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Effects of squid ink as edible coating on squid sp. *(Loligo duvauceli)* spoilage during chilled storage

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Abstract

The effects of squid ink at concentration of 0.10 and 0.25% on the total bacteria count and chemical spoilage indicator; total volatile basis nitrogen (TVBN) and trimethylamine (TMA) of squid *(Loligo duvauceli)* were analysed. The analysis were performed at interval of 5 days during 15 days of chilled storage (4°C). This studies also investigate the antioxidant capacity of the squid ink. The melanin-free squid ink were subjected to ferric reducing power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) analysis. The FRAP values found in squid ink were 0.04±0.01 µmole TE g^{-1} meanwhile DPPH values were recorded at 0.81±0.00 µmole TE g^{-1}. The squid ink at both 0.10 and 0.25% concentration showed a significantly ($p<0.05$) reduced amount of microflora in squid during 15 days of storage compared to the amount of microflora found in untreated samples. After 15 days of storage, squid coated with 0.25% squid ink showed a significantly ($p<0.05$) lower accumulation of TVBN compared to other treatments. A similar trend were found for TMA value. During 15 days of storage, the controls showed a significantly ($p<0.05$) higher accumulation of TMA compared to squid coated with 0.25% of squid ink. By using TMA analysis, squid coated with 0.25% squid ink were estimated to prolong their shelf-life for up to 9 days in chilled condition compared to untreated squid, which can stand only for 5 days. Therefore, pre-treatment with squid ink coating upon chilled storage are beneficial on keeping the quality and a potential practice for post-harvest industry.

Introduction

Fish and shellfish are highly perishable product. The shelf life of these products is limited by chemical and microbiological changes after capture. Various researches (Ke *et al*., 1984; Lakshamanan *et al*., 1993) documented that deterioration of external appearance of cephalopods are related to decrease in the skin reddish brown colour and shine. However, Lapa-Guimaraes *et al.* (2002) stated the information is controversial and suggested the deterioration of external appearance of cephalopods are due to the intensification and spreading of the pink colour instead of the decline in skin colour. Therefore, the quality of perishable products are the best be assessed by using both biochemical and microbiological indices. It has been documented elsewhere that changes of non-protein nitrogen (NPN) components during the storage, such as total volatile bases nitrogen (TVBN) and trimethylamine (TMA) are potential chemical indicator for freshness evaluation in some squid species (Ke *et al*., 1984; Lapa-Guimaraes *et al*., 2002). Paarup *et al.* (2002) added microbiological studies also beneficial for a better perception of spoilage mechanisms and shelf life prediction during squid storage.

Fish processing industry are demanding alternative methods for shelf life extension and tradeability of fresh and refrigerated fishery products. The utilization of by-products from seafood processing not only benefits to the industry, but also defeat serious ecological problems and environmental pollution. The viscera and ink sac that contain squid ink may be categorized as by-products. Sadok *et al.* (2004) stated that squid ink is produced by a side gland of the digestive tract in the mantle cavity. Squid ink is a suspension of melanin granules in a viscous colorless medium (Huazhong *et al*., 2011). Mochizuki (1979) added cuttlefish ink has delicate taste and has an antiseptic effect that may become potential edible coating for fishery products. In addition, it is also a natural and bioactive compounds that has been proved as alternative medicine and therapeutic applications (McConnell *et al.*, 1994); enhancer for growth and immunity performance in broiler chicken (Liu *et al*., 2011). Squid ink also

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potentially play a key role as antibacterial agent (Ramasamy and Murugan, 2005) and inhibit growth of *Staphylococcus aureus* (Mochizuki, 1979). Ink from *Sepia officianlis* and *Sepiella inermis* showed antioxidative and antiretroviral activities, respectively (Rajaganapathy *et al*., 2000; Lei *et al*., 2007).

This studies were conducted to investigate the effects of squid ink edible coating as pre-treatment upon 15 days chilled storage of squid on the total bacteria count and TVBN and TMA accumulation with an attempt to extent the shelf life without affecting the quality and safety of the products.

