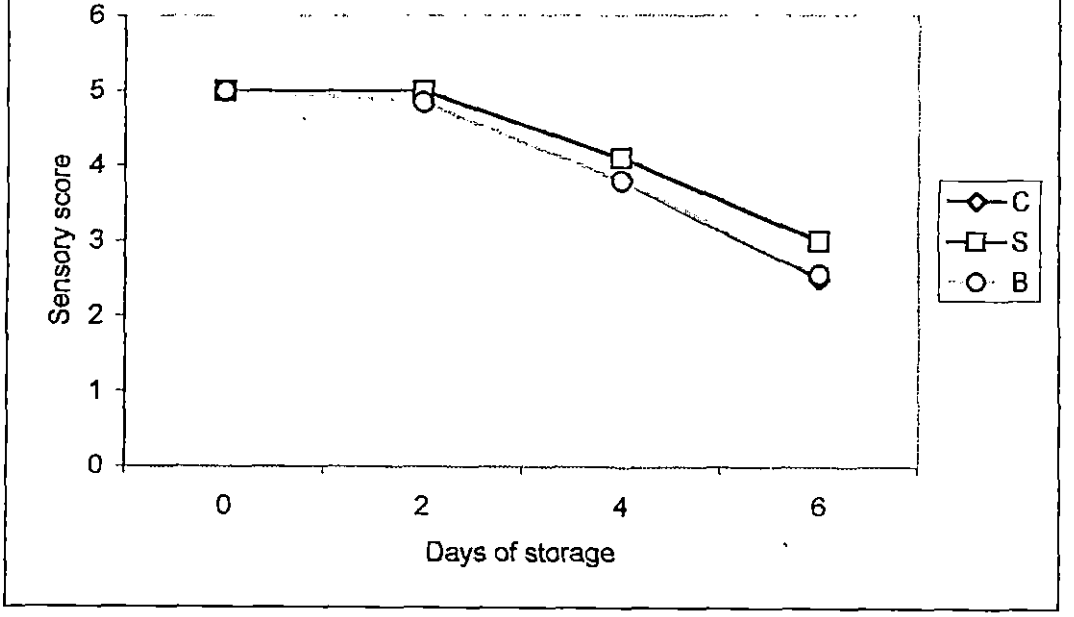
The salt + citric acid treated sample showed less loss of the sweet taste compared to the control and the BHA treated samples. The score for flavour was 5.0 for control, salt + citric acid treated and BHA treated samples, on the initial day. On the sixth day it was found to be 2.0, 2.3 ± 0.1 and 2.1, respectively.

Fig. 18. Variation of appearance of cooked cuttlefish fillets during iced storage

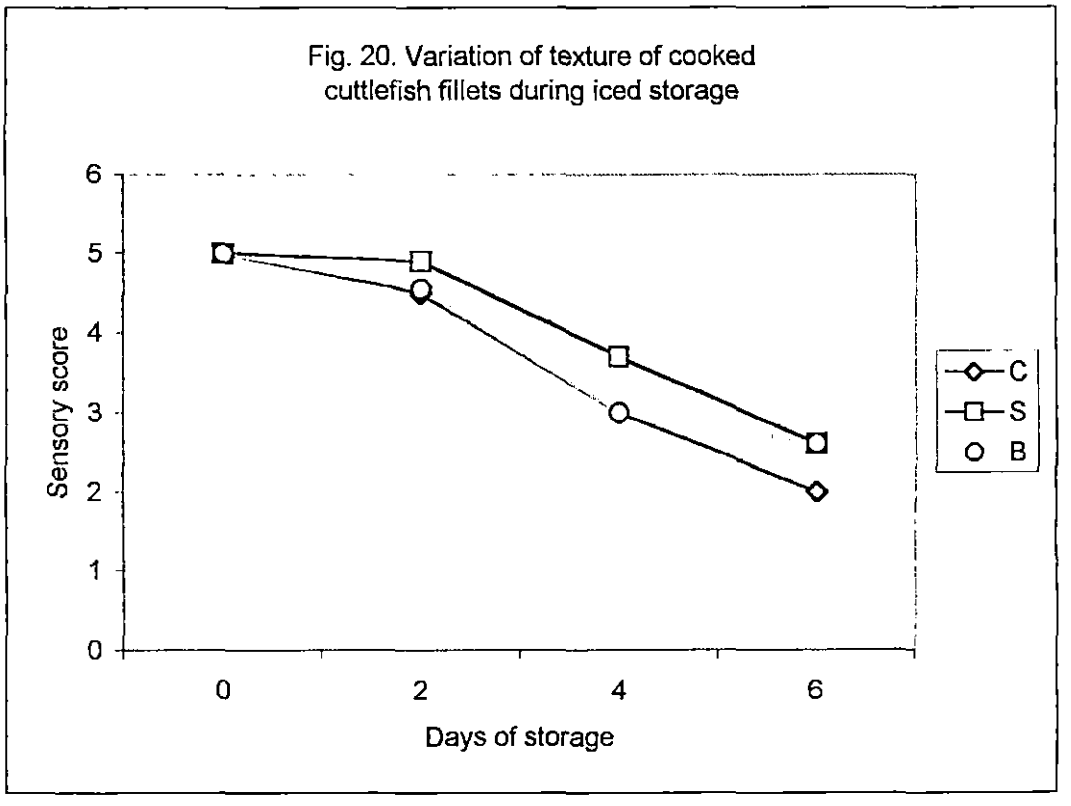


Where, C - Control, S - Salt + citric acid treated, B - BHA treated

Fig. 19. Variation of colour of cooked cuttlefish fillets during iced storage



Where, C - Control, S - Salt + citric acid treated, B - BHA treated



Where, C - Control, S - Salt + citric acid treated, B - BHA treated

These variations are shown in Fig. 21. The statistical analysis showed significant difference between the control and the salt + citric acid treated samples.

4.5. Sensory evaluation of frozen stored cuttlefish fillets

The sensory evaluation was carried out for both raw and cooked samples after thawing the sample in polythene pouches under running water.

4.5.1. Sensory evaluation of raw cuttlefish fillets.

All the three parameters, appearance, colour and texture were evaluated

4.5.1.1 Appearance of raw cuttlefish fillets

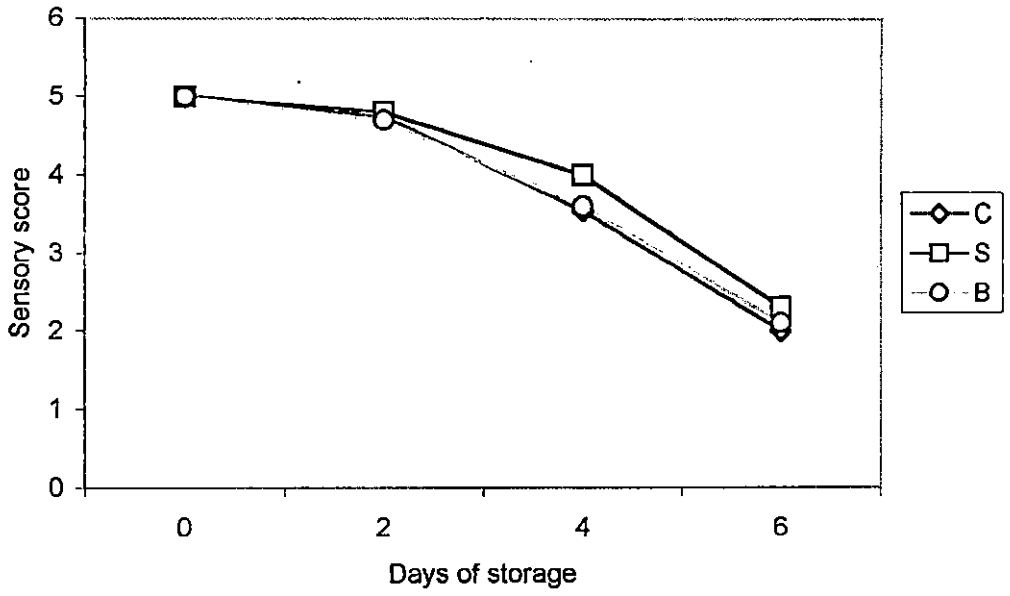
4.5.1.1.1 Zero day frozen sample.

The zero day frozen sample gave a score of 4.9 ± 0.1 in all the samples after one week of frozen storage. After the eighth week of storage, it was reduced to 3.5, 3.75 ± 0.5 and 3.6, respectively, in the control, salt + citric acid treated and BHA treated samples. These variations are shown in Fig. 22. Statistical analysis showed no significant difference between the three treatments.

4.5.1.1.2. Second day frozen sample

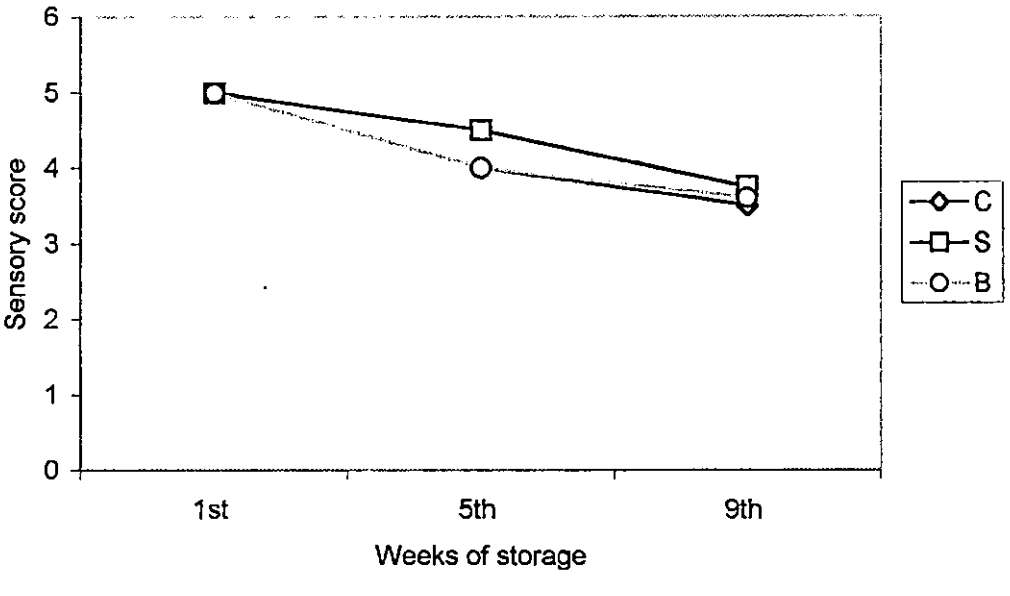
The score for appearance after one week of storage was 4.5, 4.7 and 4.5, respectively, in the control, salt+ citric acid treated and BHA treated samples. After the eighth week of storage, the score was found to be 2.5, 3.3 ± 0.1 and 2.8, respectively. The control samples turned dull white and showed signs of desiccation

Fig. 21. Variation of flavour of cooked cuttlefish fillets during iced storage



Where, C - Control, S - Salt +citric acid treated, B - BHA treated

Fig. 22 Variation of appearance of raw cuttlefish fillets - '0' day frozen



Where, C - Control, S - Salt +citric acid treated, B - BHA treated

after the eighth week. These variations are shown in Fig.23. The statistical analysis showed the control and salt + citric acid treated samples to be significantly different.

4.5.1.1.3 Fourth day frozen sample.

The score for appearance was found to be 3.0, 3.5 and 3.0, respectively, after one week of storage in the control, salt + citric acid treated and BHA treated sample. This was reduced to 1.8, 3.0 and 2.6,respectively, after the eighth week of frozen storage. The control and BHA treated samples showed signs of desiccation after the eighth week. These variations are given in Fig. 24. The statistical analysis showed the control and salt + citric acid treated samples to be significantly different.

4.5.1.2. Colour of raw cuttlefish fillets.

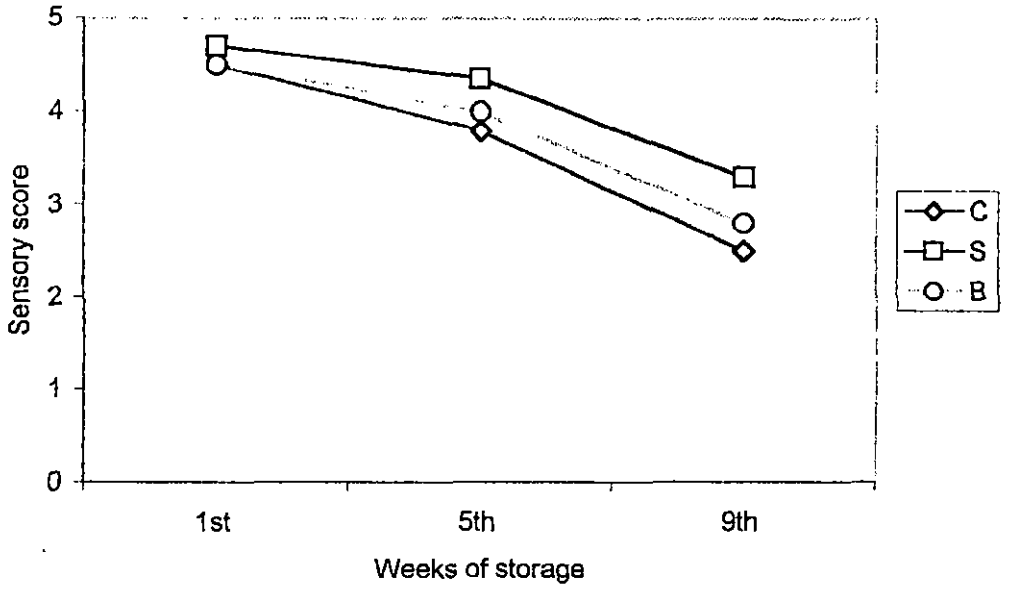
4.5.1.2.1. Zero day frozen samples.

