International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Effect of Packaging in Biogenic Amine Production in Indian Squid (*Loligo duvaucelii*) Stored in Iced Condition

Anju K A[1], Sankar T.V[2]

Central Institute of Fisheries Technology, Cochin, 682029, Kerala

Abstract: ***Changes of seven biogenic amines (putrescine, cadaverine, spermidine, spermine, Agmatine, histamine, and tyramine) in squid during ice storage with or without packaging were investigated. The highest level of biogenic amines was obtained from squid stored in ice without packet. Among the biogenic amines putrescine, cadaverine, agmatine and tyramine contents increased during the storage of packed squid held in ice, reaching levels of 107.37mg, 69.72 mg, and 125.2 mg and 2.62 mg /kg muscles at 15 days of storage, respectively. No significant differences were found in histamine concentrations within the treatments during the early stages of the storage period. However, there was a significant difference (P < 0.05) towards the end of the storage period in all treatments. Chemical, sensory and microbial qualities were measured throughout the storage time to determine the changes that took place and to evaluate the effects of both storage conditions.***

Keywords: squid, histamine, biogenic amines, LDPE packaging, ice storage

1. Introduction

Among the cephalopods, squid and cuttle fish contributed to the major export value item accounting for a share of 10.24 % of the total US $ earnings during the period 2013-14. (MPEDA, 2014). The world total catches of cephalopods (squids, cuttlefishes and octopuses) were higher than 3.3 millions of tons between 1999 and 2001 (FAO, 2001). Within the *Loliginidae* family, squids of the Loligo genera are generally regarded on the world market as more valuable, mainly due to their excellent sensory properties (Zdzislaw, 1986).

Fresh squid is a highly perishable product. The fresh squid can be stored only for a short time. For commercialization, it is essential to estimate accurately its freshness, one of the most important aspects of squid and squid products. There are few scientific studies providing information on quality changes of *Loligo duvaucelii* squid after catch and during storage. This knowledge is greatly needed to develop adequate storage methods and tests for freshness evaluation. Many chemical indices for freshness evaluation of fish, crustaceans and mollusks are based on changes of non-protein nitrogen (NPN) components during the storage, such as volatile basic nitrogen (VBN) and trimethylamine (TMA), determinations of free tryptophan (Romo et al., 1996) and urea (Romo et al., 1996; Civera et al., 1999; Paarup et al., 2002). Other studies have shown that acceptable limits are dependent on the species and storage conditions, and thus, highly variable. Sensory perception is one of the most important method for assessing freshness and quality in the fisheries sector and in fish inspection services, however it is subject specific. Different freshness or spoilage index have been established for fish as indicators of decomposition but not for squid.

Biogenic amines are non-volatile compounds, which are formed at very low levels in fresh fish, and their accumulation is related to bacterial spoilage (Mackie, 1977). Studies proposed that biogenic amine can be used as markers to evaluate fish freshness (Emborg et al., 2006; Sims et al., 1992). However many of these chemical indexes are developed for fish only but not for the cephalopods especially squid. As food safety management moves towards more risk and evidence based approaches, there is a need to develop a new quality index for squid.

Squids, as with most fish, are stored between layers of ice during transport and commercialization. Thus, the deterioration process occurs during the ice with continuous melting of water from the ice. In this storage condition, there is an increased lose of soluble components through the melted water, resulting in a loss of efficiency of many chemical tests for freshness evaluation. In such a case, when specific freshness indices for squid are evaluated, it becomes important to study the efficiency of tests in different storage conditions, and to verify their correlation with microbial contamination, to guarantee consumer safety. Therefore, the objectives of this study were to determine the biogenic amines formation and other sensory indices in two storage conditions: squids stored in direct contact with ice and squids packed in polyethylene bags and stored in ice.

2. Materials and Methods

Materials

Standard amines, containing putrescine dihydrochloride, (PUT), histamine dihydrochloride (HIS), cadaverine dihydrochloride (CAD, spermidine trihydrochloride (SPD), spermine tetrahydrochloride (SPR), tyramine (TYR) and agmatine (AGM) were obtained from Sigma. All other chemicals used were of analytical grade.

