Sensory, biochemical and bacteriological properties of octopus (*Cistopus indicus*) stored in ice

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Abstract Octopus (*Cistopus indicus*) were examined for the changes in autolytic activity, ammoniacal nitrogen, non-protein nitrogen (NPN), total volatile base nitrogen (TVBN), free fatty acid (FFA) content, aerobic plate count (APC) and sensory quality based on Quality Index Method (QIM) during ice storage. They were sensorily acceptable up to 7 days when QIM score was 10.97 out of 16.00. Autolytic activity increased from the initial value of 174 to 619 nmoles Tyr/g/h within day 3 and later decreased. There was also an increase in NPN (34.88 to 76.16 mg %), ammoniacal nitrogen (0 to 7.30 ppm) and free fatty acid content (0.35 to 1.69 % of oleic acid) during storage. TVBN values did not correlate with the spoilage, as it increased from 28 to 145 mg% within day 5, exceeding the limit of acceptability; although total QIM score was 7.47. Aerobic plate count did not show significant change suggesting that the spoilage in octopus was not microbial. The rapid spoilage in octopus was mainly due to the release of NPN compounds following autolytic activity leading to the formation of ammoniacal nitrogen, rather than microbial spoilage. Hence, ammoniacal nitrogen can be taken as an index for spoilage of ice stored octopus.

Keywords Octopus · Iced storage · QIM · Biochemical · Bacteriological

Introduction

Cephalopods constitute an important marine resource suitable for human consumption. They are nutritious with white flesh,

mild flavour and meaty texture and has more edible part (80–85 %) than that of crustaceans (40–45 %), and teleosts (40–75 %) (Kruezer 1984). Although they are consumed more in Mediterranean and Japan regions, recently they have received more attention in many countries that were not traditionally cephalopod consumers (Barbosa and Vaz-Pires 2003).

Among the cephalopods, octopus is one of the major species but it is least exploited in India. There are about 38 species of octopus recorded from Indian seas. The commonly occurring species are *Octopus dollfusi*, *O. membranaceus*, *O. globosus*, *O. aegina* and *Cistopus indicus*. The consumption of octopus is not so popular in India; therefore they are exported to countries like Thailand, China, USA, Greece, Taiwan, Vietnam, Germany, UK and Japan in frozen forms. They are also marketed in a variety of value added forms such as chilled, dried and canned products as well as readymade meals.

Cephalopods undergo very rapid spoilage due to the action of endogenous and bacterial enzymes on protein. Protease plays an important role in the breakdown of protein and its activity is higher than in various species of fish (Hurtado et al. 1999). The major quality indices used to assess freshness or spoilage of fish viz K value, TVBN, pH and polyamines (except agmatine) are not suitable quality indices for cephalopods as some of the pathways of formation of spoilage products are different (Ohashi et al. 1991).

There has been a greater focus on the freshness assessment, quality assurance and marketing of finfish and crustaceans than cephalopods. Among the cephalopods, studies are mainly focused on squid and cuttlefish; and there are only a very few reports on the iced storage of octopus (Vaz Pires and Barbosa 2004; Lougovois et al. 2008). Studies have been carried out on the chilled storage of pressurized octopus (Hurtado et al. 2001) and vacuum packaging as well as addition of oregano essential oil on the shelf life of octopus (Atrea et al. 2009).

Although the export of octopus is gaining momentum in India, the processing industry faces severe problem due to the

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rapid spoilage and off odour development of chilled and frozen octopus that has led to frequent rejection of commodities by the importing nations. Examination of routine microbiological as well as biochemical quality indices prescribed for fish by the regulating authorities has failed to judge the decomposition status of octopus. This industrial problem was hence taken up with an objective to assess the sensory, microbiological and biochemical properties of the ice stored octopus in order to formulate effective strategies for controlling spoilage and increase their shelf life.