The score for colour was found to be 4.8, 5.0 and 4.8, respectively, in the control, salt + citric acid treated and BHA treated samples. After the eighth week of storage it was reduced to 3.0, 3.5 and 3.2, respectively. These variations are given in Fig. 25. The statistical analysis showed the control and salt + citric acid treated samples to be significantly different.

4.5.1.2.2 Second day frozen sample.

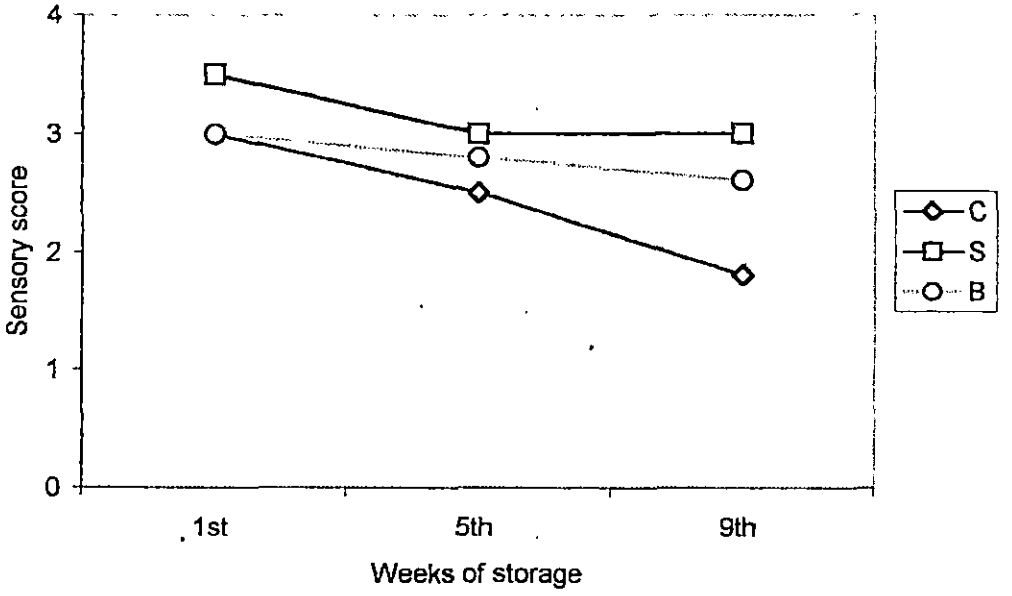
The score for colour after 1 week storage was found to be 4.5 0.1, 4.85 ± 0.1 and 4.55 ± 0.05, respectively, in the control, salt + citric acid treated and BHA treated samples. After the eighth week of storage,, it was reduced to 2.5, 2.8 and 2.55 ± 0.05, respectively. These variations are shown in Fig. 26.

Fig. 23. Variation of appearance of raw cuttlefish fillets - 2nd day frozen



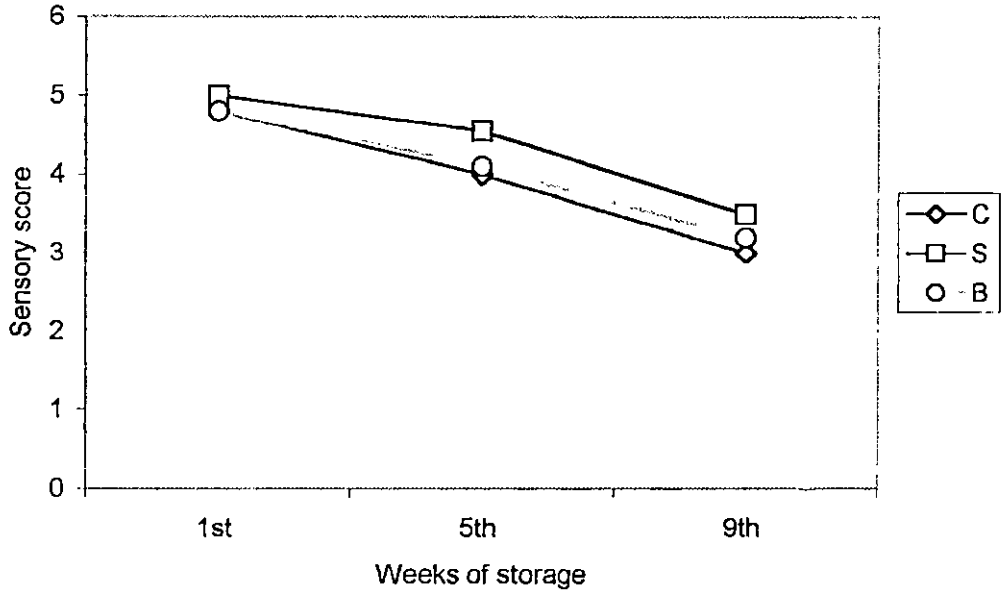
Where, C - Control, S - Salt +citric acid treated, B - BHA treated

Fig. 24. Variation of appearance of raw cuttlefish fillets - 4th day frozen



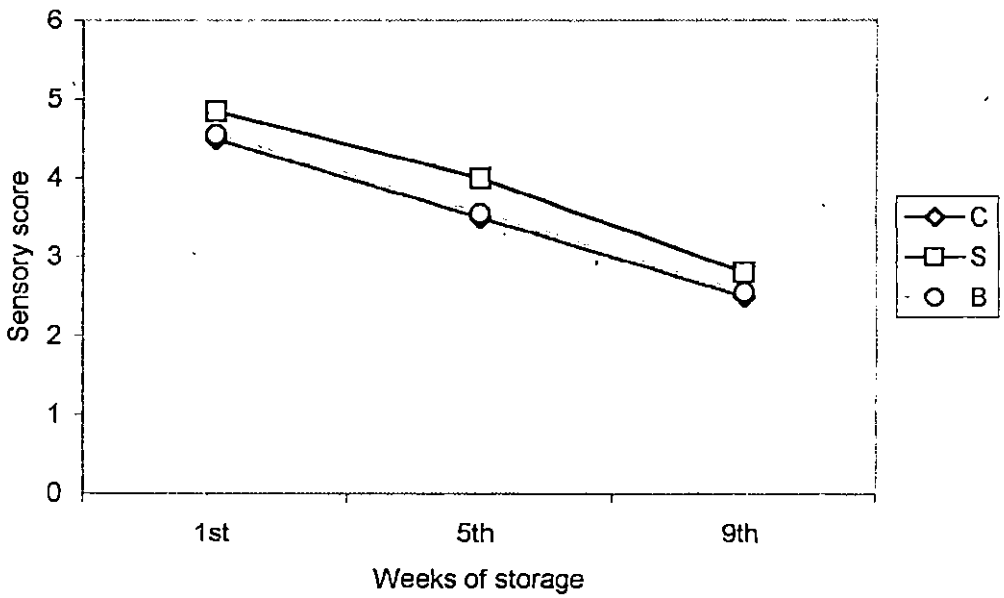
Where, C - Control, S - Salt +citric acid treated, B - BHA treated

Fig. 25. Variation of colour of raw cuttlefish fillets - '0' day frozen



Where, C - Control, S - Salt +citric acid treated, B - BHA treated

Fig. 26. Variation of colour of raw cuttlefish fillets - 2nd day frozen



Where, C - Control, S - Salt +citric acid treated, B - BHA treated

The statistical analysis showed the control, salt + citric acid treated and BHA treated samples to be significantly different. The control and salt + citric acid treated samples gave the highest difference in means.

4.5.1.2.3. Fourth day frozen sample

The control samples showed yellow discolouration on the mantle surface after the eighth week of storage. No yellow discolouration was noticed in the salt + citric acid treated and the BHA treated samples even after eight weeks. The score for colour was found to be 3.0, 3.15 ± 0.05 and 3.0 in control, salt + citric acid treated and BHA treated samples after one week of frozen storage. After the eighth week ,the scores was reduced to 1.5 ± 0.5, 2.85 ± 0.05 and 2.6, respectively.

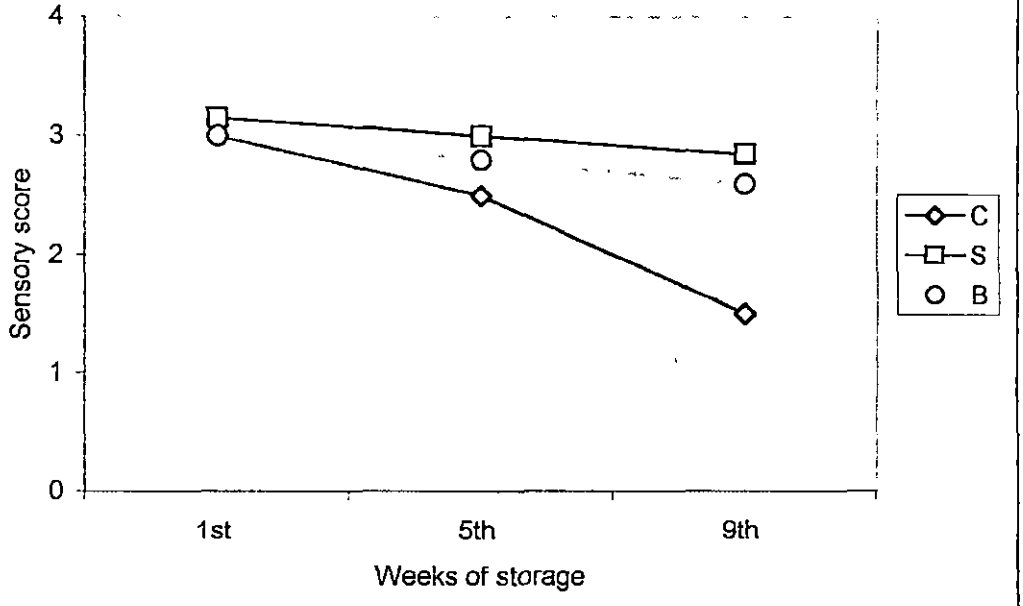
These variations are shown in Fig. 27. The statistical analysis showed significant difference between the control and salt + citric acid treated sample.

4.5.1.3. Texture of raw cuttlefish fillets.

4.5.1.3.1. Zero day frozen sample.

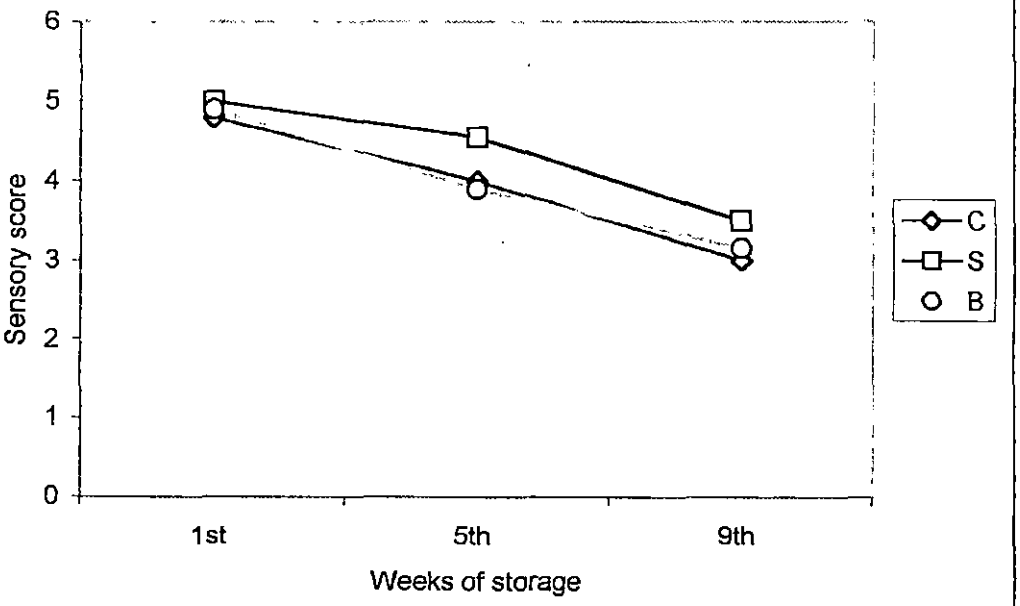
The score for texture after one week of storage was 4.8, 5.0 and 4.9, respectively in the control, salt + citric acid treated and BHA treated samples. After the eighth week it was reduced to 3.0, 3.5 and 3.15 ± 0.05, respectively. These variations are shown in Fig.28. The statistical analysis showed no significant difference among the three treatments.