Materials and Methods

Squid ink preparation

Squid ink were obtained from squid processing stall in Kuala Terengganu, Terengganu, Malaysia, placed in zip-lock bags and transported in iced storage. The squid ink were diluted tenfold using distilled water to obtained melanin-free ink. The samples were centrifuged at 27,000g for 40 minutes using centrifuge (Centrifuge 5430R Eppendorf AG, Hamburg, Germany). The supernatant were obtained and stored at -20°C upon further analysis.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assays were prepared as described by Benzie and Strain (1996). FRAP reagent contained 5 ml of 10 mmol L^{-1} 2,4,6-tripyridyl-S-triazinesolution (TPTZ) solution in 40 mmol L^{-1} hydrochloric acid, 5 ml of 20 mmol L^{-1} ferric chloride ($FeCl_3$) and 50 ml of 0.3 mmol L^{-1} acetate buffer (pH 3.6). The reagent were freshly prepared and warmed to 37°C. 100 µL sample was mixed with 3 ml of FRAP reagent and the absorbance was measured at 593 nm using a spectrophotometer (UV Mini-1240 UV-VIS Spectrophotometer Shimadzu, Japan) after incubation at 37°C for 10 minutes. The standard curve was constructed using Trolox solution 1-30 µM. The activity was expressed as 1 µmole Trolox equivalents (TE) g^{-1}.

2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay

DPPH radical scavenging activity were determined according to method by Binsan *et al.* (2008). 1.5 ml of supernatant were added to 1.5 ml of 0.15 mM 2,2-diphyneyl-1-picylhydrazyl (DPPH) in 95% (v/v) ethanol. The mixture was mixed vigorously and allowed to stand at room temperature in the dark for 30 minutes. The absorbance of resulting solution was measured at 517 nm using a spectrophotometer (UV Mini-1240 UV-VIS Spectrophotometer Shimadzu, Japan). The blank were prepared in the same manner, except that 1.5 ml distilled water was used instead of the sample. A standard curve was prepared using Trolox in the range of 0 to 50 µM. The activity was expressed as µmole Trolox equivalents (TE) g^{-1}.

Sample preparation

Fresh squid were purchased from local market in Kuala Terengganu and transported in ice at ratio of 1:2 squid to ice. Upon arrival, fresh squid were washed and dressed. Samples were subjected to soaking procedure with respective; 0.10 and 0.25% squid ink concentration for 10 min at 4°C. Meanwhile, the controls were left without squid ink coating. All samples were superchilled in blast freezer (Irinox Blast Freezer, USA) for 5 min and individually placed in polyethylene bags before vacuum packed (DZQ Vacuum Packer, China). All samples were stored in chilled temperature at 4°C prior to analysis at interval of five days within 15 days of storage. The analysis were replicated three times.

Microbiological analysis

The exterior of packed bags were disinfected with 70% ethanol, air dried and aseptically opened and all instruments were sterile before use. 10±0.1 g of squid flesh were homogenised with 90 ml maximum recovery diluent (MRD). A serial dilutions were prepared by pipette 1 ml homogenized sample into the 9 ml of MRD. The serial dilution continues diluted until appropriate dilution. Accurately, 0.1 ml of the dilution was spread on plate count agar (PCA) by using sterile glass spreader. The total bacterial counts were performed according to method described by Linton *et al*. (2003); Karim *et al.* (2011). The incubation conditions used for total aerobe counts were 30°C for 48 hours. Colonies develop on the plates were counted using colony counter (Stuart Colony Counter, UK). Plates with 30 to 300 colonies were selected and the total colonies were recorded. Microbiological counts were expressed as log colony forming units per gram of samples ($\log_{10}$CFU g^{-1}).

Total volatile basis nitrogen (TVBN) and trimethylamine (TMA) analysis

Total volatile basis nitrogen (TVBN) was determined using method published by Malle and Tao (1987) with minor modification by Karim *et al*. (2011). 100±0.1 g of squid were mixed with 200 ml of 7.5% trichloroacetic acid (TCA) and homogenised for 1 minute using laboratory blender (Waring Commercial Blender, USA) at speed 2. The homogenate was centrifuged at 3000 rpm for 5 minutes (Centrifuge 5430R Eppendorf AG, Hamburg, Germany) and the supernatant was filtered