Materials and methods

Raw material and processing

Fresh octopus, *Cistopus indicus* procured from the Fishing Harbour, Thoothukudi (Tamil Nadu, India) were brought to the laboratory in iced condition. The average weight of the octopus was 140 ± 30 g. They were washed in potable water, eviscerated, washed again and dipped in 2 ppm chlorine water for 1 min. They were then packed individually in polythene bags and kept in an insulated container over the layers of ice at a ratio of 1:1. Fresh raw octopus and octopus stored in ice are shown in Photographs 1 and 2 respectively. The insulated container was held in chill cabinet (vertical DTC 303, Sri Ganapathy Enterprises, Salem, Tamil Nadu) at 4 °C to avoid rapid melting of ice. The melt water was removed every day and fresh ice was added to compensate the loss. At the time of sampling, 3–4 packs were withdrawn for sensory analysis every day, and once in 2 days for biochemical and microbiological analysis. All analysis was carried out in triplicate (Photographs 1 and 2).

Sensory analysis

Sensory analysis was done by a panel of six judges by Quality Index Method (QIM) developed for octopus (Barbosa and Vaz-Pires 2003) as given in Table 1. The

Photograph 1 Raw material-Octopus

Photograph 2 Packed octopus for storage

attributes tested were appearance/colour, odour, mucus of skin; texture of flesh; the condition of cornea and pupil of eyes; colour, odour, mucus of mouth region and material in the sucker of arms. The average scores of individual attributes were computed and then the overall quality scores were derived for comparison.

Biochemical analysis

Autolytic activity Autolytic activity of the octopus muscle was determined by the method of Hurtado et al. (2001). The octopus muscle homogenate was mixed with five volumes of chilled 0.15 M NaCl and filtered through the moist gauze. A portion of the filtrate was incubated in a thermostatic water bath for 1 h at 40 °C and the enzyme reaction was stopped by the addition of two volumes of 10 % TCA. The mixture was incubated at 4 °C for 15 min to allow unhydrolysed protein to precipitate and again centrifuged at 6100 rpm for 15 min at 4 °C in a refrigerated centrifuge (Universal 32 R, Andreas-Hettich, Germany). The supernatant was analyzed for tyrosinase activity by the method of Lowry et al. (1951) at 660 nm by UV Vis Spectrophotometer (Model V-530, Jasco, Japan) using tyrosine as the standard. The autolytic activity was expressed in terms of tyrosinase activity as n moles Tyr/g/h.

Non-protein nitrogen Non-protein nitrogen (NPN) was estimated by following the AOAC method (AOAC 1995). The octopus muscle homogenate was mixed with five volumes of 7 % TCA to precipitate the protein and filtered through Whatman No.1 filter paper. The filtrate was digested with conc. H₂SO₄ in a Kjeltac digestion apparatus. A portion of the digested sample was distilled and the distillate was collected in boric acid, which was then titrated against 0.02 N H₂SO₄ with mixed indicator. The NPN values were calculated and expressed in mg%.

Ammoniacal nitrogen Ammoniacal nitrogen was determined by steam distillation (APHA 1992). The octopus muscle homogenate was mixed with 10 volumes of distilled water and filtered through Whatman No.1 filter paper. To a known

Table 1 QIM scheme for whole raw octopus

Freshness quality parameters		Description	QIM score
Skin	Appearance/	Very bright, well marked colours, white in the clearest parts of the body, skin elastic	0
		Colour	Bright, less coloured, slightly pink in the clearest parts of the body, skin with low elasticity
		Less bright, colourless, orange or bright spots, colour somewhat more orange, rose in the clearest parts of the body, shrunken skin	2
	Odour	Sea weedy,(sea) fresh	0
		Slightly sea weedy, slightly grassy, neutral	1
		Metallic, grassy, acid, intense	2
	Mucus	Transparent, watery	0
		Slightly milky, viscous (sticky), moderate or absent	1
	Flesh	Texture	Firm, tense
Flaccid, soft			1
Eyes	Cornea	Translucent	0
		Slightly opalescent	1
		Opalescent	2
	Pupil	Black, shining	0
		Black, dark red, muddy	1
		Dark red, opaque, normally blood stained	2
Mouth region	Colour	White, yellowish	0
		Slightly rose	1
	Odour	Sea weed or neutral	0
		Sulphurous, citric, sweet, acid	1
	Mucus	Clear	0
		Milky	1
		Yellowish	2
Arms	Material in the sucker	As a film all over the sucker	0
		Starting to agglomerate in the centre of the sucker	1
		Completely agglomerated in the centre of the sucker	2
Range of QIM score			0–16