Fig. 27. Variation of colour of raw cuttlefish fillets - 4th day frozen



Where, C - Control, S - Salt +citric acid treated, B - BHA treated

Fig. 28. Variation of texture of raw cuttlefish fillets - '0' day frozen



Where, C - Control, S - Salt +citric acid treated, B - BHA treated

4.5.1.3.2. Second day frozen sample.

After the first week of storage, the score for texture was found to be 4.0, 4.6 and 4.0, respectively, in the control, salt + citric acid treated and BHA treated samples. After the eighth week, this was reduced to be 2.4, 3.0 and 2.45 ± 0.05, respectively. The control sample was slightly sticky and soft after eight weeks of storage. These variations are shown in Fig. 29. The statistical analysis shows no significant difference between the treatments.

4.5.1.3.3. Fourth day frozen sample

The control sample was found to be soft and sticky and the BHA treated sample also was soft in texture, after eight weeks. But the salt + citric acid treated sample remained slightly firm even after the eighth week. After the one week of storage the score for texture was found to be 2.8 ± 0.1, 3.5 and 3.0, respectively in the control, salt + citric acid treated and BHA treated samples. After the eighth week it was found to be reduced to 2.0, 3.0 and 2.45 ± 0.05, respectively.

These variations are shown in Fig.30. The statistical analysis showed the control and salt + citric acid treated samples to be significantly different.

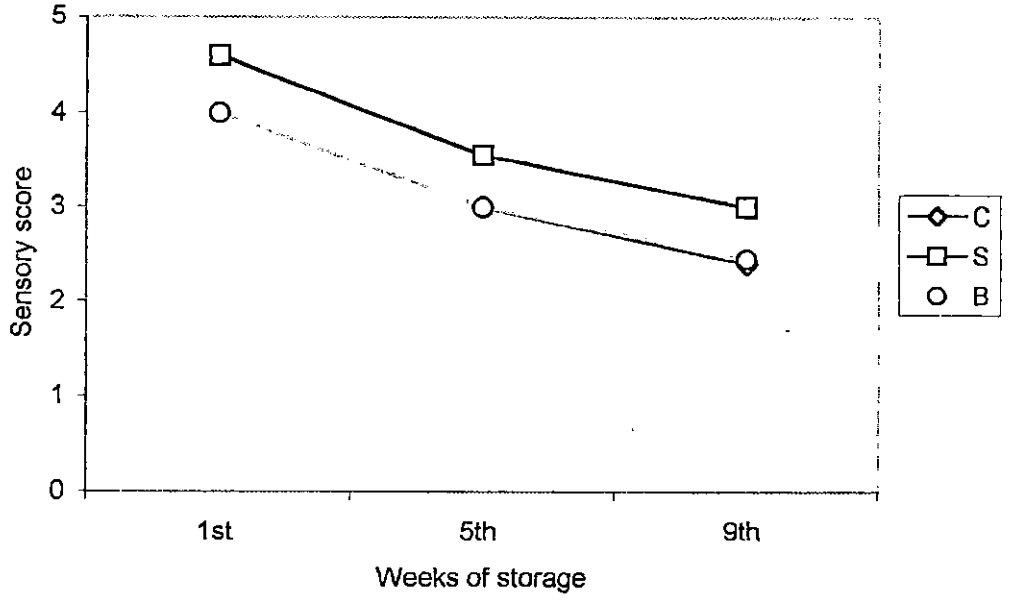
4.5.2. Sensory evaluation of cooked cuttlefish fillets

4.5.2.1. Appearance of cooked cuttlefish fillets.

4.5.2.1.1. Zero day frozen sample.

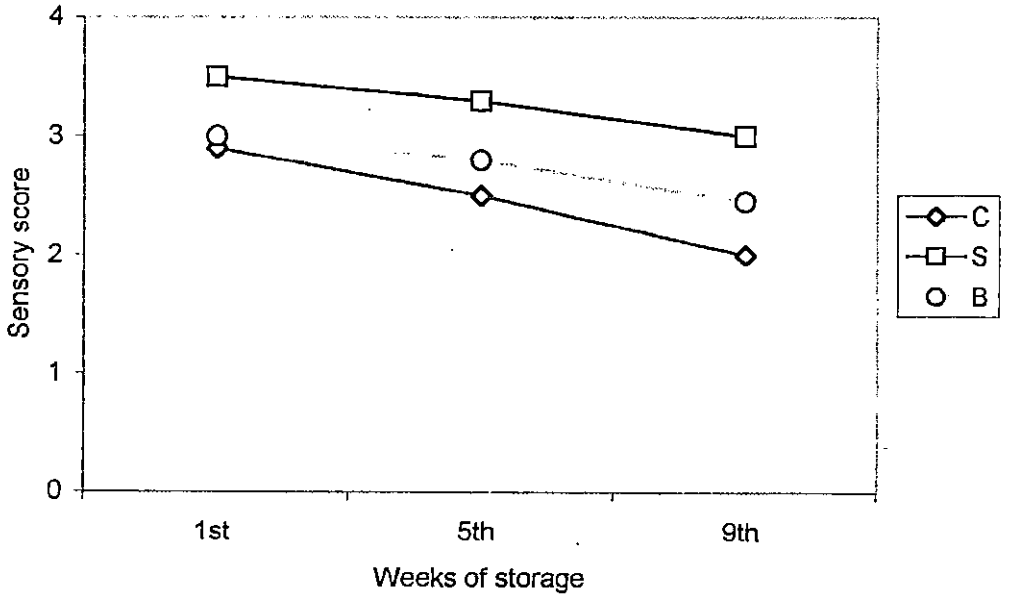
The score for appearance in the control, salt + citric acid treated and BHA treated samples was found to be 4.9, 5.0 and 4.9 after one week of storage.

Fig. 29. Variation of texture of raw cuttlefish fillets - 2nd day frozen



Where, C - Control, S - Salt +citric acid treated, B - BHA treated

Fig. 30. Variation of texture of raw cuttlefish fillets - 4th day frozen



Where, C - Control, S - Salt +citric acid treated, B - BHA treated

After the eighth week, this was found to be reduced to 3.55 ± 0.05, 3.85 ± 0.05 and 3.5, respectively. These variations are shown in Fig. 31. The statistical analysis showed no significant difference among the three treatments.

4.5.2.1.2. Second day frozen sample

After one week of storage, the control, salt + citric acid treated and BHA treated samples gave a score of 4.6, 4.8 and 4.6, respectively, which after the eighth week of storage was found to be reduced to 2.6, 3.35 ± 0.15 and 3.0, respectively. These variations are shown in Fig. 32. The statistical analysis showed significant difference between the control and salt + citric acid treated samples.

4.5.2.1.3. Fourth day frozen sample

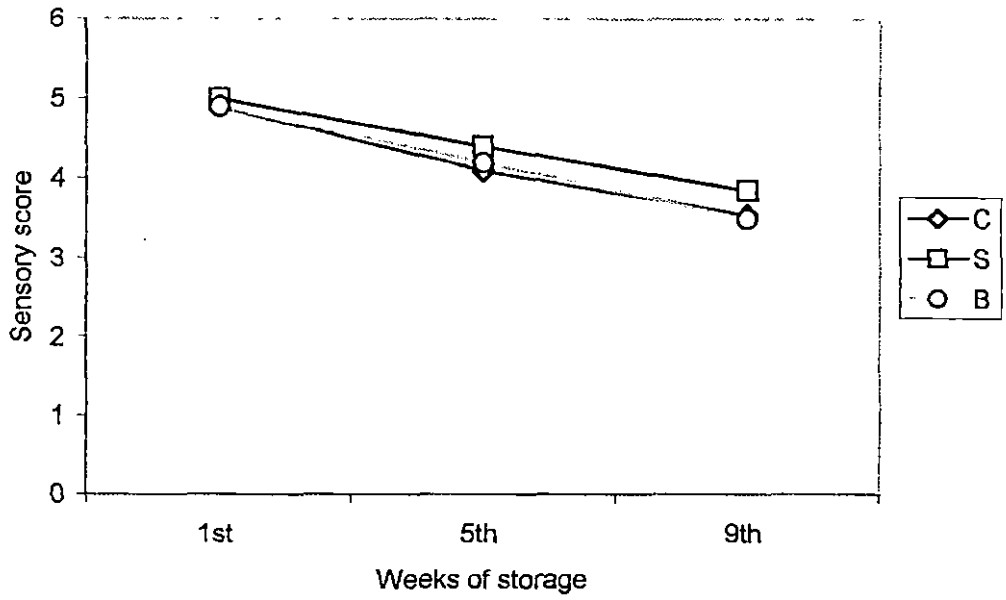
The score was found to be 3.15 ± 0.05, 3.6 and 3.1 ± 0.1, respectively, in the control, salt + citric acid treated and BHA treated samples, after one week of storage. After the eighth week it was reduced to 1.85 ± 0.05, 3.0 and 2.5, respectively. These variations are shown in Fig. 33. The statistical analysis showed no significant difference among the three treatments.

4.5.2.2. Colour of cooked cuttlefish fillets.

4.5.2.2.1. Zero day frozen sample.

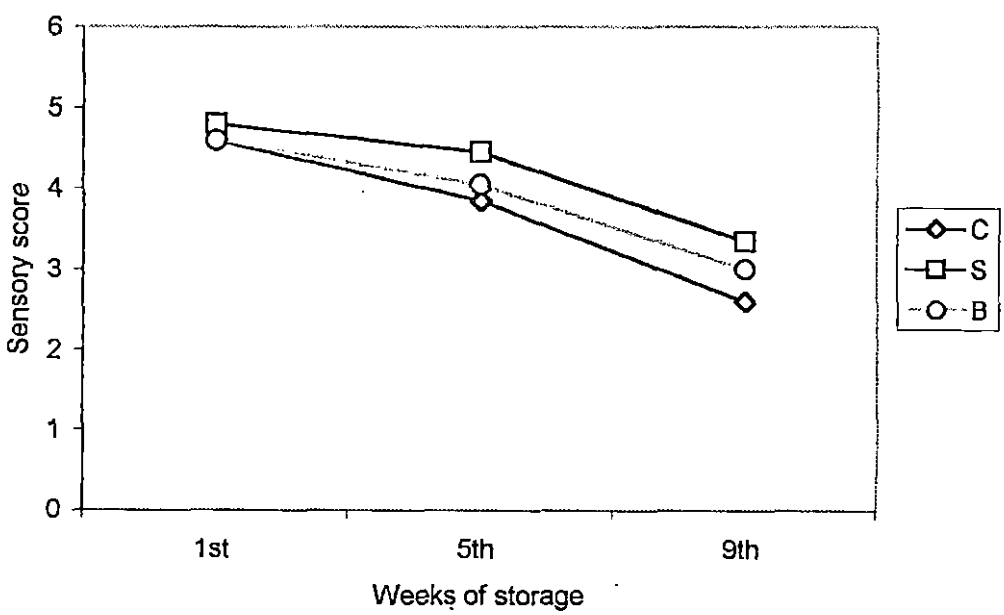
The control, salt, + citric acid treated and BHA treated samples gave a score of 4.8, 5.0 and 4.85 ± 0.05, respectively, after one week of storage. This was found to be reduced to 3.0, 3.4 and 3.05 ± 0.5,respectively, after the eighth week of storage. These variations are given in Fig. 34. The statistical analysis showed the salt + citric

Fig. 31. Variation of appearance of cooked cuttlefish fillets - '0' day frozen



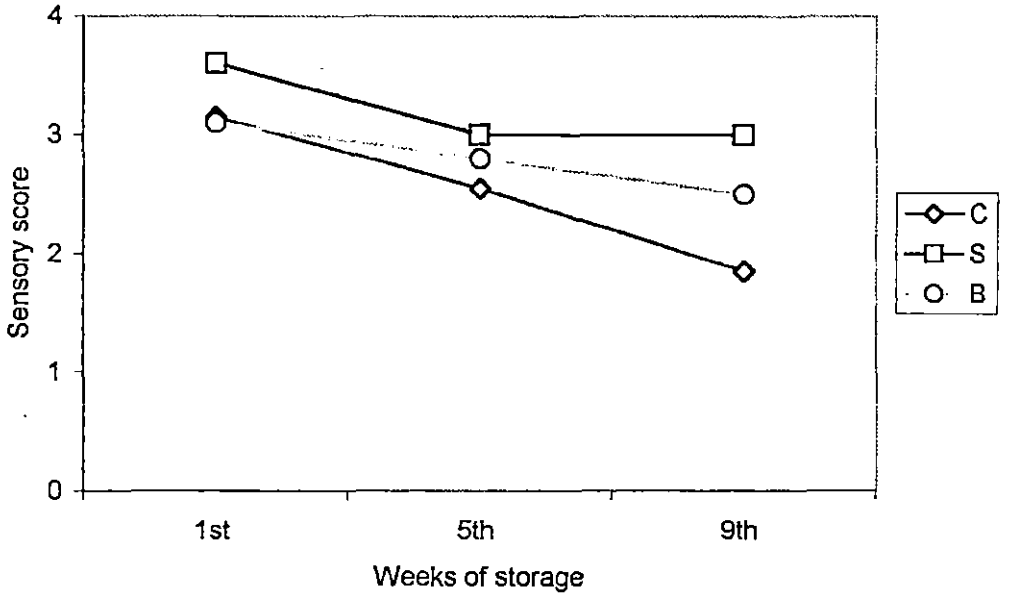
Where, C - Control, S - Salt +citric acid treated, B - BHA treated