through Whatman No.1 filter paper into the conical flask. 25 ml of filtrate was pipetted into the Kjeldahl distillation tube and 5 ml of 10% sodium hydroxide were added to the mixture. Steam distillation were performed using a vertical steam distillation unit (BUCHI Distillation Unit K-350, Switzerland) and the distillate were collected into a beaker containing 10 ml of 4% (v/v) aqueous boric acid solution and 0.04 ml methyl red and bromocresol green indicator up to a final volume of 50 ml. The distillate were titrated against 0.05 M sulphuric acid solution and shake until pink colour persist for 15 seconds. The quantity of TVB-N was determined in mg from the volume of sulphuric acid (n ml),

$$TVB\text{-}N = n^{*}16.8 \text{ mg nitrogen } 100\ g^{-1}$$

The analysis for trimethylamine (TMA) were done in the same manner as TVBN analysis except that 20 ml of 35% formaldehyde was added to the distillation tube to block the primary and secondary amines, while leaving the tertiary amines to react. The quantity of TMA was determined in mg from the volume of sulphuric acid (n ml),

$$TMA = n^{*}16.8 \text{ mg nitrogen } 100\ g^{-1}$$

Statistical analysis and shelf life estimation

The entire experiment was replicated three times. The data were analysed statistically using ANOVA to compare the significant differences among groups at 0.05 level of probability. All statistical analysis were done using the IBM SPSS Statistic software (Version 20). The estimation shelf life mean of each treatment and result of analysis were based on linear regression. The microbial shelf life was taken as the time to reach 10^7 CFU g^{-1}, as recommended by International Commission on Microbiology Specification for Food (ICMSF, 1986). Meanwhile the chemical shelf life was taken as the time to reach 35 and 15 mg N 100 g^{-1} for TVBN and TMA value, respectively (Connell and Shewan, 1980).

Result and Discussion

Antioxidant capacity of squid ink

The antioxidant active compounds from melanin free squid ink were found to be recorded at 0.04±0.01 and 0.81±0.00 µmole TE g^{-1} for FRAP and DPPH values, respectively (Table 1). Previous research by Vate and Benjakul (2013) documented that FRAP and DPPH value in squid ink were 171.2±7.3 and 179.6±2.1 µmole TE g^{-1}, respectively. Interestingly, Liu *et al.* (2011) documented that crude squid ink formulated in basal diet fed to chicken broiler showed a significant elevated total superoxide dismutase (SOD) activity but no significant changes in malonedialdehyde (MDA) activity in serum. However, they added that both antioxidant activity indicator (SOD and MDA) did not induce any significant changes at low dose of squid ink. Chen *et al.* (2007) documented that melanin of squid ink can catalyse O^{2-} to H_2O_2 and thus avoid the free radical chain reaction triggered by O^{2-}. Melanin of squid ink act as superoxide dismutase that play a key role as the first line of defence in antioxidant system of living cells and works at preventing the production of free radicals. Consequently, current studies showed a significantly low value of antioxidant activity in melanin-free squid ink.

Table 1. Ferric reducing power (FRAP) assay and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay of squid ink

Antioxidant activity indicator	Melanin-free squid ink (µmole TE g^{-1})
FRAP	0.04± 0.01
DPPH	0.81± 0.00

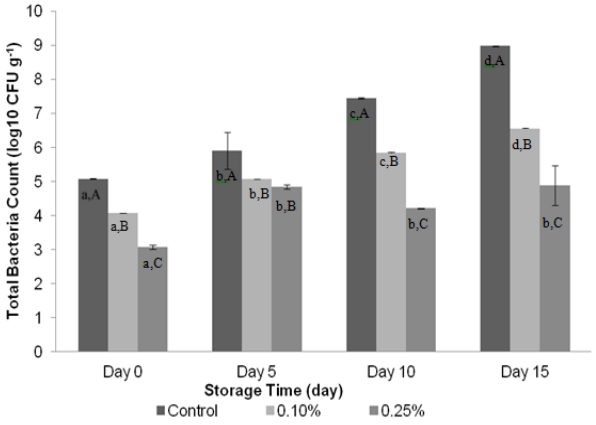

Figure 1. Total bacteria count of squid coated in three different squid ink concentration; 0.00 (controls), 0.10 and 0.25 % during 15 days of chilled storage
Different small letters (a, b, c) refer to similar treatments at different storage day indicate significant differences ($p<0.05$).
Different letter (A, B, C) refer to similar storage day at different treatments indicate significant difference ($p<0.05$).
Values are given as mean ± SD