volume of filtrate, five portions of borate buffer were added and the pH was adjusted to 9.5 using 6 N NaOH. From this, 5 ml of the aliquot was distilled for 10 min in a Kjeldal distillation apparatus to liberate nitrogen and the distillate was collected in 2 % boric acid, which was then titrated against 0.02 N H₂SO₄ using mixed indicator. The amount of ammoniacal nitrogen was calculated and expressed as ppm.

Total volatile base nitrogen Total Volatile Base Nitrogen (TVB-N) was determined by the steam distillation method described by Antomaropoulos and Vyncke (1989). The octopus muscle homogenate was mixed with five volumes of 6 % perchloric acid to precipitate the muscle protein and centrifuged at 4000 rpm for 5 min at 5 °C in a refrigerated centrifuge. Then, 5 ml of the filtrate was distilled for 10 min in a Kjeldal distillation apparatus and the distillate was collected in 2 % boric acid, which was then titrated against the 0.05 N HCl using mixed indicator. The amount of TVB-N was then calculated and expressed as mg %.

Free fatty acid Free fatty acid (FFA) content was determined by the method of Takagi et al. (1984). The octopus muscle homogenate was mixed with 15 g of anhydrous sodium sulphate to remove the moisture and then extracted with three volumes of chloroform: methanol mixture (2:1). The amount of FFA present in a portion of extract was measured by titration with 0.05 N NaOH solution using metacresol purple as indicator. The FFA values were calculated and expressed as % of oleic acid.

Bacteriological analysis

Aerobic Plate Count of octopus muscle was determined by standard methods (APHA 1995). The muscle, 10 g was homogenized, with 90 ml of sterile physiological saline. Serial dilutions were then made subsequently with the same diluents. Suitable dilutions were pour plated onto Plate Count Agar and incubated at room temperature for 48 h. The counts were expressed as the log of colony forming units per g (CFU/g).

Results and discussion

Sensory quality

Quality Index for octopus stored in ice The overall average quality index scores of ice stored octopus are given in Fig. 1. The QIM being a demerit score method, the score increased with the increase in storage time as the sensory quality deteriorated ($r^*=0.99$). The skin was initially very bright, with well marked colour, elastic and white, (Photograph 3) and later turned slightly pink with loss of brightness (Photograph 4) on day 5 and finally became dull and pinkish on day 8 (Fig. 2). The formation of pink colour is found to be a common phenomenon in cephalopods. The chromatophores in the skin break down easily during storage, leading to pink discolouration (Botta et al. 1979; Ohmori et al. 1975; Lapa-Guimaraes et al. 2002). In addition, stacking and abusive handling can also lead to pink colouration (Sungsri-in 2010). The mucus in the skin was transparent initially which started becoming sticky from day 5 to.

The texture of the muscle was initially firm and elastic, which became soft from day 6 onwards. The endogenous proteases play an important role in softening of cephalopod tissue, including octopus (Hurtado et al. 1999; Hatate et al. 2000). The cornea was initially translucent which became slightly opalescent on day 2 and completely opalescent from day 5 onwards. The pupil was black and shining initially. The shininess was lost on day 1 and the pupil became opaque from day 5 onwards. The initial fresh and seaweedy odour of the sample vanished on the day 4 and the odour became neutral on day 5 and later turned ammoniacal on day 9, ie on the day of rejection. In support of our findings, Barbosa and Vaz-Pires (2003) perceived slightly unpleasant odour in ice stored octopus between days 4 and 5, which later became unacceptable on day 8. Lougovois et al. (2008) observed sour and rotten odour but not ammoniacal odour during the spoilage of whole musky octopus stored in ice. As the octopus stored in ice was wrapped in polythene bags, the ammonia liberated due to

Photograph 3 Fresh octopus

autolytic degradation might have got trapped within the bags instead of getting dissolved in ice water and this had led to the formation of ammoniacal odour.

