Indicators of protein spoilage in fresh and defrosted crustaceans and cephalopods stored in domestic condition

Serena Altissimi, Maria Lucia Mercuri, Marisa Framboas, Mauro Tommasino, Stefania Pelli, Ferdinando Benedetti, Sara Di Bella, Naceur Haouet

Institute for Experimental Veterinary Medicine of Umbria and Marche, Perugia, Italy

Abstract

In relation to consumer demand, crustaceans and cephalopods are sold as both fresh and defrosted. It is well known that total volatile basic nitrogen (TVB-N) and volatile amine values, especially, biogenic amines and biogenic amine index, are expression of freshness of fish products, but there is a lack of knowledge of their acceptability limits, for crustaceans and cephalopods. In order to assess these limits, real-time shelf life tests were carried out, relating the results of TVB-N, biogenic amines and BAI to the sensory evaluation of crustaceans and cuttlefishes, both fresh and defrosted. TVB-N and biogenic amines have been analysed in many shrimp species and cuttlefishes purchased in Perugia (Central Italy), and BAI was calculated as the ratio between different biogenic amines. The results show levels of TVB-N and spermine different between shrimp and cuttlefish (TVB-N: 37 *vs*. 14 mg/100 g; spermine: 4 *vs*. 14 mg/kg, respectively) while the other biogenic amines and BAI are close to zero in both. Among biogenic amines, cadaverine and even more putrescine significantly affect BAI values and seem to be the most effective in assessing limits of acceptability during storage.

Introduction

Fisheries and aquaculture play an important role of international trades and food sector and supplied the world with about 128 million tones of fish in 2010 as food for people (Jinadasa, 2014). With sustained growth in fish production and improved distribution channels, world fish food supply has grown dramatically in the last five decades, with an average annual rate of 3.2 percent in the period 1961-2013, double that of population growth, resulting in increasing average per capita availability (FAO, 2016).

Crustaceans and cephalopods often come from fishing areas located far from seafood markets, so they are treated with additives to limit the onset of melanosis and immediately frozen (Smaldone *et al.*, 2011). In relation to consumer demand, they are then often sell as defrosted. However, fresh crustaceans and cephalopods are also present on the market.

Fish and shellfish are highly perishable, and prone to vast variations in quality due to differences in species, environmental habitats, feeding habits and action of autolysis enzymes as well as hydrolytic enzymes of microorganisms on the fish muscle (Venugopal, 2002).

In assessing the shelf life of food products, indicators of deterioration and spoilage should be identified as well as their levels of acceptability. Protein and lipid degradation molecules are the main indicators typically used (Haouet *et al.,* 2006). In crustaceans and cephalopods, the low amount of fat makes difficult the evaluation of lipid degradation, while for protein spoilage there is a lack of data in literature.

The quality and the freshness of fish can be estimated by sensory tests, microbiological methods or by chemical methods such as measuring volatile compounds, lipid oxidation, determination of ATP breakdown products and the formation of biogenic amines (Gulsun *et al*., 2009).

Volatile amines are the characteristic molecules responsible for the fishy odour and flavour present in fish several days after the catch and they are commonly used as criteria for assessing the fish quality. Ammonia is present in freshly caught fish muscle at an average concentration of 10 mg/100 g wet weight and increases during chilled storage by endogenous and bacterial enzymes deamination activities. According to some authors, ammonia is a poor indicator of fish freshness and cannot be considered as an effective marker of fish spoilage (Paarup *et al.,* 2002).

Enzymatic and bacteriologic activity can rapidly decrease the protein content and quality of seafood that become stale; some ammonia, trimethylamine, dimethylamine and others volatile basic nitrogenous compounds are produced, which together are called TVB-N (Fallah *et al*., 2015; Wu and Bechtel, 2008). Total volatile basic nitrogen (TVB-N) is considered as an important characteristic for the assessment of quality in seafood products and appears as the most common chemical indicators of marine fish spoilage (Amegovu *et al*., 2012, Wu and Bechtel, 2008). During decomposition of seafood, various amounts of biogenic amines are also usually produced, depending on the fish species (Lehane and Olley 2000; Prester *et al.,* 2011) and have been proposed as a freshness indicator of fish products, including cuttlefish and crustacean. The most common biogenic amines associated with seafood spoilage are histamine, tyramine, putrescine and cadaverine (Lehane and Olley, 2000). They are formed by bacteria naturally present in decomposed fish that decarboxylate the corresponding free amino acids (Halász *et al*., 1994; Önal, 2007).