Microbiological analysis

The most abundant total bacteria count were found to be in controls; 8.98±0.01 $\log_{10}$ CFU g^{-1} after 15 days of chilled storage. The total bacteria count showed least increased in squid coated with 0.25% squid ink compared to other two treatments during 15 days of storage (Figure 1). Total bacteria count in

Table 2. Total volatile basic nitrogen (TVBN) value of squid coated with 0.00, 0.10 and 0.25 % squid ink during 15 days of chilled storage

Storage time (day)	TVBN value (mg N 100 g^{-1})		
	Coated with 0.00 % squid ink (Controls)	Coated with 0.10 % squid ink	Coated with 0.25 % squid ink
0	11.20 ± 1.94^{a,A}	4.48 ± 1.94^{a,B}	3.36 ± 0.00^{a,B}
5	17.92 ± 1.94^{b,A}	12.32 ± 1.94^{b,B}	8.96 ± 1.94^{a,B}
10	31.36 ± 1.94^{c,A}	25.76 ± 1.94^{c,B}	22.40 ± 1.94^{b,B}
15	48.16 ± 1.94^{d,A}	35.84 ± 1.94^{d,B}	26.88 ± 3.36^{b,C}

Different capital letters (a, b, c) in the same column indicate significant differences ($p<0.05$). Different letter (A, B, C) in the same row indicate significant difference ($p<0.05$).
Values are given as mean ± SD from triplicate determinations.

Table 3. Trimethylamine value of squid coated with 0.00, 0.10 and 0.25 % squid ink during 15 days of chilled storage

Storage time (day)	TMA value (mg N 100 g^{-1})		
	Coated with 0.00 % squid ink (Controls)	Coated with 0.10 % squid ink	Coated with 0.25 % squid ink
0	7.84 ± 1.94^{a,A}	4.48 ± 1.94^{a,A}	3.36 ± 0.00^{a,B}
5	12.32 ± 3.88^{a,A}	7.84 ± 1.94^{a,A}	5.60 ± 1.94^{a,A}
10	28.00 ± 1.94^{b,A}	26.64 ± 1.94^{b,A}	19.04 ± 1.94^{b,B}
15	39.20 ± 5.13^{c,A}	28.00 ± 1.94^{b,B}	21.28 ± 1.94^{b,B}

Values are given as mean ± SD from triplicate determinations.
Different capital letters (a, b, c) in the same column indicate significant differences ($p<0.05$). Different letter (A, B, C) in the same row indicate significant difference ($p<0.05$).

both controls and squid coated with 0.10% squid ink showed a significant ($p<0.05$) increasing trend parallel with storage duration. However, squid soaked at 0.25% squid ink did not show any significant changes after 5 days of storage until the end of storage day (Figure 1). At a higher concentration of melanin-free squid ink showed a relatively decelerate bacterial growth during squid storage due to the activation of antimicrobial properties.

Previous studies by Vaz-Pires and Seixas (2006) documented that shelf life of squid stored in ice were up to 9 days. The relatively low number of bacteria in current works meets an agreements with Vaz-Pires *et al*. (2008). It had been documented elsewhere that the putrefaction of cephalopods mainly due to the rapid enzymatic action (Ohashi *et al*., 1991; Hurtado *et al*., 1999; Hurtado *et al*., 2001; Lapa-Guimaraes *et al*., 2002). It is well known that the spoilage process in cephalopod 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 (Vaz-Pires and Seixas, 2006)

Total volatile basic nitrogen (TVBN) analysis

Total volatile basic nitrogen (TVBN) accumulated in squid were higher in controls compared to the treated samples after 15 days of chilled storage (Table 2). TVBN value increased significantly ($p<0.05$) with storage duration in all samples. However, squid coated with 0.25% of squid ink showed a gradual increased ($p<0.05$) in TVBN value after the 5th day of storage. In addition, samples coated with 0.25% squid ink did not reach the unacceptable value for consumption (35 mg N 100 g^{-1}) within 15 days of storage (Table 2).