In the mouth region, there was a change in colour from white to pink on the day of rejection. The odour was sweetish on the day 5, after which it turned acidic and ammoniacal on day 8 (Fig. 3). Thus pink discolouration and ammoniacal odour were the predominant sensory changes noticed during ice storage of octopus.

Proximate composition

The proximate composition of octopus (*Cistopus indicus*) is as given in Table 2.

Biochemical quality

Autolytic activity Autolytic activity is generally expressed in terms of tyrosinase activity because L-tyrosine is the major monophenolic substrate in animals, including fish. Octopus initially had an autolytic activity of 174 n mol tyrosinase / g / h, which increased significantly ($p<0.05$) to a maximum of 620 n mol tyrosinase / g / h within day 3 of storage (Table 3). Later on, the activity decreased gradually and continued to remain till the day of rejection. This was because of the higher

Fig. 1 Overall Quality Index Score for octopus stored in ice

Photograph 4 Spoiled octopus

Fig. 2 Quality Index Score for skin, flesh and eyes of octopus stored in ice. S. App/Colour- skin appearance and colour; S. Mucus- skin Mucus; S. Odour- skin odour

proteolytic activity during initial stages of spoilage and subsequent reduction in the substrate protein. With respect to squid, Sungsi-in (2010) reported that the autolytic activity was high on day 4 of storage, followed by a reduction on day 7 and later increased on day 10. The initial increase was also attributed to the variation in muscle protease activity affected by post rigor condition and muscle softening. The final increase was thought to be due to the presence of microbial proteases Gomez-Guillen et al. (2003). Vaz Pires et al. (2008) stated that the autolytic activity of endogenous enzymes rather than microbial enzymes was mainly responsible for the changes in sensory attributes. Hurtado et al. (2001) however observed no change in the autolytic activity values of untreated and pressurized octopus muscle during chilled storage (3 °C) and attributed that pressurization at 400 MPa had inhibited the activity of muscle proteases.

Non - protein nitrogen NPN compounds such as free amino acids, betaines, TMAO, TMA, agmatine, adenine, adenylic phosphoric acid, choline, hypoxanthine, methyl agmatine

Fig. 3 Quality Index Score for mouth and skin of octopus stored in ice. M. Colour- mouth colour; M. Odour- mouth odour; M. Mucus- mouth mucus; A. Sucker Mat.- sucker material in arms

Table 2 Proximate composition of octopus, *Cistopus indicus*

Parameters	Composition (%)
Moisture	84.15±0.59
Protein	13.33±0.09
Lipid	0.64±0.01
Ash	0.31±0.01
Carbohydrate	0.05±0.00

and urea have been identified in muscle of various octopuses (Asano 1957). These compounds increased consistently in octopus stored in ice from 34.88 mg% to 76.16 mg% (Table 3). This was in accordance with the report of Capillas et al. (2002) who had stated that there was an increase in NPN extractives of valador, puta and octopus wrapped in polyurethane films and held in ice. However, Venkatappa (2006) have reported a decrease in the NPN values with an increase in storage of octopus held in alternate layers of ice in an insulated box. Wrapping of octopus in polymer films could have trapped most of NPN compounds within the pack, while direct storage in ice could have aided in leaching of these compounds in the ice melt water.

Ammoniacal nitrogen Ammoniacal nitrogen is responsible for the development of ammoniacal odour in the seafood and hence considered as an important index of decomposition. Ammonia is derived from enzymatic deamination of free amino acids or from decomposition of nucleic bases (Huang et al. 1993). The ammoniacal nitrogen was not detected in the iced octopus until day 3, and it appeared in slightly lower concentration on day 5 and thereafter increased around five times on day 7, the day until it was sensorily acceptable (Fig. 4). The increase was attributed to the production of ammonia due to the bacterial deamination of proteins, amino acids and other basic nitrogenous compounds in the flesh of cephalopods (Lougovois et al. 2008). Paarup et al. (2002) reported an increase in ammoniacal nitrogen of squids stored in ice similar to this finding.