Fig. 32. Variation of appearance of cooked cuttlefish fillets - 2nd day frozen



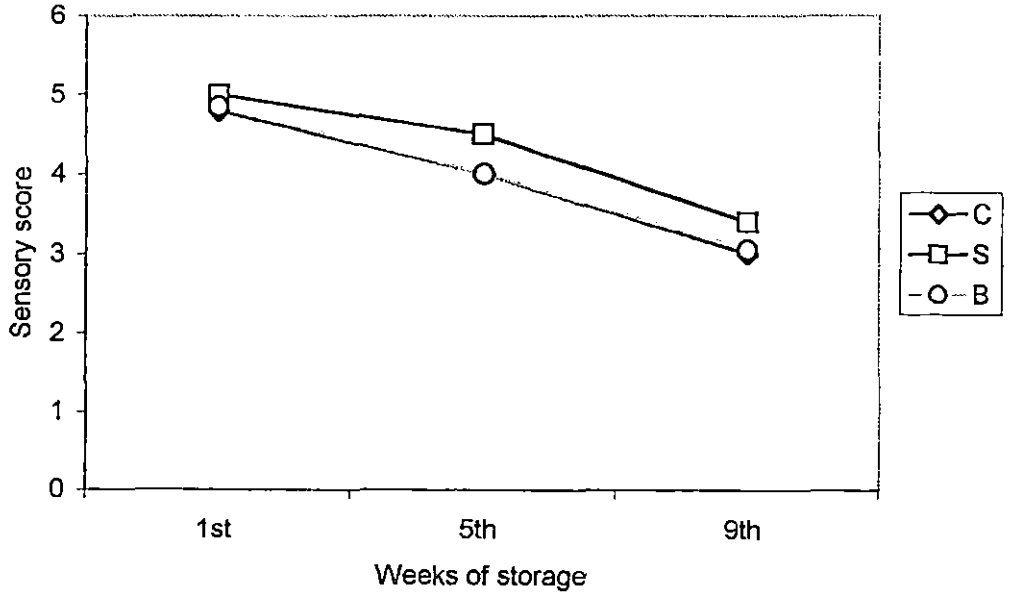
Where, C - Control, S - Salt +citric acid treated, B - BHA treated

Fig. 33. Variation of appearance of cooked cuttlefish fillets - 4th day frozen



Where, C - Control, S - Salt +citric acid treated, B - BHA treated

Fig. 34. Variation of colour of cooked cuttlefish fillets - '0' day frozen



Where, C - Control, S - Salt +citric acid treated, B - BHA treated

acid treated sample to be significantly different from the control and BHA treated sample.

4.5.2.2.2. Second day frozen sample.

After one week of storage the score was 4.6, 4.8 and 4.6, respectively, in the control, salt + citric acid treated and BHA treated samples. After the eighth week, this was freduced to 2.45 ± 0.05, 2.9 and 2.5,respectively. These variations are shown in Fig. 35. The statistical analysis showed significant variation between the control and salt + citric acid treated samples.

4.5.2.2.3. Fourth day frozen sample

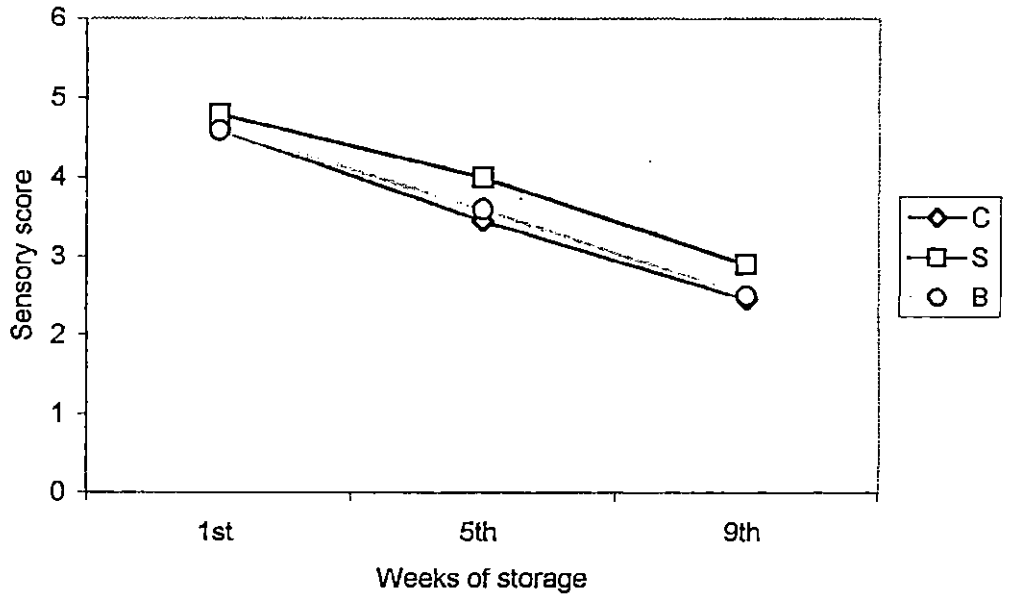
The score for colour after one week of storage was found to be 3.1 ± 0.1, 3.35 ± 0.15 and 3.0, respectively in the control, salt + citric acid treated and BHA treated sample. After the eighth week of storage this was reduced to 1.5 ± 0.5, 3.0 and 2.55 ± 0.05, respectively. These variations are shown in Fig. 36. The statistical analysis showed no significant variation among the three treatments.

4.5.2.3. Texture in cooked cuttlefish fillets

4.5.2.3.1 Zero day frozen sample

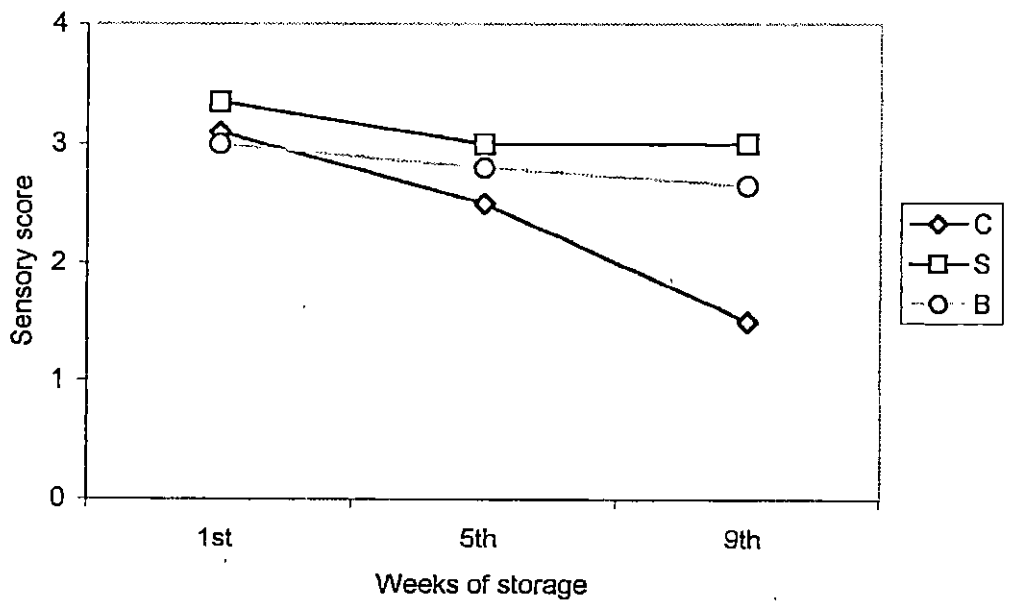
After one week of storage, the score was found to be 4.85 ± 0.05, 5.0 and 4.8,respectively, in the control, salt + citric acid treated and BHA treated samples. This was reduced to 3.1, 3.75 ± 0.05 and 3.05 ± 0.05, respectively, after the eighth week of storage. These variations are shown in Fig. 37. The statistical analysis showed no significant difference among the three treatments.

Fig. 35. Variation of colour of cooked cuttlefish fillets - 2nd day frozen



Where, C - Control, S - Salt +citric acid treated, B - BHA treated

Fig. 36. Variation of colour of cooked cuttlefish fillets - 4th day frozen



Where, C - Control, S - Salt +citric acid treated, B - BHA treated

4.5.2.3.2. Second day frozen sample

The score was found to be 4.6, 4.8 and 4.6, respectively, after one week of storage, in the control, salt + citric acid treated and BHA treated samples. This was reduced to 2.45 ± 0.05, 2.9 and 2.5 after the eighth week of storage. The control and the BHA treated samples were found to be hard to chew after the eighth week. These variations are shown in Fig. 38. The statistical analysis shows significant difference between the control, salt + citric acid treated samples.

4.5.2.3.3. Fourth day frozen sample

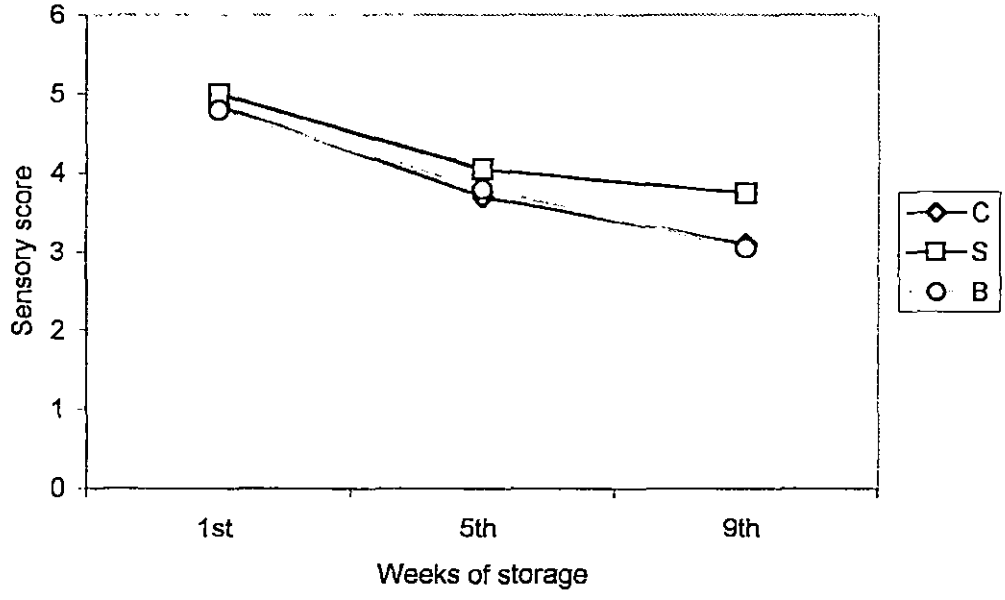
The control, salt + citric acid treated and BHA treated samples gave a score of 2.8, 3.4 ± 0.1 and 3.0, respectively, after one week of storage. This was reduced to 2.0, 3.0 and 2.3,respectively, after the eighth week of storage. The control and the BHA treated sample were hard to chew and showed loss of juicieness. These variations are shown in Fig. 39. The statistical analysis shows significant difference between the control, salt + citric acid treated samples.

4.5.2.4. Flavour of cooked cuttlefish fillets

4.5.2.4.1. Zero day frozen sample

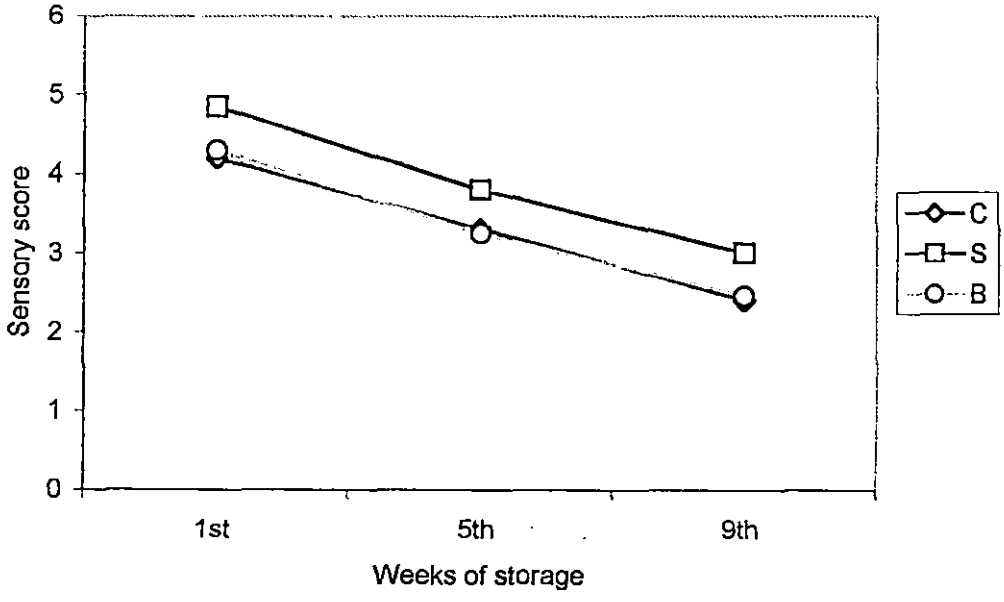
After one week of storage it was found to be 4.9, 4.95 ± 0.05 and 4.9, respectively, in the control, salt + citric acid treated and BHA treated samples. After the eighth week, it was found to be 3.3 ± 0.1, 3.8 and 3.3, respectively. These variations are shown in Fig 40. The statistical analysis showed the salt + citric acid treated sample to be significantly different from the control and BHA treated sample.