Previous studies by Tomac *et al.* (2014) found that the initial TVBN value was 15.20±0.20 mg N 100 g^{-1}. These finding were similar to Ruiz-Capillas

Table 4. Shelf life prediction of squid coated with 0.00, 0.10 and 0.25 % squid ink during 15 days of chilled storage

Treatments	Estimated shelf life using microbiology analysis (days to reach 10^7 CFU g^{-1})	Estimated shelf life using TVBN value (days to reach 35 mg N 100 g^{-1})	Estimated shelf life using TVBN value (days to reach 20 mg N 100 g^{-1})	Estimated shelf life using TMA value (days to reach 15 mg N 100 g^{-1})
Coated with 0.00 % squid ink (Controls)	8	11	5	5
Coated with 0.10 % squid ink	17	15	8	7
Coated with 0.25 % squid ink	23	19	10	9

et al. (2002) as they documented 12-18 mg N 100 g^{-1} TVBN were accumulated in cephalopods species. Current study showed at the same range of TVBN value in controls (11.20±1.94 mg N 100 g^{-1}) but revealed a significantly ($p<0.05$) lower TVBN value; 4.48±1.94 and 3.36±0.00 mg N 100 g^{-1} for squid coated with 0.10 and 0.25% squid ink, respectively (Table 2). In contrast, Zaragoza *et al*. (2015) recently published that the initial TVBN value were very high (91.93±1.41 mg N 100 g^{-1}) and increasing at the end of storage (12 days) to reach values of 138.85±35.15 mg N 100 g^{-1}.

Trimethylamine (TMA) analysis

The accumulation of trimethylamine (TMA) in all squid samples were significantly ($p<0.05$) increased only after day 5 (Table 3). However, TMA value in squid coated with 0.10 and 0.25% squid ink increased but not significantly ($p>0.05$) from day 10 until the end of storage day. Within 10 days of storage, the TMA value showed no significant different ($p>0.05$) between the controls and samples treated with 0.10% of squid ink. However, squid treated at both 0.10 and 0.25% squid ink effectively ($p<0.05$) reduced the TMA accumulation during 15 days of storage (Table 3).

Studies by Ruiz-Capillas *et al.* (2002) published that TMA value in fresh cephalopods were less than 1 mg N 100 g^{-1}. A similar trend were also documented by Vaz-Pires *et al*. (2008), where TMA level of cephalopods were recorded at range of 0.1 to 10.00 mg N 100 g^{-1} within 13 days of iced storage. In current studies, the initial level of TMA were recorded at 7.84±1.94 mg N 100 g^{-1}. Meanwhile a lower level of TMA found in samples treated with 0.10 and 0.25% of squid ink.

Shelf life prediction

The prediction of shelf life for squid in iced (controls) were predicted to spoil at the 8th day of storage according to the microbiology analysis, when the total bacteria counts reach a maximum level of 10^7 CFU g^{-1}. However, with regards to the chemistry analysis; when TVBN and TMA value reach the rejection point of 35 and 15 mg N 100 g^{-1}, respectively, shelf life of squid in iced (controls) were spoiled after 11 and 5 days of chilled storage, respectively. Meanwhile squid coated with 0.10% squid ink were unacceptable to consume at 17th day of storage according to microbiological analysis and may extend at only on 15th and 7th days regards to TVBN and TMA analysis prediction. In addition, samples were predicted unacceptable to consume according to the microbiology, TVBN and TMA value were at day 23, 19 and 9, respectively (Table 4).

Interestingly, it has been documented elsewhere suggesting shelf life prediction were according to microbiology analysis, but not for squid shelf life estimation. Current studies suggesting TMA value are considered more reliable and useful spoilage indicator for squid. Squid are spoil due to the autolytic changes by action of bacterial enzymes on the trimethylamine oxide that produce TMA, that are responsible for the unpleasant odours. Meanwhile, shelf life prediction for squid according to microbiology analysis were not reflected as it gives poor correlation of TMA value and bacterial counts, as agreed with Huss (1995); Ozyurt *et al.* (2009); Feuntes *et al.* (2011). With regards to TVBN value for shelf life prediction,

current studies suggesting a level of 20 mg N 100 g^{-1} were considered as the acceptability limit to evaluate squid shelf life. This are coincide with shelf life prediction with regards to the TMA value. The predicted shelf life according to TVBN value; reach at rejection point of 20 mg N 100 g^{-1} were 5, 8 and 10 for controls, squid coated at 0.10 and 0.25 % squid ink, respectively (Table 4).