Total volatile base nitrogen Total volatile base nitrogen (TVB-N) primarily includes trimethylamine (TMA), ammonia and dimethylamine (DMA). This index reflects later stages of spoilage rather than freshness. On day 1, the average TVB-N value was 23.5 mg %, which sharply increased significantly ($p<0.05$) to 144 mg % within day 5 and later declined gradually on day 9 to 105 mg % (Table 3). There are few reports that there was a steady increase in the TVB-N levels in octopus stored in ice until the day of rejection (Atrea et al. 2009). Paarup et al. (2002) proposed the limit of acceptability for TVB-N as 45–60 mg/100 g for pressurised vacuum packed squid. However, Ohashi et al. (1991) found that TVB-N values were not suitable as freshness indicators for the quality

Table 3 Changes in biochemical and bacteriological quality of octopus during iced storage

Days/ Parameters	Autolytic activity n mol tyrosinase / g / h	NPN mg %	TVB-N mg %	APC CFU/g
0	174±38 ^a	34.88±2.13 ^a	23.50±4.20 ^a	4.023±0.14 ^a
1	223±45 ^a	32.48±2.43 ^a	28.00±4.37 ^a	4.176±0.25 ^a
3	619±59 ^b	43.44±5.05 ^b	58.00±9.94 ^b	4.377±0.47 ^a
5	470±36 ^c	49.28±6.40 ^b	144.90±8.41 ^c	4.835±0.29 ^b
7	432±40 ^c	45.92±4.25 ^b	136±8.90 ^c	4.607±0.23 ^b
9	346±27 ^d	76.16±5.96 ^c	105±3.79 ^d	3.096±0.21 ^c

All values are mean±standard deviation of triplicate analysis ($n=3$). Different superscripts in the same column indicate significant differences ($P<0.05$)

of octopus, as some of the pathways are different. Our findings also supported this fact, as TVB-N values increased initially on storage and later decreased in the decomposed octopus.

Free fatty acid value FFA is an index of hydrolytic rancidity due to the formation of free fatty acids from triacylglycerides by the action of autolytic lipases. The lipid content of the octopus was quite low (0.64 %) compared to other teleosts. In this study, the FFA was detected initially at low concentration until day 3 and later increased during storage with the significant increase ($p<0.05$) noticed between days 5 and 7 (Fig. 5). The increase in FFA was also reported in octopus stored in ice Venkatappa (2006), and cuttlefish fillets stored in ice (Joseph and Sherief 2003). They observed changes in FFA irrespective of the method of icing the octopus ie. with or without overwrapping with polythene films.

Bacteriological quality

Aerobic plate count (APC) provides an estimate of the total number of aerobic micro organisms in foods. The initial microbial load in octopus was log 4.023 CFU/g, which slightly reduced ($p>0.05$) on day 1, and then remained more or less constant (Table 3). Rapid chilling of octopus after harvest and

maintenance of low temperature during subsequent storage had prevented the growth of bacteria. This result clearly demonstrated that the spoilage of octopus was mainly autolytic and not microbial. Hurtado et al. (1999) also stated autolysis was more intense in octopus than microbial action. The total aerobic plate count at the point of rejection of fish products is usually log 7–9 CFU/g (Huss et al. 1997; Olafsdottir et al. 1997) and such higher counts were not obtained even on the day of rejection in the iced octopus. To supplement this, Ohashi et al. (1991) had reported that total aerobic bacteria count did not change significantly during 2 weeks of storage of squids in ice.

Conclusion

The octopus had a short shelf life of only 7 days in ice when wrapped with polythene bags. Pink discolouration and ammoniacal odour were the predominant sensory changes noticed during ice storage of octopus. Total volatile base nitrogen values cannot be a quality index for ice stored octopus. Ammoniacal nitrogen increased steadily during ice storage and also coincided with changes in the sensory attributes analysed by QIM and can be considered as suitable index to monitor the quality in seafood industries.

Fig. 4 Changes in ammoniacal nitrogen of octopus stored in ice **Fig. 5** Changes in free fatty acid of octopus stored in ice

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