Fig. 37. Variation of texture of cooked cuttlefish fillets - '0' day frozen



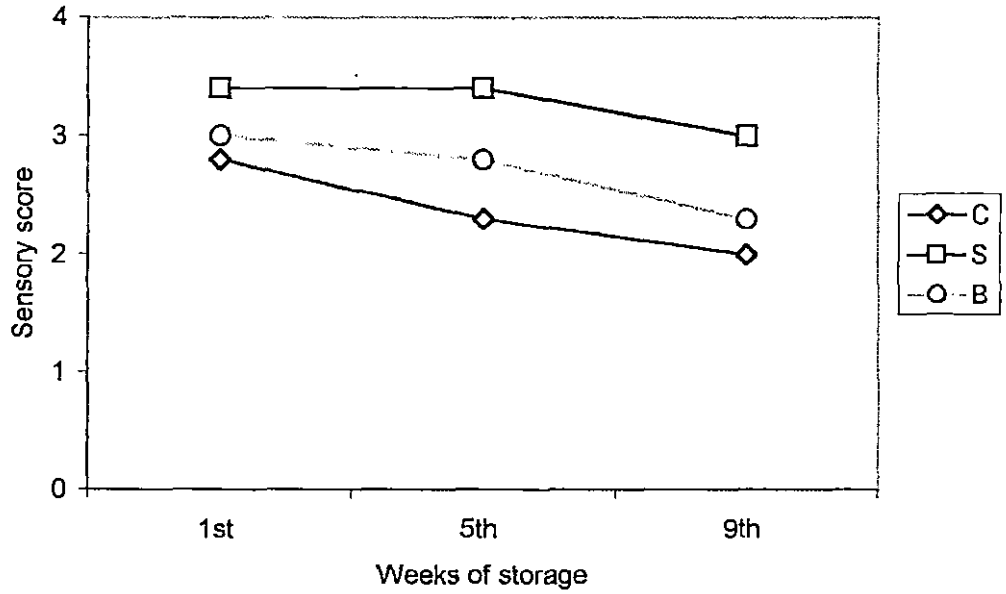
Where, C - Control, S - Salt +citric acid treated, B - BHA treated

Fig. 38. Variation of texture of cooked cuttlefish fillets - 2nd day frozen



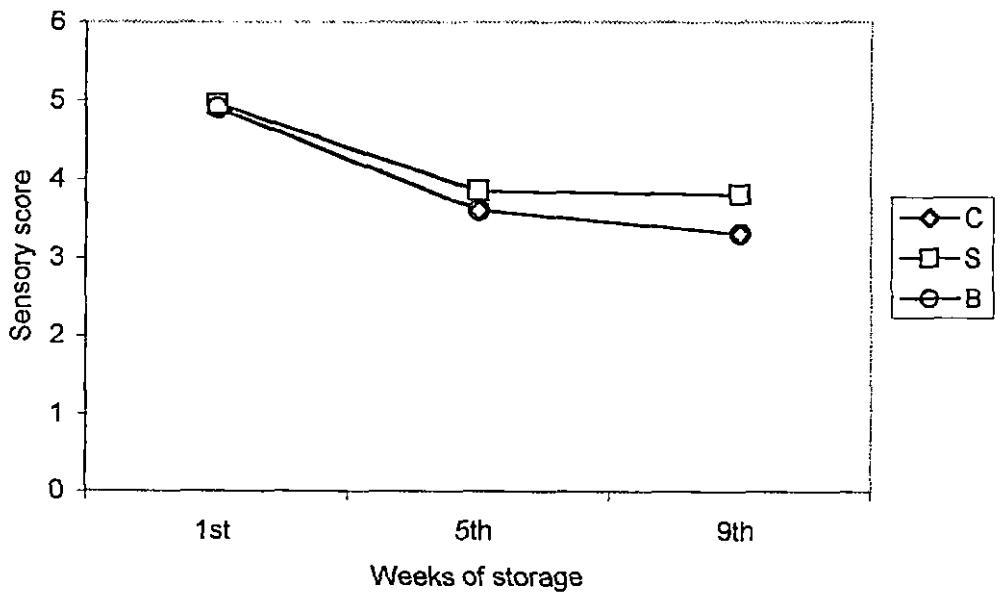
Where, C - Control, S - Salt +citric acid treated, B - BHA treated

Fig. 39. Variation of texture of cooked cuttlefish fillets - 4th day frozen



Where, C - Control, S - Salt +citric acid treated, B - BHA treated

Fig. 40. Variation of flavour of cooked cuttlefish fillets - '0' day frozen



4.5.2.4.2. Second day frozen sample

The score for flavour after one week of storage was found to be 4.45 ± 0.05, 4.75 ± 0.05 and 4.5, respectively in control, salt + citric acid treated and BHA treated sample. After the eighth week it was found to be 2.8, 3.0 and 2.9, respectively. These variations are shown in Fig. 41. The statistical analysis showed the salt + citric acid treated sample to be significantly different from the control and BHA treated samples.

4.5.2.4.3. Fourth day frozen sample.

The control, salt + citric acid treated and BHA treated samples after one week of storage gave a score of 2.8, 3.3 ± 0.1 and 2.9, respectively. After the eight week, it was found to be reduced to 2.1, 2.45 ± 0.05 and 2.2, respectively. The characteristic flavour and sweet taste was completely lost in the control and the BHA treated samples after the eighth week. These variations are shown in Fig. 42. The statistical analysis shows control, salt + citric acid treated samples to be significantly different.

The result of statistical analysis of sensory scores for iced and frozen samples are given in Table 16 and 17 respectively.

The sensory observations for iced and frozen cuttlefish fillets are given in Table 18, 19, 20 & 21.

Fig. 41. Variation of flavour of cooked cuttlefish fillets - 2nd day frozen

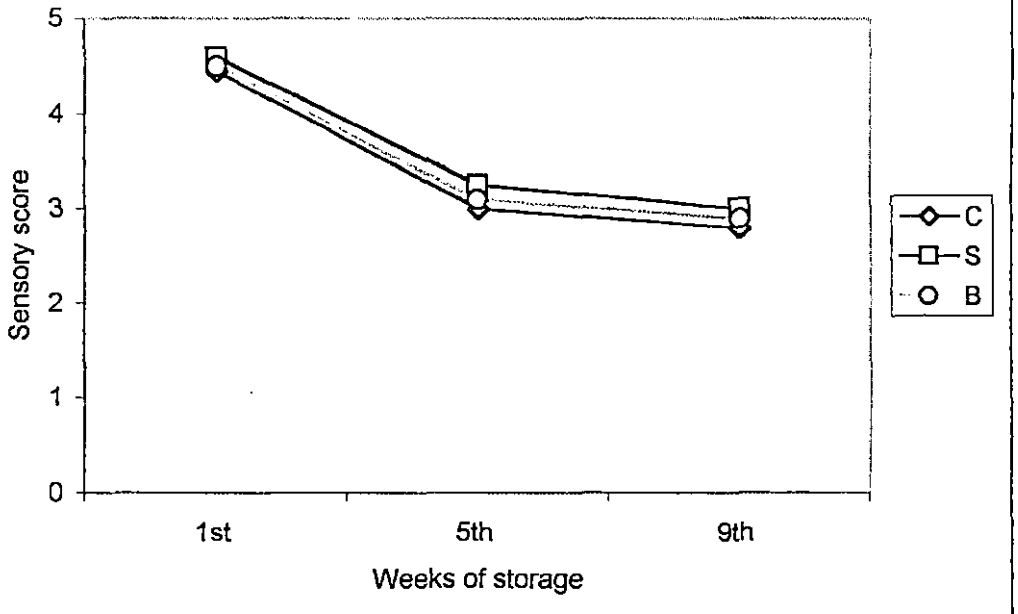
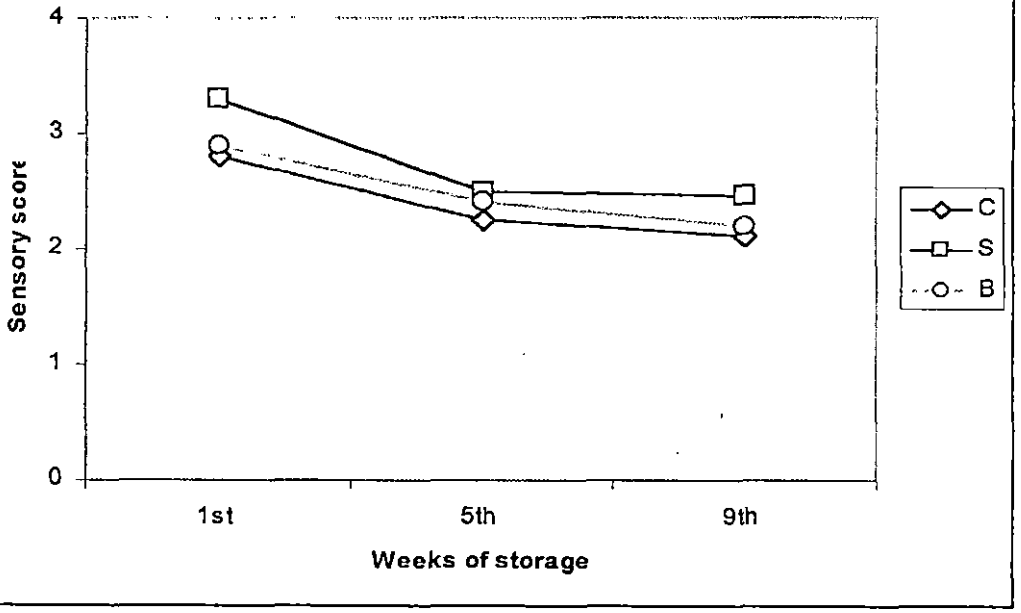


Fig. 42. Variation of flavour of cooked cuttlefish fillets - 4th day frozen



Where, C - Control, S - Salt +citric acid treated, B - BHA treated

Table 16 : Result of statistical analysis of sensory scores of ice stored cuttlefish fillets

Parameters	F -value (Raw sample)	F-value (Cooked sample)
Appearance	6.942*	2.743
Colour	5.615*	5.078
Texture	6.09*	5.313*
Flavour	-	6.09*

Table 17 : Result of statistical analysis of sensory scores of frozen stored cuttlefish fillets

Parameters	1st day frozen		2nd day frozen		4th day frozen	
	Raw	Cooked	Raw	Cooked	Raw	Cooked
Appearance	2.714	4.916	10.231*	8.312*	8.312*	6.94*
Colour	10.231*	9.75*	21*	8.312*	8.312	5.634
Texture	5.142	4.0	6.5	4.22	12*	12*
Flavour	-	12*	-	12.144*	-	12*

* Significantly different at 5% level

Table 18 : Sensory observations of ice stored cuttlefish fillets

Days	Treat-ments	Appearance and Colour	Texture	Flavour(cooked)
0	C	White colour with sheen	Firm & elastic	Characteristic flavour with sweet taste
	S	White colour with sheen	Firm & elastic	Characteristic flavour with sweet taste
	B	White colour with sheen	Firm & elastic	Characteristic flavour with sweet taste
2	C	White with slight sheen	Firm	Fairly good flavour and taste
	S	White with sheen	Firm	Good flavour with sweet taste
	B	White with slight sheen	Firm	Fairly good flavour and taste
4	C	Dull white with no sheen	Fairly firm	Loss of characteristic flavour and taste
	S	White with sheen	Firm	Fairly good flavour and taste
	B	Dull white with slight sheen	Fairly firm	Loss of characteristic flavour and taste
6	C	Dull white with loss of sheen, thin mantle	Slightly soft, difficult to chew	Slight after - taste
	S	White with slight sheen	Fairly firm	No off flavour or taste
	B	Dull white with no sheen	Slightly soft	Bland taste

C-Control, S-Salt+citric acid treated, B - BHA treated

Table 19 : Sensory observations zero day frozen

Treat-ments	Appearance and colour			Texture			Flavour(cooked)		
	1st week	5th week	9th week	1st week	5th week	9th week	1st week	5th week	9th week
C	White, smooth and firm good sheen	Slight loss of white colour and sheen	Dull white loss of sheen	Smooth and firm	Less firm	Less firm	Fresh characteristic flavour and slight sweet taste	Less characteristic flavour, slight sweet taste	Loss of sweet taste and chara-cteristic flavour
S	White, smooth and firm good sheen	White colour and sheen	White slight loss of sheen	Smooth and firm	Smooth and firm	Firm	Fresh characteristic flavour and slight sweet taste	No loss of sweet taste	Slight sweet taste
B	White, smooth and firm good sheen	Slight loss of white colour and sheen	Dull white loss of sheen	Smooth and firm	Less firm	Firm	Fresh characteristic flavour and slight sweet taste	Less characteristic flavour, slight sweet taste	Loss of sweet taste and chara-cteristic flavour