Although some levels were proposed for TVN-B and biogenic amines as indicators of freshness and decomposition, there is a general lack of data in literature and no limits are established and recognized.

The aim of the study was to assess limits of acceptability of TVB-N and biogenic amines, as indicators of freshness, in crustaceans and cephalopods, comparing their concentrations with sensory evaluation.

Materials and Methods

A total of forty-nine crustacean and cephalopod samples were purchased in different days in several fish shops and warehouse stores sited in Perugia (Central Italy).

Crustaceans were represented by nine batches of fresh deep-water rose shrimps (*Parapenaeus longirostris*), nine batches of defrosted argentine red shrimp (*Pleoticus*

Correspondence: Naceur Haouet, Institute for Experimental Veterinary Medicine of Umbria and Marche, Via Salvemini 1, 06126 Perugia (PG), Italy.
Tel.: +39.075.343259 - Fax: +39.075.3433060.
E-mail: mn.haouet@izsum.it

Key words: Crustaceans; Cephalopods; TVB-N; biogenic amines.

Contributions: the authors contributed equally.

Conflict of interest: the authors declare no potential conflict of interest.

Received for publication: 17 August 2017.
Revision received: 25 September 2017.
Accepted for publication: 26 September 2017.

Italian Journal of Food Safety 2017; 6:6921
doi:10.4081/ijfs.2017.6921

muelleri) and seven batches of frozen Norway lobster (*Nephrops norvegicus*). Cephalopods were all represented by common cuttlefish (*Sepia officinalis*) of which nine batches were fresh and twelve defrosted. One sample of each batch was immediately analysed and the other stored at temperature ranging between 2 and 4°C, reflecting domestic storage conditions. Each day following purchase, one sample was collected and submitted to evaluation.

Analytical determinations of TVB-N and biogenic amines (putrescine, cadaverine, histamine, tyramine, spermine and spermidine) were carried out. BAI was calculated following the formula (Haouet 2001):

$$\frac{\text{putrescine + cadaverine + histamine}}{\text{1 + spermine + sperimidine}}$$

The sensory evaluation was performed by five trained assessors following a modification of the Quality Index Method (QIM), assigning to the whole seafood product a score based on a scale from 0 to 3 demerit points (0: fresh; 1: onset of decay; 2: significant decay; 3: advanced decay). The sample storage condition score was based on the following attributes: for both cuttlefish and crustaceans, appearance, colour, odour, texture of the flesh, cornea and pupil of the eyes; for cuttlefish, mucus of the skin, colour, odour and mucus of the mouth region and presence of material in the sucker of arms; for crustaceans, melanosis appearance.

The limits of acceptability of TVB-N, biogenic amines and BAI were assessed at score 1 of the sensory evaluation.

Each sample was analysed in triplicate (three times with independent analysis per sample).

Statistical analysis of data was performed using the one-way analysis of variance (Little and Hills, 1978). Correlations between sensorial scores and analytical results were evaluated performing a linear regression plot of data (Figures 1 and 2) and using the Bravais-Pearson correlation coefficient.

TVB-N was performed following a modification of the method of Regulation EC N. 2074 (2005). 0.1 N sulphuric acid solution was obtained from Carlo Erba (Italy); 2% boric acid solution, 3 mg mL^{-1} ammonium chloride solution, silicone as an anti-foam, Tashiro indicator and magnesium oxide were obtained from Sigma-Aldrich (Switzerland); pumice stone was purchase from Fluka (USA); Milli-Q water was produced from a Milli-Q system (Millipore, USA).