Sample preparation

Freshly landed squid (L. *duvaucelii*) weighing approximately 150g and of 55 cm in length were purchased from a commercial fish landing centre, Kochi, India. The duration between catch and arrival of the squid at the laboratory was less than one hour where they were always kept in ice. To ensure uniformity of the sample characteristics, all squid were bought from the same supplier and were treated in the same manner. Upon arrival, the whole squid were washed under running tap water, headed, gutted, skinned and rinsed to remove ink and any other extraneous material. Then, they were randomly divided into two homogenous groups. One lot was iced in boxes and another was packed in polyethylene pouches (30-35 cm) of approximately 4 numbers each, and then placed into boxes with flaked ice. One pack was analyzed immediately and the data collected was labeled as the data for 0 day. All other packed and unpacked samples were stored in a chill room in iced condition (2 ± 2 °C) for 15 days. Both the squid were sampled every 3 day for the sensory, biochemical and microbiological analyses.

Biogenic amine quantification

Squid samples were homogenized in a Waring blender for 2 min. Ground samples (5 g) were transferred to 50 ml centrifuge tubes and homogenized with 25 ml 5% TCA solution. The homogenates were then centrifuged at 8000g for 10 min in a refrigerated high speed centrifuge and the supernatant was filtered through Whatman No. 1 filter paper. The filtrates were then placed in volumetric flasks and 5% TCA was added to a final volume of 50 ml. After which, an aliquot of each extract was diluted using methanol (ratio 1:10).

Diluted samples were directly injected and analyzed without further sample preparation. Chromatographic separation and MS detection of biogenic amine were performed by using an API 4000 QTRAP LCMSMS coupled with Waters Aquity UPLC system. For the LC method Acetonitril with 0.005% TFA and water with 0.005% TFA were used as mobile phases in gradient mode. The column (Aquity BEH C18 1.7μm 2.1x50mm) was kept at 55°C with a flow-rate of 0.3mL/min. The total analysis time was set to 7 min.

Sensory analysis

On each sampling day, packed and unpacked squid were separated from the original lot and used for descriptive sensory evaluation by 8 trained panelists according to Quality Index method for shortfin squid (Vaz-Pires et al., 2008) with slight modifications. In the present study the sensory characteristics of squid were based on 4 quality attributes and 8 parameters. The parameters were scored from 0 to 1, 0 to 2 or 0 to 3, depending on the different characteristics. The sum of the protocol scores totaled 16 demerit points, where 3 points regarded the skin appearance, 6 regarded the odour, 1 regarded the mucus, 3 regarded the texture, 6 regarded the eye appearance and shape, and 7 regarded the mouth odour and appearance. Each panelist analyzed the samples individually and recorded his/her score for each parameter of the QI protocol.

Total plate count (TPC)

Twenty-five grams of squid slices were aseptically weighed and homogenized in stomacher bags with 225 ml sterile phosphate buffer for 1 min. The homogenized sample was serially diluted using 9 ml phosphate buffer. Further serial dilutions were made and 0.1 ml of each dilution was pipetted onto the surface of the plate count agar (Merck), in triplicates, after which they were incubated for 2 days at 37 ^{0}C (AOAC, 2002).

Statistical analysis

Descriptive measures expressed as means and standard deviations were used for the bacteriological, physical and chemical parameters. Bacteriological data were expressed as log CFU/g.

3. Results and Discussion

Biogenic amine analysis

Biogenic amine production (histamine, putrescine, tyramine, and cadaverine) correlated well with microbial load in squid (Sims et al., 1992). Table 2&3 show the production of biogenic amines in packed and unpacked squid muscles stored in ice storage. In the presents study, the biogenic amine content increased with the storage period. Large changes in the contents of putrescine and cadaverine (Table. 1&2) were observed throughout the storage period of both the squid samples. Putrescine was detected in small amounts at the stage of initial decomposition and increased rapidly at the stage of advanced decomposition. The concentration of putrescine in unpacked squid sample held in ice reaching maximum levels of 196.8mg/kg and 107.37mg/kg in packed squid muscles on 15th day of storage period. The putrescine content of unpacked squid samples in ice was significantly ($P < 0.05$) higher compared to packed samples. This could be attributed to the effect of packaging responsible for the formation of biogenic amines. This amine might be involved in the putrid smell.