C-Control, S-Salt+citric acid treated, B - BHA treated

Table 20 : Sensory observations second day frozen

Treat-ments	Appearance and colour			Texture			Flavour(cooked)		
	1st week	5th week	9th week	1st week	5th week	9th week	1st week	5th week	9th week
C	Slight loss of sheen and white colour	Loss of white colour, slight loss of sheen	Dull white, loss of sheen and desiccation	Firm & rubbery	Slightly soft	Slightly sticky and hard to chew	Fairly sweet taste	Bland taste	Loss of characteristic flavour, bland taste
S	White colour with sheen	White colour with sheen	Slight loss of white colour and sheen	Firm & rubbery	Firm,	Slightly firm	Fairly good flavour and taste	Loss in characteristic fresh flavour	Faint, sweet taste
B	Slight loss of sheen and white colour	Loss of white colour, slight loss of sheen	Dull white, loss of sheen	Firm & rubbery	Less firm	Soft	Fairly sweet taste	Loss of characteristic flavour,	Bland taste

C-Control, S-Salt+citric acid treated, B - BHA treated

Table 21: Sensory observations fourth day frozen

Treat-ments	Appearance and colour			Texture			Flavour(cooked)		
	1st week	5th week	9th week	1st week	5th week	9th week	1st week	5th week	9th week
C	Dull white loss of sheen	Dull white no sheen, mantle thin	Yellow discolouration on mantle surface, desiccation	Loss of firmness, hard to chew	Soft, difficult to chew	Soft and sticky, hard to chew	Loss of characteristic flavour and sweet taste	Loss of characteristic flavour and sweet taste	Slight after taste
S	White with slight sheen	White with slight sheen	White loss of sheen	Firm	Sligtly firm, not hard to chew	Slightly firm	Faint sweet taste and flavour	Faint sweet taste and flavour	Very faint sweet taste
B	Dull white slight sheen	Dull white, no sheen, mantle thin	Dull white, desiccation	Less firm	Soft	Soft and hard to chew	Loss of characteristic flavour and sweet taste	Loss of characteristic flavour and sweet taste	Bland taste

C-Control, S-Salt+citric acid treated, B - BHA treated

4.6. Proteolytic activity

The muscle tissue from the anterior, middle region, lateral side and posterior region showed the maximum proteolytic activity at pH 7, and the least at pH 3. Among these the lateral and the posterior region showed the highest proteolytic activity.

The μg tyrosine liberated /ml/h is given in Table 22. The statistical analysis showed the proteolytic activity at pH 7 to be significantly different from those at pH 3, 4 and 8. The proteolytic activity at pH 3 and 8 are not significantly different from the activity at pH 4 and 8. The proteolytic activity at the anterior region was significantly lower than that at the lateral and posterior region. The result of statistical analysis is given in Table 23.

Table 22 : µg Tyrosine liberated/ml/h from different regions of the cuttlefish mantle.

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

Table 23: ANOVA for proteolytic activity of cuttlefish fillets at the different regions of the mantle.

Source of variation	D.F.	S.S.	M.S.S.	F. ratio
Between treatments	3	16211.989	5403.996	44.055*
Between blocks	3	5008.519	1669.506	13.610*
Error	9	1103.982	122.664	
Total	15	22324.49		F(5%)=3.29

* Significantly different at 5% level

DISCUSSION

5. DISCUSSION

The control and treated samples of iced and frozen cuttlefish fillets were subjected to different biochemical tests to measure the extent of spoilage and oxidative rancidity. Coupled with this, the sensory evaluation of the raw and cooked samples was also carried out for both iced and frozen cuttlefish fillets. The results were interpreted to find out the reason for yellow discolouration and to suggest a suitable method for preventing it in frozen stored cuttlefish fillets

5.1. Biochemical evaluation of ice stored cuttlefish fillets

5.1.1 Alpha amino nitrogen

Free amino acids have been considered to contribute to, or at least enhance, the "faint sweet flavour of really fresh fish' (Tarr, 1965). Simidu and Takeda (1952) found the mono amino nitrogen content to be abundant in squid so long as the meat tasted sweetish.

Joseph *et al.*, (1977) reported the alpha amino nitrogen content in squid to decrease after two days of storage in ice. Sastry and Srikar (1985) also observed considerable fluctuation in the total free amino acid content in ice stored cuttlefish (*Sepia aculeata)*. The total free amino acid content after recording the highest value on the second day in ice, showed a rapid decrease thereafter, and the lowest value of 2.21 mg/g was observed at the end of 12 days.

In the present study also, the alpha amino nitrogen content showed a steady decrease during the period of storage. Compared to the salt + citric acid treated

sample, the alpha amino nitrogen content became very low by the fourth day of iced storage in the control and the BHA treated sample. By the sixth day, the content showed a rapid decrease in all the three samples. The organoleptic characteristics also showed marked changes by the fourth day of storage. It has been reported that the capacity of fish muscle to bind water, a property reflected in drip, is highest in unfrozen fish before rigor sets, in and decreases with the storage time on ice (Anon, 1958 - 59).

The decrease in alpha amino nitrogen can hence be attributed to leaching that occurs during iced storage. The statistical analysis also showed significant difference among the control, salt + citric acid treated sample and the BHA treated sample.

Subba Rao & Heerdt (1955) also reported that the addition of salt to fish muscle improves the moisture retention. The chelating action of citric acid serves to increase the water retention of the products treated with this substance (Vaessen-schoemaker, 1966). The results of the present study also confirm this. The leaching of amino compounds observed during iced storage of the salt +citric acid treated sample was not so high as in the other two till the fourth day. So the treatment may be considered as effective in preventing leaching through the moisture retention action of salt +citric acid.

5.1.2. Non protein nitrogen

Konosu *et al.*, (1958) reported that cuttlefish and squid contain high quantities of free amino acids and the decrease observed in the NPN content in these during iced storage was attributed to the leaching of free amino acids during storage.

Toyo-o-Takahashi (1965) and Joseph *et al.*, (1977) also reported that the squid meat contains high percentage of NPN and that it decreased during iced storage. Borgstrom (1965) reported high content of NPN in squid. Sastry and Srikar (1985) found the NPN content in cuttlefish (*Sepia aculeata*) to show a steady decrease during iced storage. The NPN content was found to decrease from an initial value of 19.11% of total nitrogen to a final value of 9.1% of total nitrogen at the end of 14 days of storage.

Raghunath (1984) reported a decrease of NPN from 0.72% to 0.43% in squid stored in crushed ice and water in the ratio of 1 : 2 : 0.2, after 8 h of storage. The sweet taste of the meat, which is linked to the NPN fraction, was also found to be affected adversely.

In the present study the NPN content was found to decrease steadily as the storage progressed. The salt + citric acid treated sample showed less decrease in NPN compared to the control and the BHA treated samples as in the case of the alpha amino nitrogen content. This further confirms the water retention action of salt + citric acid. The result is in agreement with that of Subba Rao and Heerdt (1955).

5.1.3. Total volatile base nitrogen

The TVBN, a measure of ammonia, TMA and DMA, has been used to determine early stages of fish spoilage (Wittfogel, 1958). It can also be used for products not containing TMAO (e.g. fresh water fishes). Increase of the TVBN content with fish spoilage has been reported in many studies (Farber, 1965).

The TVBN and TMA values were found to give satisfactory correlation with the organoleptic assessments by using the parametric two way analysis. These were found to be the most suitable quality indices for chilled squid (Kreuzer, 1984). Woyewoda and Ke (1980) suggested limits of acceptability for TVBN as 30-45 mg/100g.

Joseph *et al.*, (1977) found the TVBN content in ice stored squid to increase steadily from an initial value of 3.21 mg/100g. to 18.21 mg/100g by the sixth day. The TVBN in dressed cuttlefish subjected to direct icing and ice-water slurry and packed in polystyrene container, was found to reach the acceptable limit on the tenth day of storage (Anon, 1994b)

In the current study the TVBN content was found to increase considerably after the second day on ice. The control showed the highest TVBN content of 16.619 ± 0.394 by the sixth day of storage. The TVBN content in all the three samples was found to be within the acceptable limit as suggested by Woyewoda and Ke (1980), but organoleptically the meat showed considerable change by the sixth day. The low value of TVBN can be suggested to be due to the increase in the moisture content of the meat during iced storage, which leads to dilution of the solid content, and hence, may mislead if taken as indicators of freshness, when the TVBN is analysed on wet weight basis (Young *et al.*, 1973).

The statistical analysis showed the salt + citric acid treated sample to be significantly different from the control and the BHA treated sample. The salt + citric acid sample showed the lowest value of TVBN even on the sixth day of storage compared to the control and the BHA treated sample. This may be due to the

combined effect of the salt and citric acid in preventing the biochemical degradation of the nitrogenous compounds in the meat, which may be through, the inhibitory action of salt on the microbial growth and /or the enzyme inhibitory action of citric acid by decreasing the pH.

5.1.4. Trimethylamine

The bacterial reduction of the trimethylamine oxide (TMAO), was found to be the source of TMA in fish (Tarr, 1940). This oxide is apparently found only in marine fishes (Castell, 1946). Although the reduction of TMAO to TMA is primarily attributed to the activity of spoilage bacteria, some fishes are reported to have enzymes capable of reducing TMAO, to formaldehyde (FA) and dimethylamine (DMA) rather than to TMA. (Yamada and Amano, 1965). Sikorski *et al.*, (1976) reports that the conversion of, TMAO to DMA and FA is catalysed by an enzyme present in the sarcoplasmic fraction of fish, squid and clams.

Woyewoda and Ke (1980) suggested the limit for TMA in cephalopods as 3-10 mg N/100g. In iced cod stored for 20-25 days, the TMA content was found to increase to 50 mgN/100g, which was the upper limit of acceptability for this fish (Kreuzer, 1984). A number of reports have appeared in which it is found that the content of TMA is not a reliable or reproducible index of spoilage since its increase occurred at later stages of spoilage, or there are variations in the levels between species, or the ranges of values are very wide, or no appreciable change takes place in its content.

In the current study, the TMA content was found to increase rapidly after the second day of storage in ice. The salt + citric acid treated sample showed less

increase compared to the control and the BHA treated samples until the fourth day of storage. By the sixth day, the TMA content was almost the same in all the three samples. The control gave a value of 9.495 ± 0.315 mg%, the salt + citric acid treated sample 8.64 ± 0.362 mg % and the BHA treated sampled 8.825 ± 0.0915 mg%. These values are within the limit suggested by Woyewoda and Ke (1980) eventhough, the control sample was nearing the limit of acceptability The organoleptic evaluation also showed this to be true, since the quality of the control was considerably lowered by the sixth day. The formation of DMA from TMAO can be another reason for the reduced values of TMA content, preventing the increase in TMA (Joseph *et al.*, 1985). It has also been suggested that TMA is a product of early stages of spoilage (Hess, 1941) and that it may be lost indiscriminately during storage.

The statistical analysis showed significant difference among the control and the two treatments. The increase in TMA content was less pronounced in the case of salt + citric acid treated sample, as observed in the case of TVBN content, suggesting again the microbial inhibition by salt and enzyme inhibition by citric acid.

5.1.5. Free fatty acids.

Lipid hydrolysis is a common post mortem feature in fish. Prior to the appearance of oxidative rancidity in lean fish, there is a rise in lipid hydrolysis that leads to a build up of non-esterified ('free') fatty acids, which are more readily oxidised than the esterified fats. It has been commonly observed, that the FFA production is triggered by low temperatures (Dyer *et al.*, 1956; Jonas and Tomilson, 1962). It is most rapid at temperatures just below freezing (Lovern and Olley, 1962),

occurs in firmly frozen material (Wood, 1959) and is catalysed by endogenous enzymes (Olley and Lovern, 1960). Lovern (1961) observed that microbial enzymes contribute little to the process either in iced or frozen fish.

The FFA along with TMA and TVBN has been used in Canada to estimate the changes in squid quality (Kreuzer, 1984). In the current study, up to the second day the BHA treated sample gave very low values for FFA compared to the control and the salt + citric acid treated samples. By the fourth day, the FFA content in the control, salt + citric acid treated and BHA treated sample were found to be 42.097 ± 1.174%, 31.32 ± 0.0987% and 25.173 ± 0.673 %, respectively.

By the sixth day, the control and salt + citric acid treated samples showed a decrease in FFA to 35.807 ± 0.807% and 28.875 ± 0.875% respectively. This may be due to the utilisation of FFA in oxidative changes taking place, thereby lowering the percentage FFA.

The changes in FFA during iced storage have not so far been reported in squid and cuttlefish. The increase in percentage FFA observed in the present study is however in concurrence with those observed in fishes (Jonas, 1969).

5.1.6. Peroxide value.

The estimation of the content of fatty peroxide or hydroperoxides has been used as a measure of rancidity. The hydroperoxides may be regarded as among the early participants in the oxidative chain reaction and often may be detected before any rancidity becomes definitely evident. Depending on the conditions of storage

and the type of fish, the level of peroxide determined at which rancidity becomes sensorily detectable, may vary within rather wide limits (Farber, 1965).