Five g of homogenised sample was transferred into a test tube and 70 mL of MilliQ water, some drops of silicone as anti-foam and few pieces of pumice stones were added; after a maceration phase of 30 minutes, a receiver flask containing 25 mL of boric acid at 2% and three drops of Tashiro indicator was prepared. After the maceration phase, 1-2 g of magnesium oxide was added to the test tube containing the sample. The receiver flask and the tube containing sample were then arranged into the distillation unit's positions and the instrument was started. At the end of distillation, the solution turned to an emerald green colour, titrated with 0.1N sulphuric acid until colour turned to violet. A parallel blank solution was performed using all reagents and Milli-Q water instead of sample.

The calculation of TVB-N (mg/100 g) was done with the following formula:

$$\frac{[(V_1 - V_0) \times 14 \times 0.1 \times 100]}{M}$$

where V_1 is the volume in mL of sulphuric acid used for sample titration; V_0 is the volume in mL of sulphuric acid used for blank titration; M is the sample weight; 14 is the molecular weight of nitrogen; 0.1 is the normality of sulphuric acid.

The determination of biogenic amines concentrations was performed following modifications of the procedures described by Zhai *et al.* (2012) and Rea et al. (2005). The reference materials (histamine dihydrochloride 99%; tyramine hydrochloride 99%; cadaverine dihydrochloride 99%; spermine tetrachloride 99%; putrescine dihydrochloride 99% and spermidine trihydrocloride 98%), dansyl chloride (Dns-Cl, 99%) for dansylation, 1,7-diaminoheptane (98%) as internal standard (IS), acetonitrile, HPLC grade, and acetone, HPLC-grade, were obtained from Sigma-Aldrich (USA). Trichloroacetic acid (TCA) was purchased from Carlo Erba (Italy) and deionized water was produced from a Milli-Q system (Millipore, USA).

Stock solution of the biogenic amines were prepared to concentrations of 10 mg/mL in Milli-Q water. A working solution (20 ppm) was prepared by diluting 200 µL of intermediate solution of 1 mg/mL in Milli-Q water to a final volume of 10 mL. The internal standard solution was prepared by dissolving 25 mg of 1,7- diaminoheptane in 25 mL Milli-Q water. The Dns-Cl solution (10 mg/mL) was prepared by dissolving 500 mg of Dns-Cl in 50 mL of acetone. All solutions were kept at a temperature of 4°C prior to use.

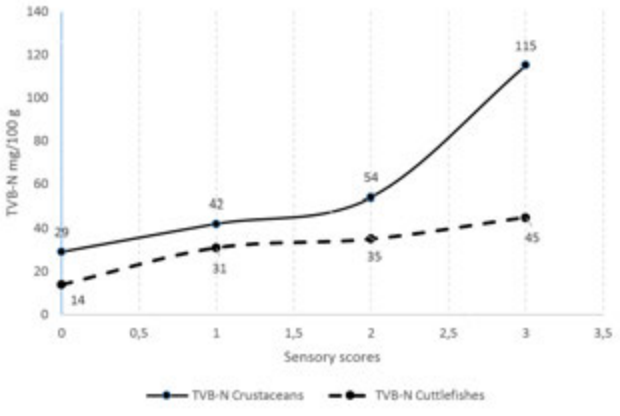

Figure 1. Levels of total volatile basic nitrogen in crustaceans and cuttlefishes stored at 2-4°C among time.

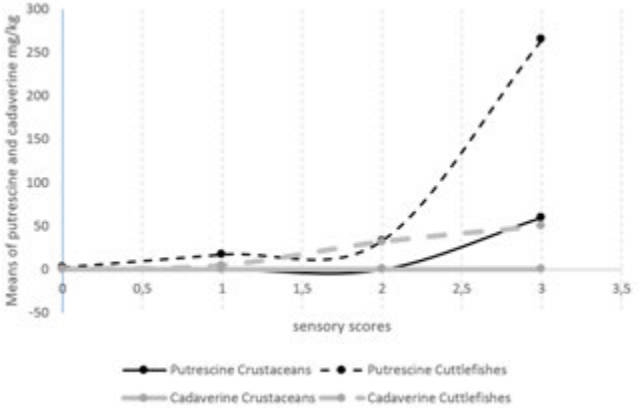

Figure 2. Levels of putrescine and cadaverine in crustaceans and cuttlefishes stored at 2-4°C among time.