Putrescine and cadaverine increases sharply as freshness decreases, and it could therefore be used to replace the traditional quality indices. Cadaverine levels reached the maximum level of 91.4mg/kg for unpacked squid and 69.72mg/kg in packed sample at the end of the storage. Significant differences were found in the levels of cadaverine among the two treatments. Similar results were reported for different squid species *Loligo subulata* (Ozogul, 2004). *Todarodes pacificus* (Yamanaka, H., 1987). Among the biogenic amines, histamine is potentially hazardous and is believed to be the causative agent in scombroid poisoning (Arnold & Brown, 1978). In the present study, histamine was detected from 6th day onwards in packed squid and 9th day onwards in the unpacked sample (Table 2&3). At the end of storage, squid samples direct contact with ice presented histamine levels of 1.85g/kg and packed sample showed the level of 1.74 g/kg. However the production of histamine is negligible compared to the scombroid fishes, because of the low level of aminoacid histidine in squids (Yamani 1989). Authors (Ozogul et al., 2002) have suggested that histamine could be a good quality index for scombroid fishes stored in ice. The European Community has set the maximum average histamine level for scombroid and scombroid-like fish to 100 mg kg-1, whereas the US Food and Drug Administration has set this level to 50 mg kg-1 (FDA 1996). Currently there is no legal limit for histamine or other biogenic amines in any cephalopod species. The low level of histamine found in the study was consistent with some other studies on squid (Prester et al., 2009) under different storage conditions.

No significant changes in the spermidine and spermine (Fig. 6) levels throughout the storage period in the two different conditions. The spermidine values were less than 9mg/kg throughout the storage period, where as spermine showed high value in the range of 140 to 270mg/kg in both storage conditions. Ozogul et al. (2002) reported that ice storage inhibited the formation of these two amines in sardine and modified atmosphere packed herring. In both storage condition, the agmatine content was observed from 1st day onwards, and it was produced throughout the storage period in both storage conditions, whereas the production of agmatine was low in packed squid sample, indicating the effectiveness of packing in inhibiting the production of agmatine. The amino acid arginine is extremely abundant in free state in invertebrates (Ohashi et al., 1991; Yamanaka et al., 1987), in which it plays a role as phosphate source for ATP synthesis (Contreras, 2002). Agmatine can be easily formed from arginine decarboxylation, which could be an explanation for the predominant presence of agmatine in squid since the beginning of the storage. High agmatine level of 163.4mg/kg was observed for squid samples direct contact with ice at the 6th day after that there was slight decrease in the production of agmatine. The same pattern was shown in other sample also. Yamanaka H, 1987, reported agmatine appeared to be most useful as a potential index for freshness of common squid. Paulo Vaz-Pires et al. (2008) agmatine produced could be, subsequently, converted in putrescine, spermidine and spermine, making any increase less noticeable.

Tyramine is another important biogenic amine which has vasoactive and psychoactive properties along with its adverse reactions involving monoamine-oxidase inhibitor drugs. In the present study, tyramine appeared from 0th day onwards and the production of tyramine concentration was low compared to the other biogenic amines. Similar results were reported for carps (Krizek et al., 2002) and sardine (Ozogul., 2004) stored under different conditions. There was a significant correlation between the storage condition, time and amine content (histamine, putrescine, tyramine, Agmatine and cadaverine) in squid samples.

Table 2: Changes in the biogenic amine (mg/kg) in squid during ice storage in indirect contact with ice

Days	Putrescine	Cadaverine	Histamine	Agmatine	Tyramine	Spermidine	Spermine
0	ND	2.77±0.12	ND	5.9±0.02	2.31±0.02	6.35±0.16	266±0.15
3	5.23±0.6	10.92±0.1	ND	21±0.16	2.73±0.1	4.585±0.02	221±0.11
6	24.74±0.2	24.32±0.03	ND	41.2±0.13	2.6±0.13	3.245±0.03	252±0.08
9	36.17±0.12	37.22±0.16	1.27±0.23	79.5±0.26	2.5±0.11	4.715±0.11	191±0.12
12	102.37±0.16	55.82±0.04	1.37±0.08	142.8±0.06	2.55±0.07	3.695±0.6	231±0.21
15	107.37±0.06	69.72±0.21	1.74±0.01	125.2±0.13	2.62±0.06	4.74±0.21	246±0.04