The present study showed the BHA treated sample to give the lowest value of PV during the period of storage. This shows the protective effect of BHA against oxidative changes. BHA is a known effective antioxidant commonly added to food and it also comply with the safety regulations of the Federal Food and Drug Administration of the US. After the second day of storage the increase in PV was at a slow rate. The control gave a value of 11.805 ± 0.6904 milliequivalent/kg fat on the second day, which then increased to 12.517 ± 0.210 milliequivalent/kg fat on the fourth day, which then reduced to 12.132 ± 0.368 milliequivalent/kg fat by the sixth day. This may be due to the peroxide being an intermediary compound in the oxidative changes.

In the salt + citric acid treated sample, the PV was 7.856 ± 0.714 milliequivalent/kg on the second day, which then increased to 9.999 ± 0.909 milliequivalent/kg on the sixth day. The lower value given by the salt + citric acid treated sample compared to the control may be due to the antioxidant property of the citric acid. The citric acid glaze has been reported to extent the storage life of surmai (*Cybium commersonii*) over 5 months (Jadhav and Magar, 1970).

5.1.7. Thiobarbituric acid reactive substances (TBARS)

In fishes, a number of studies on the application of TBA have been carried out as measure of fat rancidity. Ryan and Stansby, (1959) and Andersson and Danielson, (1961) have reported direct correlation of TBA with the sensory judgement of herring rancidity.

The TBARS showed a steady increase in all the samples. This increase in TBARS was the least in BHA treated sample compared to the salt + citric acid treated sample and the control. This shows the protective effect of BHA against oxidative changes. BHA is an antioxidant with free radical scavenging activity. It reacts directly with free radicals formed during oxidation reactions and converts them to less reactive compounds by donating a hydrogen atom rather than blocking the initial free radical generation reaction (Mahoney and Graf, 1986). Till date no literature is available on the effect of BHA treatment during iced storage of squid and cuttlefish. However, Kamasastri, *et al.*, (1967) has reported the BHA treatment to be beneficial in retarding rancidity in frozen pomfrets.

When compared to the control, the increase in TBARS was less in salt +citric acid treated sample. This is because of the retardation of lipid autooxidation through the metal chelating action of citric acid. The presence of transition metal ions such as iron and copper in trace amounts in most foods are well known to act as catalyst of lipid oxidation (Castell and Spears, 1968; Cillard et al., 1980a; b; Mahoney and Graf, 1986). Metal chelating agents such as citric acid and EDTA, chelate metal ions or suppress reactivity by occupying all co-ordination sites on the metal ion (Mahoney and Graf, 1986) and therefore, may be effective agents in retarding metal - catalysed lipid oxidation (Lemon et al., 1950). Metal - catalysed lipid oxidation may be more pronounced in cephalopod meat as cephalopods are reported to accumulate a number of heavy metals (Kreuzer, 1984; Falandysz, 1989 and Oehlenschlaeger, 1991).

5.2. Sensory evaluation of iced stored cuttlefish fillets.

Sensory or organoleptic tests are subjective methods and determine the state of quality and for approximately how long the product will be fit for consumption. The objective methods measure certain components of the products and hence are used as supplement to organoleptic tests in basic storage studies. A value expressed by a number is attributed to each discernable step in spoilage to allow statistical evaluation.

The organoleptic quality with reference to texture and overall acceptance of the ice-stored cuttlefish (*Sepia aculeata*) was adjudged to be in good condition for 2 days, fair upto 4 days and acceptable upto 6 days of storage in ice (Sastry and Srikar, 1985). Hence, for the effective utilisation of cuttlefish it was suggested that, the material should be preserved by freezing within 2 days of storage in ice. Joseph *et al.* (1985) also reported a similar suggestion that based on the biochemical and organoleptic changes, the squid meat cannot be kept in ice in prime condition for more than 2 days. In another study, the dressed cuttlefish subjected to direct icing and in iced water slurry packed in polystyrene container was found to be in acceptable condition even on the tenth day of storage with respect to the odour, texture and flavour. But the whole cuttlefish subjected to the same storage condition was found to be acceptable only upto the eighth day (Anon, 1994b).

In the present study, the salt + citric acid treated sample was found to be organoleptically, in good condition for 4 days and 'fair' upto 6 days of storage with respect to appearance, texture and colour. The control and the BHA treated samples was found to be 'fair' upto the fourth day and by the sixth day of storage they

reached the limit of acceptability with respect to appearance, texture, colour and flavour.

During the period of iced storage, no yellow discolouration was observed in any of the samples. However, the control and the BHA treated samples were found to lose their original sheen, white colour and firm texture with the increase in storage days compared to the salt + citric acid treated sample. This may be attributed to the increased moisture retention and consequent texturisation of the muscle due to salt + citric acid treatment. The better colour of the salt + citric acid treated sample may be due to the effect of citric acid. (ADB/Infofish, 1985).

5.3. Biochemical evaluation of frozen stored cuttlefish fillets

5.3.1. Alpha amino nitrogen

Albette *et al.*, (1986) suggested the decrease in alpha amino nitrogen and salt soluble nitrogen during frozen storage to be due to the protein degradation and loss in thaw drip. Joseph and Perigreen (1988), observed the alpha amino nitrogen in frozen stored cuttlefish (*Sepia aculeata)* to decrease from an initial value of 252 mg/100g to 140 mg/100g by the end of the storage period of 16 months. They also observed the sweet taste of the samples to decrease proportionally, which indicated the possibility of high leaching of amino acid responsible for the sweet taste.

Selvaraj *et al.*, (1991, 1992) and Joseph *et al.*, (1985) also observed the alpha amino nitrogen content in squid to decrease with increased frozen storage duration. Joseph *et al.*,(1985) observed that the dressed mantles of squid kept in ice for one day and then frozen and stored at -20 ± 1^0C, to give the lowest value of alpha amino

nitrogen compared to the dressed mantles which were washed, frozen immediately and stored at -20 ± 1^{0}C. Hence, this showed that the dressed mantles could not be kept in direct contact with ice for long periods because of the rapid leaching of soluble protein and NPN components. The frozen storage study in squid by Selvaraj *et al.*, (1992), showed the polyphosphate treated and the ascorbic acid treated squid to show less decrease in the alpha amino nitrogen content compared to the control.

In the present study, the fourth day frozen sample showed the lowest alpha amino nitrogen content during the period of storage. This can be due to the longer storage duration of the fillets in direct contact with ice (Joseph *et al.*, 1985; Raghunath, 1984). 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 eight weeks of frozen storage compared to the control and the BHA treated sample This can be attributed to the increased water holding capacity of the salt +citric acid treated sample and lower leaching of the free amino acids.

5.3.2. Non protein nitrogen.

Joseph and Perigreen (1988) observed the NPN content in frozen stored cuttlefish fillets (*Sepia aculeata*) to decrease from 28.63 (% of total nitrogen) in the first month to 20.84 (% of total nitrogen) by the end of 16 months storage. Increased drip loss and lower moisture content was also observed after the tenth month. Hence, the loss in water holding capacity and increased leaching of the NPN fraction in the thaw drip was suggested to be the reason for the decrease in the NPN content.

Selvaraj *et al.*, (1991) attributed the decrease in NPN in frozen stored squid to the loss of soluble nitrogen fraction in the thaw drip. They also observed the decrease

in NPN in the ascorbic acid treated sample to be less than that in the control and suggested it to be due to the increased water holding capacity of the treated sample.

In the present study, among the zero day, second day and fourth day frozen samples, the salt + citric acid treated samples showed less decrease in NPN than the control and the BHA treated sample. This can hence be attributed to the increased water holding capacity of the muscle and less leaching of the NPN fractions in the salt + citric acid treated sample. This is in conformity with the results already reported.

5.3.3. Total Volatile Base nitrogen.

The TVBN content in frozen stored squid and cuttlefish has been reported to increase with storage time. In frozen stored squid, Joseph, *et al.*, (1977) found the TVBN content in the control, salt treated and polyphosphate treated samples in the zero week to be 3.2 mg/100g, 5.3 mg/100g and 4.6 mg/100g, respectively. By the nineteenth week, the TVBN content was estimated to have increased to 7.2 mg/100g, 12.8 mg/100g and 9.2 mg/100g. Joseph and Perigreen (1988) also observed a similar increase in TVBN content in frozen stored cuttlefish fillets (*Sepia aculeata*) from an initial value of 3.26 mg/100g to 16.8 mg/100g by the end of 16 months. Selvaraj *et al.*, (1991) also observed the TVBN content to increase in frozen stored squid. They found the ascorbic acid treated sample to show lower values than the control.

In the current study also the TVBN content was found to be increasing steadily in the control, salt + citric acid treated and BHA treated sample. The TVBN content were within the limit even for the four day iced and frozen samples stored for eight weeks. But the control sample was found not acceptable organoleptically i.e.

with regard to texture, flavour and appearance. The value of TVBN in the control even when it was not acceptable may be attributed to its loss by leaching in the thaw drip as suggested by Joseph and Perigreen (1988).

The statistical analysis showed significant difference among the control and the two treatments. The increase in TVBN was significantly less in salt + citric acid treated sample indicating the inhibitory action of salt and citric acid in the biochemical degradation of nitrogenous compounds.

5.3.4. Trimethylamine

Castell *et al.*, (1958) found that the TMA values had a significant correlation with assigned sensory scores of fresh and frozen fish, although, seasonal fluctuations and a species difference in the degree of correlation was noted. Castell *et al.*, (1968) observed that TMA formation increased with frozen storage duration and that it was not due to bacterial action alone. The increase in TMA was also found to increase with rise in storage temperature

Sastri and Srikar (1985) observed an increase in TMA in frozen stored cuttlefish (*Sepia aculeata*). Selvaraj *et al.*, (1991) found the TMA content in frozen stored squid to increase gradually in both the control and in the ascorbic acid treated samples. The ascorbic acid treated sample was found to give an initial value of 1.4 mg%, which increased to 14 mg % by the ninth month of storage.

In the present study also, the TMA content was found to increase gradually in all the three samples. Among the fourth day frozen samples, the control showed the highest TMA content, (9.05 ± 0.017 mg%) after eight weeks, nearing the limit of

acceptability. The salt + citric acid treated and BHA treated samples gave values of TMA within the acceptability limit. The statistical analysis also showed the control to be significantly different from the two treatments. There was no significant difference between the BHA and the salt + citric acid treatment.

The results thus indicate that the production of TMA in the salt + citric acid treated and the BHA treated samples are less compared to control. The salt + citric acid treatment may be inhibiting the bacterial degradation of TMAO to TMA whereas BHA may be acting through its antioxidant property checking TMAO to donate oxygen for autoxidation thus releasing less TMA.

5.3.5. Free fatty acids

The release of FFA by lipid hydrolysis during frozen storage is considered to be one cause of undesirable textural changes, the major quality loss seen in frozen stored lean fish (Gould and Peters,1971) The increase in FFA in frozen stored fish is considered primarily as a phenomenon having a strong bearing on protein extractability, a commonly used index of textural deterioration. Castell *et al.*, (1968) reported the FFA to increase in frozen cod muscle irrespective of the storage temperature

In the present study, the percentage FFA was found to increase gradually in all the three samples. The treated samples showed less rise in percentage FFA compared to the control. The statistical analysis also showed significant difference among the control and the two treatments. No report is available on the changes in FFA during frozen storage of squid and cuttlefish fillets. However, experiments conducted on fish show that FFA increases during frozen storage and treatments with

NaCl and ascorbic acid solutions can check the increase in FFA levels during frozen storage (Joseph and Perigreen, 1988).

Free fatty acids are formed in fish tissue primarily from phospholipids by the action of phospholipases (Bligh, 1961). Temperature has a marked effect on the hydrolysis of lipids in fish tissues. At temperature around the freezing point the rate of hydrolysis increases with decreasing temperature until, such as in cod, a maximum rate is obtained at -4^{0}C (Lovern and Olley, 1962). At lower temperature the rate falls off in a very marked fashion but is still measurable at -30^{0}C.

The results of the present study are in conformity with the above studies. There is an increase in free fatty acids 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.

5.3.6. Peroxide value

The peroxides are found to develop in cold stored flesh and are considered as precursors of break down products that cause rancid flavours in fat. The PV becomes significantly measurable, only after a considerable reduction in quality has occurred and hence is not considered a good index. It is still used nevertheless, as a rough guide to the keeping quality of fresh fish (Liljemark *et al.*, 1959), and to gauge the success of antioxidant glazes (Tarr,1944).

Selvaraj, *et al.*, (1991) observed the PV to increase gradually during frozen storage of squid, and the increase was found to be less in ascorbic acid treated

samples than in the control. They observed a PV of 4.7 milliequivalent/kg fat initially which increased to 39.1 milliequivalent/kg fat by the end of the 9th month.

In the present study, the PV increased gradually in all the three samples. 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, this may be due to the progressive break down of peroxides taking place whereby, the PV showed no much increase. However, the control gave the highest value of PV, 15.277 ± 0.277 milliequivalent/kg fat after eight weeks of storage.