Conclusion

The present studies using combination of edible coating; 0.25% squid ink and low temperature (4°C) for squid storage gives a significant benefits for reducing the accumulation of TVBN and TMA. The shelf life extension were up to 9-10 days. Both TVBN and TMA value are more reflect in squid shelf life prediction. The acceptability limit for TVBN value were suggested at 20 mg N 100 g^{-1} for squid shelf life estimation. Meanwhile, squid coated with 0.25% squid ink showed a relatively low bacterial growth during 4°C storage.

References

Benzie, I.F.F. and Strain. J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP influenced by different freeze-thaw cycles. Food Chemistry 72: 70-76.

Binsan, W., Benjakul, S., Visessanguan, W., Roytakul, S., Tanaka, M. and Kishimura, H. 2008. Antioxidativve activity of mungoong an extract pastr from cephalothorax of white shrimp *(Litopenaeus vannamei)*. Food Chemistry 106: 185-193.

Chen, S.G., Xue, C.H., Xue, Y., Li, Z.J. Gao, X. and Ma, Q. 2007. Studies on the free radical scavenging activities of melanin from squid ink. China Journal of Marine Drugs 26(1): 24-27.

Connell, J.J. and Shewan, J.M. 1980. Past, present, and future of fish science. In: Connell, J.J. (Eds). Advances in Fish Science and Technology, pp 133-164. Farnham, Surrey, U.K.: Fishing News Books, Ltd. 4th Edition.

Feuntes, A., Fernandez-Segovia, I., Barat, J.M. and Serra, J.A. 2011. Influence of sodium replacement and packaging on quality and shelf-life of smoked sea bass (*Dicentrarchus labrax* L.). LWT-Food Science and Technology 44: 16-23.

Huazhong, L., Luo, P., Chen, S. and Shang, J. 2011. Effects of squid ink on growth performance, antioxidant functions and immunity in growing broiler chicken. Asian-Australia Journal Animal Science 24(12): 1752-1756.

Hurtado, J. L., Borderı´as, J., Montero, P. and An, H. 1999. Characterization of proteolytic activity in octopus *(Octopus vulgaris)* arm muscle. Journal of Food Biochemistry 23: 469-483.

Hurtado, J.L., Montero, P. and Borderias, J. 2001. Chilled storage of pressurized octopus *(Octopus vulgaris)* muscle. Journal of Food Science 66: 400-406.

Huss, H.H. 1995. Quality and quality changes in fresh fish. In Fisheries Technical Paper, No. 348. pp. 132. Rome: Food and Agriculture Organisation of United Nations.

International Commission on Microbiology Specifications for Foods (ICMSF). 1986. Sampling plans for fish and shellfish. In: Microorganisms in Foods 2, Sampling for microbiological analysis: Principles and Specific Applications. pp 181-196. Toronto, Canada: Blackwell Scientific Publication University of Toronto Press.

Karim, N,U., Kennedy, T., Linton, M., Watson. S., Gault, N. and Patterson, M.F. 2011. Effect of high pressure processing on the quality of herring *(Clupea harengus)* and haddock *(Melanogrammus aeglefinus)* stored in ice. Food Control 22: 476-484.

Ke, P.J. Burns, B.G. and Woyewoda, A.D. 1984. Recommended procedures and guidelines for quality evaluation of Atlantic short-fin squid (Illex illecebrosus). Food Science and Technology 17(5): 279-281.

Lakshamanan, P.T., Varme, P.R.G. and Iyer, T.S.G. 1993. Quality of commercially frozen cephalopod products from India. Food Control 4: 159-164.

Lapa-Guimaraes, J., Silva, M.A.A., de Felicio, P.E. and Guzman, E.C. 2002. Sensory, colour and psychrotrophic bacterial analysis of squids *(Loligo plei)* during storage in ice. Food Science and Technology 35: 21-29.