Biogenic amines were extracted transferring a 5 g slurry of each sample into a centrifuge tube containing 20 mL of 5% (w/v) trichloroacetic acid (TCA) and 100 µL of 1,7-diaminoheptane. The mixture was vortexed for 15 min and then centrifuged at 10,000 g for 5 min at 4°C. The supernatant was collected and the residue was extracted again with the same volume of TCA. The supernatant was collected and the residue was extracted again with others 10 mL of TCA. One mL of sample solution was then mixed with 200 µL of 2M sodium hydroxide and 300 µL of saturated sodium carbonate. Under the alkaline conditions, the mixture was derivatives by the addition of 2 mL Dns-Cl. After incubation for 45 min at 40°C, 100 mL of 30% ammonium hydroxide was added to remove any residual Dns-Cl. Finally, the mixture was centrifuged at 6000 g for 5 min, and the supernatant was filtered through 0.45 mm-pore-size filters prior to HPLC analysis. Quantification of the biogenic amines was carried out by using a reverse phase HPLC UV-DAD and HP ChemStation software (AGILENT 1200). A Grace Smart C18 column (250 mm_4.6 mm, 5 mm) was used for separation. Water Milli-Q (solvent A) and acetonitrile (solvent B) were used as mobile phases with a gradient system of eluition. The flow rate was 1.0 mL/min and the temperature was 30°C. The sample was detected at 520 nm (emission wavelength) with an injection volume of 20 µL.

Results and Discussion

Fish quality is a complex concept involving a whole range of factors, which for the consumer include for example: safety, nutritional quality, availability, convenience and integrity, freshness eating quality and the obvious physical attributes of the species, size and product type. Information about handling, processing and storage techniques, including time/temperature histories that can affect the freshness and quality of the products is very important for the partners in the chain (Abbas *et al*., 2008). The influence of processing such as chilling, ice storage, slurry ice, freezing, cooking, canning and packaging including modified atmosphere packaging are of concern.

Fish decomposition is very complex (Hungerford, 2010). The sensory evaluation of most of the fishes is not sufficient to detect the quality of fish; therefore, chemical testing is required (Prester, 2011). Volatile amines are commonly used as criteria for assessing the fish quality. Besides, formation of biogenic amines in fish is species specific; it is much higher in histidine-rich (dark muscle) fish than in histidine-poor fish (white muscle) (Auerswald *et al*., 2006; Prester, 2011).

For crustaceans, the scores of the sensory evaluation were 1 in four just purchased samples (25%) and 0 in all the 12 others (75%), and 1 in three defrosted specimens (33%) and 0 in the six others (67%). For cuttlefish, the sensory evaluation score was 0 for all just acquired samples. The score of 1 (onset of decay) was generally observed after few days of storage at the temperature of 2-4°C and was necessary to assess limits of acceptability of TVB-N, biogenic amines and BAI. Interestingly, it should be noted that in advanced condition of decay, a strong ammonia smell was associated with high values of TVB-N while a fecal odour was associated with a wide increase of putrescine and cadaverine.

The results of TVB-N, putrescine and cadaverine in the different specimens of crustaceans and cuttlefishes collected are reported in Table 1, and those obtained for the total of crustaceans and cuttlefishes are shown in Table 2.

We can observe that the obtained levels of both TVB-N and biogenic amines are lower in defrosted products than in fresh one (Table 1). Among fresh crustaceans, the basal levels of TVB-N (just acquired) are significantly lower in lobster than in rose shrimp and argentine red shrimp 18 *vs*. 34 mg/100 g). Several other authors have suggested that TVB-N could be a good quality indicator for crustaceans; in Australia and Japan it is commonly used as a quality index for shrimps, for trade purposes, setting a limit of acceptability at 30 mg/100 g (Smaldone *et al.,* 2011). Instead, in our study, the level of TVB-N in samples at the onset of decay was higher

Table 1. Total volatile basic nitrogen (TVB-N), putrescine and cadaverine values in crustaceans and cuttlefish just purchased and at the onset of organoleptic alterations.