The statistical analysis also showed the control to be significantly different from the two treatments. The BHA treated sample gave the lowest mean value showing its antioxidant property.

5.3.7. Thiobarbituric acid reactive substances

Selvaraj, *et al.*, (1991) observed the TBA values to increase during frozen storage of squid. The increase was found to be less in ascorbic acid treated sample, which gave a value of 3.8 mg/100g meat by the end of nine months of storage. This was attributed to the preventive action of ascorbic acid on lipid oxidation in frozen stored squid.

In the present study, the BHA treated samples gave the lowest values of TBARS during the period of storage. This shows the antioxidant property of BHA. But the fourth day frozen samples gave almost near values for TBARS in the BHA treated and salt + citric acid treated samples after eight weeks of storage. The 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.

5.4. Sensory evaluation of frozen stored cuttlefish fillets

The organoleptic evaluation of frozen squid and cuttlefish are invariably carried out along with the biochemical evaluations in order to find out the shelf life. The high content of NPN and alpha amino nitrogen content has been found to correlate well with the sweetish taste of the fresh squid and cuttlefish (Simidu and Takeda, (1952); Joseph *et al.*, (1977); Joseph and Perigreen 1988). In fresh fishes also, the free amino acids have been considered to contribute to, or at least enhance, the faint sweet flavour (Tarr, 1965). In addition to this, the loss of moisture as thaw drip and cook drip has also been related to the fall in textural qualities and juiciness of the raw and cooked meat (Selvaraj *et al.*, 1991, 1992).

Joseph and Perigreen (1988) while studying the frozen storage characteristics of cuttlefish fillets observed that, initially the fillets were white in colour, firm and chewy. Signs of desiccation was observed by the fourth month, which increased on further storage and the fillets were finally observed to be dull white with yellow discolouration inside and the texture changed to rubbery by the end of the storage period of 16 months and the desiccation made the product unacceptable aesthetically.

In another study, Selvaraj *et al.*, (1991) observed that the ascorbic acid treated squid to be acceptable even after nine months of storage while, the control was found to be unacceptable after 6 months. Selvaraj *et al.*, (1992) also observed that the polyphosphate treated squid remained in acceptable condition throughout the 9 months of storage, whereas, the control became unacceptable by the eighth month of

storage. Joseph *et al.*, (1997) found the control samples of squid to be unacceptable after 15 weeks of storage whereas the salt treated and polyphosphate treated samples remained in good conditions for 19 weeks.

In the current study, the zero day frozen samples of the control, salt + citric acid treated and the BHA treated were found to remain in fair and acceptable condition even after 8 weeks of storage. The biochemical evaluation of the zero day frozen samples also supports this finding. The statistical analysis showed the salt + citric acid treated sample to be significantly different from the control and the BHA treated sample with respect to colour and flavour. This can be reasoned as to the property of the particular treatment in improving the moisture retention property and white colour enhancing quality.

In the case of the second day frozen samples, the salt + citric acid treated sample remained in fair and acceptable condition with respect to the appearance, texture and flavour even after eight weeks of storage. These samples did not lose much of their sheen, firmness of texture and the slight sweetish taste and hence remained in acceptable condition. On the other hand, the BHA treated sample and the control was found to remain in fair and acceptable condition only upto four weeks of storage. After the eighth week of storage they gave a sensory score of below '3' (a score of '3' indicates 'fair and acceptable') however, they were evaluated to be above the borderline of acceptability indicated by the sensory score of '2'. The changes noticed in the BHA treated and in the control samples after the eighth week was, loss in the 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 the eighth week.

The fourth day frozen sample showed the poorest quality after the eighth week in the case of control. In respect to appearance, colour, texture and flavour, the control samples was found to be nearing the limit of acceptability after 4 weeks of storage itself. 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. The texture was found to be soft and sticky, when raw and on cooking it was found to be hard to chew with loss of juiciness. Desiccation of the samples was also observed. Hence the control samples frozen on the fourth day, were found to be unacceptable aesthetically also by the end of the storage period of eight weeks.

In case of the BHA treated samples and the salt + citric acid treated samples no yellow discolouration was noticed ever after 8 weeks of storage. The BHA treated sample after the eighth week, compared to the salt + citric acid treated sample, was found to give a score of below '3' for all the attributes even though, it did not reach the borderline of acceptability. In case of flavour and texture it was found to near the borderline of acceptability. Desiccation was also noticed in the BHA treated samples after the eight week of storage.

The salt + citric acid treated sample remained in fair and acceptable condition with respect to texture and colour even after the eighth week of storage. No desiccation was noticed in this sample. The 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.

The earlier findings also are in accordance with the present observations. Joseph *et. al.*, (1985), has observed that by dressing the squid immediately after capture, the pink and yellow discolouration could be avoided. But, it could not be kept in direct contact with ice for longer periods because of the rapid leaching of the soluble protein and non-protein component, as a result of which the characteristic sweet taste of the squid was lost within two days in ice.

From the present study also, it can be seen that the iced storage of the dressed cuttlefish can help to retard the onset of yellow discolouration, which is clear from the fact that the iced fillets did not show any yellow discolouration in any of the samples during the period of 6 days, but the prime quality of the material was lost as the storage progressed. Further more, the zero day frozen samples also did not show any yellow discolouration even after eight weeks of storage and were found to be of better quality than the second day and fourth day frozen samples. However, yellow discolouration was observed in the fourth day frozen sample after eight weeks of storage. This indicates that storage in ice for a prolonged period and subsequent frozen storage leads to yellow discolouration. This can be checked by treatment with salt + citric acid more effectively than BHA treatment.

5.5. Proteolytic activity

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. 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.

Pink disclouration in skin-on squid/cuttlefish muscle during iced storage is attributed to the penetration of the chromatophore pigment of the skin into the muscle (Dhananjaya *et al.* 1987). In the present study, the deskinned cuttlefish subjected to ice storage did not show discolouration of any sort during the period of study. In the case of frozen stored cuttlefish fillets, the untreated sample frozen on fourth day of iced storage showed yellow discolouration after eight weeks of storage, but the treated samples showed no discolouration. The salt + citric acid treatment was found to be better than BHA treatment. The former treatment prevents protein degradation as well as lipid autooxidation, supporting the Olcott's hypothesis that the yellow disclouration 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 autooxidation. Surface desiccation occurring during frozen storage may accelerate a Maillard type reaction between the amino and carbonyl compounds leading to yellow discolouration.

SUMMARY

6. SUMMARY

1. The objective of the study was to find out the cause of yellow discolouration in iced and subsequently frozen cuttlefish fillets of *Sepia aculeata*. The samples were subjected to the following treatments (1) control (2) a dip in 2% salt + 0.2% citric acid solution for 10 minutes and (3) a dip in 0.01% BHA solution for 10 minutes, to study the effect of these on spoilage and oxidative changes.

2. The biochemical parameters analysed were alpha amino nitrogen content, non-protein nitrogen content, total volatile base nitrogen content, trimethylamine content, percentage free fatty acid, peroxide value and thiobarbituric acid reactive substances. The sensory evaluation of the raw and cooked samples was also simultaneously carried out. The biochemical composition and proteolytic activity of the mantle was also evaluated on the initial day.

3. Both in the iced and frozen stored samples, the alpha amino nitrogen and non protein nitrogen content were found to decrease as the storage progressed. The iced storage for four days prior to freezing was found to have a detrimental effect on the frozen stored material in respect of the two parameters. This decrease could also be correlated with the decrease in flavour and sweet taste of the material. Among the treatments, the salt + citric acid treated sample was found to be significantly different from the control and the BHA treated sample. The decrease in alpha amino nitrogen and non protein nitrogen was found to be significantly less in this treatment.

4. The salt + citric acid treatment, hence, confirmed its effectiveness in preventing leaching through its moisture retention action. The chelating action of citric acid also served to increase the water retention of the products.

5. The TVBN and TMA content were found increasing steadily during the period of storage in both iced and frozen stored materials. However, their contents were found to be within the limits of acceptability and hence could not be correlated in the case of the fourth day frozen samples, to the sensory scores obtained. This can be attributed to the leaching of the components in melting ice and thaw drip.

6. The salt + citric acid treatment showed less increase in TVBN and TMA content during the period of storage in iced and frozen material, which suggests the inhibitory action of salt on the microbial growth and/or the enzyme inhibitory action of citric acid.

7. The BHA treated sample showed less rise in FFA, PV, and TBARS compared to the salt + citric acid treated and the control confirming its antioxidant property. In the case of salt + citric treated samples, the extent of increase in these parameters was less pronounced than in the untreated sample. This probably is on account of the inhibition of phospholipases either by salt and / or citric acid, providing less FFA for auto oxidation.

8. In the ice stored fillets, no yellow discolouration was noticed during the period of study. The untreated and BHA treated samples were found to lose their original sheen, white colour and firm texture compared to the salt + citric acid treated sample. The better colour and texture of salt + citric acid treated sample may be attributed to

the increased moisture retention, texturisation of the muscle and colour improvement action of the treatment.

9. In the case of frozen stored samples, the fourth day frozen untreated sample showed yellow discoluration after eight weeks of frozen storage. This indicates that prolonged storage in ice and subsequent frozen storage leads to yellow discolouration. The salt + citric acid treatment can be considered to be more effective than the BHA treatment in maintaining the organoleptic and biochemical quality of the material.

10. The location where yellow discolouration occurred was found to be high in proteolytic activity

11. The results indicate that the yellow discolouration in frozen stored cuttlefish may be due to a Maillard or an aldehyde amine type reaction as suggested by Olcott (Schultz *et al.*, 1962) in the case of rusting of frozen fish. Treatment of the fillets with salt + citric acid was found to be better than treatment with BHA indicating the possible role of desiccation in yellow discolouration reaction.

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CAUSE OF YELLOW DISCOLOURATION IN ICED AND FROZEN CUTTLEFISH FILLETS AND ITS CONTROL

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ABSTRACT

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The cause of yellow discolouration in ice stored and subsequently frozen cuttlefish fillets of *Sepia aculeata* was investigated in this study. The fillets were subjected to the following treatments (1) control (2) dip in 2% salt + 0.2% citric acid solution for 10 min. and (3) dip in 0.01% BHA solution for 10 min., prior to ice storage for a duration of six days. The iced fillets were frozen on the zero day, second day and fourth day of iced storage. Both the iced and frozen samples were periodically subjected to various biochemical and sensory evaluations.

The NPN and alpha amino nitrogen contents of the salt + citric acid samples, both in the iced and the frozen samples, were found to be higher than those of the other two. This suggests that salt + citric acid are capable of retaining moisture and preventing the leaching of NPN compounds. This is supported by the sensory evaluation results, which gave higher scores salt + citric acid treatment. The increase in TVBN and TMA contents were found to be less pronounced in the salt + citric acid treated samples suggesting the microbial and enzyme inhibitory action of the salt and citric acid. The increase in the PV and TBARS were also less in this treatment when compared to the control, showing antioxidant property of citric acid. The BHA treated samples showed the least rise in PV and TBARS both in iced and frozen material, indicating its effectiveness as an antioxidant.

Sensory evaluation of the iced samples showed no discolouration during the period of six days, but the fourth day frozen untreated samples showed yellow discolouration after the eight weeks of storage. Since the salt + citric treatment and BHA treatment are effective in retarding the yellow discolouration, a Maillard or an

aldehyde amine type reaction is postulated as the possible cause of yellow discolouration in frozen stored cuttlefish. As the proteolytic activity at the site of appearance of yellow discolouration was high, the amino compounds formed by the enzyme action may be taking part in the aldehyde - amine reaction, the aldehyde being produced by autoxidation of the unsaturated phospholipids of the meat.

Appendix 1

A copy of guideline used to evaluate the sensory quality of cuttlefish fillets

Numerical rating	5	4	3	2	1
Appearance	Slightly glossy. Surface smooth and firm	Slight loss of sheen. Very slightly bruised	Slightly sheen. Slightly scabby and slightly bruised outside. Slightly chalky and slightly curdy inside.	Bruised and moderately scabby outside. Chalky and curdy inside. No sheen	Blemished and scabby. Washed out. Brushed outside. Very chalky and very curdy inside
Colour	White	Off white	Dull white	Yellow to light brown	Pinkish brown
Texture	Smooth. Firm and rubbery (outside) spongy (inside)	Rubbery. Firm. Slightly chalky and slightly crumbly (inside). Slightly tough (outside) on cooking	Sticky and gluey (outside), chalky and crumbly (inside)	Slightly mushy (outside). Slightly blemish and scarred. Mushy (inside) and slimy	Outside mushy with curdy interior. Very crumbly. Highly blemished and scarred.
Flavour	Fresh (Seawater) flavour. Sweet taste	Slight fresh flavour. Slight cabbage flavour. Very slight after-taste	Cabbage flavour. Slight after - taste	Slightly bitter, cheese -like flavour. After - taste	Very bitter. Very cheesy and offensive. Foul, putrid and sickening

From Botta, et al., (1979)
Overall acceptability : 5 - Very good; 4 - Good; 3 - Fair; 2 - Slightly spoiled; 1 - Spoiled