Table 3: Changes in the biogenic amine in squid (mg/kg) during ice storage in direct contact with ice

Days	Putrescine	Cadaverine	Histamine	Agmatine	Tyramine	Spermidine	Spermine
0	ND	4.05±0.15	ND	8.3±0.12	2.31±0.21	6.35±0.11	266±0.32
3	14.1±0.11	13.1±0.14	ND	68.8±0.08	2.88±0.08	8.7±0.2	255±0.12
6	38.8±0.2	32.9±0.06	1.07±0.6	163.4±0.01	3.13±0.12	9.2±0.05	144±0.43
9	99.7±0.21	47.6±0.12	1.44±0.6	139.2±0.13	2.93±0.02	8.8±0.04	168±0.12
12	164.5±0.03	65.46±0.11	1.76±0.12	137.1±016	2.91±0.23	8.05±0.15	225±0.16
15	196.8±0.21	91.4±0.08	1.85±0.32	133.4±0.02	6.03±0.14	7.82±0.22	233±0.51

Sensory analysis

In the present study the sensory characteristics of squid are based on the QIM developed for raw squid consisted of 4 quality attributes and 8 parameters .During the storage period, squid showed gradual and consistent changes for all the parameters of sensory evaluation, reaching a total score of 16 demerit points. Rejection, which was based on acceptability of external sensorial attributes of squid, occurred at 12 days of storage in the case of unpacked squid and 15 days in the case of packed squid, which is in accordance with the findings of Hurtado et al. (1999). According to these authors, the shelf-life of octopus is extremely limited, typically 12 days in the case of whole squid and 15days for gutted and cleaned squid, after catch at low storage temperature of 2.5 ^{0}C. The rates of change of the different parameters were not the same (Fig.3). All parameters considered show a clear variation within the first 8 days in ice. Some increase very rapidly in the first period, like skin colour and eyes; some others vary more clearly near the end of this period, e.g. mouth region odour, mucus and flesh texture. The earliest and most pronounced changes were found in the eyes (cornea showed changes at the first day of storage). Cornea and pupil changes can be irregular on both eyes of the same squid, probably due to physical damages caused, among other possible reasons, by contact with ice. All the parameters were considered to be useful to distinguish the freshness of squid. Slightly unpleasant odours started to be perceived around storage 9, but became unacceptable at day 12 in the case of unpacked squid, but in the case of packed squid the spoilage started at the day 9th and become unacceptable at the day of 15. This connected with colour of the skin which becomes pink and intense ammoniacal odour and flappy texture were the main parameters to define rejection.