Lei, M., Wang, J.F., Wang, Y.M., Chen, S.G. and Xue, C.H. 2007. Effects of sepia on the metabolization of blood lipid and antioxidant ability in hyperlipidemia rats. China Journal of Marine Drugs 3: 30-33.

Linton, M., McClements, J.M. and Patterson, M.F. 2003. Changes in the microbiological quality of shellfish brought about by treatment with high hydrostatic pressure. International Journal of Food Science and Technology 38: 713-727.

Liu, H., Luo, O., Chen, S. and Shang, J. 2011. Effects of squid ink on growth performance, antioxidant functions and immunity in growing broiler chickens. Association of Animal Production Society 24(12): 1752-1756.

Malle, P. and Tao, S.H. 1987. Rapid quantitative determination of trimethylamine using steam distillation. Journal of Food Protection 50: 756-760.

McConnell, O., Longley, R.E., Koehn, F.F. and Gullo, V.P. 1994. In: Gullo V.P. (Eds). The discovery of natural products with therapeutic potential. pp 109-174. Boston: Butterworth-Heinemann.

Mochizuki, A. 1979. An antiseptic effect of cuttlefish ink. Bulletin Japan Society Science Fisheries 45: 1401-1403.

Ohashi, E., Okamota, M., Ozawa, A. and Fujita, T. 1991. Characterization of common squid using several freshness indicator. Journal of Food Science 56: 161-163.

Ozyurt, G., Kuley, E., Ozkutuk, S. and Ozogul, K. 2009. Sensory, microbiological and chemical assessment of the freshness of red mullet *(Mullus barbatus)* and

goldband goatfish *(Upeneus moluccensis)* during storage in ice. Food Chemistry 114: 505-510.

Paarup, T. Sanchez, J.A. Pelaez, C. and Moral, A. 2002. Sensory, chemical and bacteriological changes in vacuum-packed pressurised squid mantle *(Todaropsis eblanae)* stored at 4°C. International Journal of Food Microbiology 74: 1-12.

Rajaganapathy, J., Thyagarajan, S.P. and Edward, J.K. 2000. Study on the cephalopods ink for anti-retoviral activity. Indian Journal Experimental Biology 38: 519-520.

Ramasamy, M.S. and Murugan, A. 2005. Potential antimicrobial activity of marine molluscs from Tuticorin, southern coast of India against 40 biofilm bacteria. Journal Shellfish Research 24: 243-251.

Ruiz-Capillas, C., Moral, A., Morales, J. and Montero, P. 2002. Characterization of non-protein nitrogen in the cephalopods volados *(Illex coindetii)*, pota *(Todaropsis eblanae)* and octopus *(Eledonecirrhosa)*. Food Chemistry 76: 165-172.

Sadok, S., Abdelmoulah, A. and Abed, A.E. 2004. Combined effect of sepia soaking and temperature on the shelf life of peeled shrimp *Penaeus kerathurus*. Food Chemistry 88: 115-122.

Tomac, A., Mascheroni, R.H. and Yeannes, M.I. 2014. Modelling total volatile basic nitrogen production as a dose function in gamma irradiated refrigerated squid rings. LWT-Food Science and Technology 5: 533-536.

Vate, N.K. and Benjakul, S. 2013. Antioxidative activity of melanin-free ink from splendid squid *(Loligo formosana)*. International Aquatic Research 5: 9-20.

Vaz-Pires, P. and Seixas, P. 2006. Development of new quality index method (QIM) schemes for cuttlefish *(Sepia officinalis)* and broadtail shortfin squid *(Illex coindetii)*. Food Control 17: 942-949.

Vaz-Pires, P., Seixas, P., Mota, M., Lapa-Guimarães, J., Pickova, J., Lindo, A. and Silva, T. 2008. Sensory, microbiological, physical and chemical properties of cuttlefish *(Sepia officinalis)* and broadtail shortfin squid *(Illex coindetii)* stored in ice. LWT-Food Science and Technology 41(9): 1655-1664.

Zarogoza, P., Fuentes, A., Ruiz-Rico, M., Vivancos, J.L. Fernandez-Segovia, I., Jose, V., Barat, J.M. and Martinez-Manez, R. 2015. Development of colorimetric sensor array for squid spoilage assessment. Food Chemistry 175: 315-321.