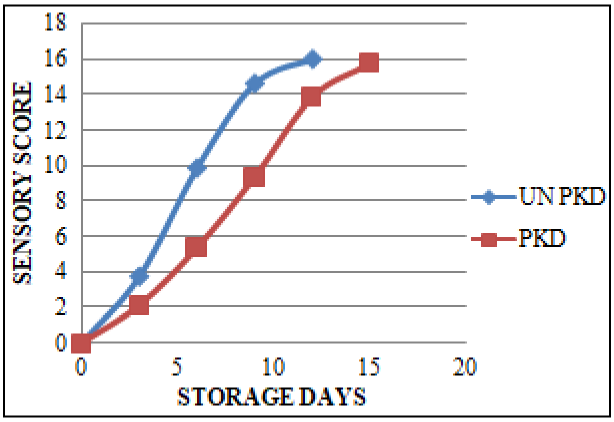

Figure 3: Changes in the sensory score of squid sample in ice storage

Microbial analysis

Squids, analyzed in initial day after catch, showed a TPC equal to $5.5x10^3$ cfu/g of sample (Table.1). After that there was a reduction of TPC in the first two days and the number gradually increased with storage days in unpacked squid. From 6th to 15th day, a steady increase in bacterial load was seen. Initial reduction in TPC was due to leaching and chilling effect of ice on bacteria of ice on bacteria. These data confirm results of other studies in different species of squid. (Judite Lapa-Guimaraes 2005). TPC increased with increasing ice storage time and decreased the quality of cephalopod (Civera 2000). *Shewanella putrefaciens* , *Pseudoalteromonas sp and Pseudomonas sp* dominated in spoiled gutted squid (Paarup, 2002). In squid loligo species, TPC analysis revealed an initial bacterial flora lower around $5.5x10^3$ and 7.4x105cfu/g in packed sample at rejection (15^{th} day). It is well known that the spoilage process in cephalopod molluscs is different from fish due, among many less clarified reasons, to thinner and fragile skin, nutritional composition much more favourable to enzymatic degradation, shorter and less pronounced rigor mortis, and initial autolytic degradation for a longer period (Hurtado et al., 1999; Vaz-Pires et al., 2008). The results obtained in this work suggest that these differences exist and are normally observed. The relatively low number of spoilage bacteria (as measured in this work) at the point of rejection (which is around 10^7-10^9 cfu/g for many fish and fish products) (Huss et al., 1997) supports the common idea of the enzymatic action being more rapid and effective in cephalopods (Hurtado et al., 1999; Ohashi et al., 1991). Gram-negative bacteria are the predominant amine-forming bacteria in fish, cephalopods and shellfish (Lakshmanan, R., 1993). The study was found that that the packed sample shows comparatively less microbial count than the other one.

Table 1: Changes in the total bacterial count in ice storage

Storage Days	Total Plate Count (Packed)	Total Platecount (Unpacked)
0th	$5.5x10^3$	$5.5x10^3$
3rd	$6.2x10^3$	$3.3x10^4$
6th	$3.3x10^4$	$7.82x10^4$
9th	$7.8x10^4$	$4.54x10^5$
12th	$3.14x10^5$	$8.34x10^5$
15th	$7.4x10^5$	$2.1x10^6$

4. Conclusion

In conclusion, squid sample packed in polythene pouches showed beneficial effects in extending the quality attributes compared to sample stored direct contact with ice. By using polythene pouches the squid samples retained quality up to 15 days and other one up to 12 days only. Inhibition of bacterial decarboxylase activity and prevention of bacterial growth are very crucial to control biogenic amine production. The present study indicates that the use of polythene pouches for packing samples coupled with the proper maintenance of chilled storage temperature helps in reducing the formation of biogenic amines to a greater extent and also reduces the risk of bacterial toxin.

5. Acknowledgement

Authors would like to thank Director, Central Institute of Fisheries Technology, Cochin, India for providing facilities to carry out this work.

References

[1] Alia bano et al.,Sadiqa Shakir., Askari Begum and R B Quadri (1992). Aminoacids content of sea squids. Journ .Chemi.soc.Pak. vol.14 No.3
[2] AOAC. (2002). Official methods of analysis, standard methods for the examination (17th ed.). Washington, DC: Association of Official Analytical Chemists, APHA.
[3] Arnold, S. H., & Brown, W. D. (1978). Histamine toxicity from products. In C. O. Chichester, E. M. Mark, & G. F. Stewart (Eds.). Advances in food research (Vol. 24 pp. 113–154). New York: Academic Press.
[4] Civera, T., Grassi, M.A. and Pattono, D., (2000) Chemical and Microbial characteristics of Cephalopods during storage in ice. Industrie. Alimentari. 38(384), 933-937.
[5] Civera, T., Grassi, M.A., Pattono, D. (1999). Ca ratteristiche chimiche e microbioligiche di molluschi cfalopodi nel corso della conservazione. Industrie Alimentari, 38,933-93
[6] Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs [displayed 8 November 2010]. Available at http://www.fsai. ie/uploadedFiles/Reg2073_2005(1).pdf.
[7] Contreras, G. E. (2002). Bioqumica de pescados e invertebrados. Santiago de, Chile: Centro de Estudios em Cieˆncia y Tecnologia de Alimentos. p. 309
[8] Emborg, J., Dalgaard, P. (2006). Formation of histamine and biogenic amines in cold-smoked tuna: an investigation of psychrotolerant bacteria from samples implicate in cases of histamine fish poisoning. J. Food Prot. 69:897–906.
[9] FAO (2001). Year book of Fishery Statistics: summary tables 2001. Fish, crustaceans, mollusks, etc. Capture production by group of species. Available: ftp.fao.org/fi/stat/summ-01/ala.pdf
[10] Food and Drug Administration (FDA), 1996. Decomposition and histamine in raw, frozen tuna and mahi-mahi, canned tuna and related species. Compliance Policy Guides 7108.240, Sec. 504-535.
[11] Hurtado, L., Borderias, Montero, P., An H., (1999). Characterization of proteolytic active in Octopus (Octopus vulgaris) arm muscle. J.Fd. Biochem. 469-483.
[12] Huss, H. H., Dalgaard, P., & Gram, L. (1997). Microbiology of fish and fish products. In J. B. Luten, T. Borresen, & J. Oehlenschlager (Eds.), Seafood from producer to consumer, integrated approach to quality. 413-430.
[13] Judite Lapa-Guimaraes., Pedro Eduardo de Felıcio., Emılio Segundo Contreras Guzman. (2005). Chemical and microbial analyses of squid muscle (*Loligo plei*) during storage in ice Food Chemistry 91, 477–483.
[14] Křížek, M., Pavlíček, T. & Vácha, F. (2002). Formation of selected biogenic amines in carp meat. Journal of the

Science and Food Agriculture, 82, 1088-1093. http://dx.doi.org/10.1002/jsfa.1154

[15] Lakshmanan, P.T., Varma, P.R.G. and Iyer, T.S.G., (1993). Quality of Commercially frozen cephalopods products from India. J. Food control. 4 (3), 159-164.

[16] Mackie, M. and Fernandez, J. (1977). Histidine metabolism in fish. Urocanic acid in mackerel (*Scomberscombrus).* J. Sci. Food Agric. 28, 935-940.

[17] MPEDA (2014), http://mpeda.gov.in/MPEDA/annual_reports.php

[18] Ohashi, E., Okamoto, M., Ozawa, A., & Fujita, T. (1991). Characterization of common squid using several freshness indicators. Journal of Food Science, 56, 161-163, 174.

[19] Ozogul, F., K.D.A Taylor, Quantick and Y Ozogul (2002). Biogenic amines formation in Atlantic herring (Clupea harengus) stored under modified atmosphere packaging using a rapid HPLC method.37, 515-522.

[20] Ozogul, F., Polat, A., & Özogul, Y. (2004). The effects of modified atmosphere packaging and vacuum packaging on chemical, sensory and microbiological changes of sardines (Sardina pilchardus). Food Chemistry, 85, 49–57.

[21] Paarup, T., Sanchez, J.A., Moral, A., Christensen, H., Bisgaard, M., Gram, L. (2002). Sensory, chemical and bacteriological changes during storage of iced squid (*Todaropsis eblanae*). J. Appl. Microbiol. 92:941-50.

[22] Prester Lj, Macan J, Varnai VM, Orct T, Vukušić J, Kipčić D. Endotoxin and biogenic amine levels in Atlantic mackerel (Scomber scombrus), sardine (Sardina pilchardus) and Mediterranean hake (Merluccius merluccius) stored at 22 °C. Food Add Contam 2009;26:355-62

[23] Romo, C., Astudillo, J., Munoz, O., & Contreras, E. (1996). Determination the ındices bioqumicosy funcionales relevantes paraevaluar laconservacio´n dejibia (Dosidicus gigas) a bordo. Santiago. Proceedings of the workshop on fish and mollusc larviculture (pp. 197-213).

[24] Sims, G.G., Farn, G., York, R.K. (1992). Quality index for tuna: correlation of sensory attributes with chemical indices. J. Food Sci.57, 1112–1115.

[25] Vaz-Pires Paulo., Pedro Seixas., Micaela Mota., Judite Lapa-Guimaraes., Jana Pickova., Andreia Lindo., Teresa Silva. (2008). Sensory, microbiological, physical and chemical properties of cuttlefish (*Sepia officinalis*) and broadtail shortfin squid (*Illex coindetii*) stored in ice; Food Science and Technology. 41, 1655-1664

[26] Yamanaka, H., Shiomi, K., Kikuchi, T. (1987). Agmatine as a Potential Index for Freshness of Common Squid (Todarodes pacificus).Journal of Food Science. 52(4): 936–938.

[27] Zdzislaw,E.,Sikorski., Hona kolodziejska. (1986). The composition and properties of squid meat. Food Chemistry.20, 213-224.