Time	Parameters, specimen	TNB-N (mg/100 g)		Putrescine (mg/kg)		Cadaverine (mg/kg)	
		Mean	SE	Mean	SE	Mean	SE
Just purchased	Fresh rose shrimps	34^{A}	0.7	0	0	0	0
	Defrosted argentine red shrimp	34^{A}	0.7	0	0	0	0
	Frozen Norway lobster	18^{B}	0.7	0	0	0	0
	Fresh cuttlefish	15^{A}	0.7	2	0.5	1^{A}	0.3
	Defrosted cuttlefish	13^{B}	0.7	3	0.8	0^{B}	0
Alteration onset	Fresh rose shrimps	45^{aA}	1	0	0	0	0
	Defrosted argentine red shrimp	39^{bB}	1	0	0	0	0
	Frozen Norway lobster	42^{cA}	1	0	0	0	0
	Fresh cuttlefish	38^{A}		15^{A}	0.7	11^{A}	0.7
	Defrosted cuttlefish	26^{B}		19^{B}	0.5	1^{B}	0.5

SE, standard error. ^{abc}Significant differences with P≤0.05; ^{ABC}significant differences with P≤0.01; ^{ABC}significant differences with P≤0.001.

Table 2. Differences of levels of total volatile basic nitrogen (TVB-N), putrescine and cadaverine between crustaceans and cuttlefishes.

Fishes		Parameters, specimen (mg/100 g)		Putrescine (mg/kg)		Cadaverine (mg/kg)	
		Mean	SE	Mean	SE	Mean	SE
Crustaceans	Just purchased	29.3^{A}	1.0	0.0^{A}	0.0	0.0	0.0
	Alteration onset	41.9^{B}	0.6	0.0^{A}	0.0	0.0	0.0
Cuttlefishes	Just purchased	$14,3^{B}$	0.3	2.3^{A}	0.5	0.4^{A}	0.2
	Alteration onset	30.7^{A}	1.1	17.1^{B}	0.5	4.8^{B}	0.8

^{ABC}Significant differences with P≤0.01; ^{ABC}significant differences with P≤0.001.

resulting 42 mg/100 g in crustaceans (Table 2) with mean values varying between samples (P≤0.05); especially defrosted argentine red shrimp shows in Table 1 the lowest content (39 *vs*. 45 and 42 mg/100 g; P≤0.001). In cuttlefish, the levels of TVB-N started from a basal level of 14 mg/100 g in fresh samples and raised to 30.7 mg/100 g at the onset of deterioration (Table 2), with the mean result significantly lower in defrosted samples than in fresh one (Table 1: 26 *vs*. 38 mg/100 g; P≤0.001). However, the basal values of TVB-N observed in fresh specimens (30 mg/100 g in crustaceans and 14 mg/100 g in cuttlefish) were similar to those obtained in other studies (Sykes *et al.,* 2009; López-Caballero *et al*., 2007) and the level of 30 mg/100 g in shrimp is usually found in high-quality fresh crustacean (López-Caballero *et al*., 2007).

Biogenic amines and BAI cannot represent in crustaceans indicators of spoilage as the levels resulted generally not detected and only putrescine is observable in a phase of high alteration. Instead, in cuttlefish, both putrescine and cadaverine can represent indicators of freshness as they present mean values significantly (P≤0.001) higher in samples at the onset of decay (score 1 of sensory evaluation) than in those just acquired (17 and 5 mg kg^{-1} *vs*. 2.3 and 0.4 mg kg^{-1}, for putrescine and cadaverine respectively; Table 2). Both putrescine and cadaverine increase over time but while cadaverine shows a gradual and moderate growth, putrescine has a sudden and wide raise at score 2 of organoleptic evaluation (significant decay) (Figure 2).

Also TVB-N shows a gradual and continuous increase over time, with higher mean values observable in crustaceans than in cephalopods (Figure 1).

The correlation coefficients were all positive and close to 1.

As for biogenic amines, agmatine has been proposed as a freshness indicator of cuttlefish (Sepia officinalis) (Vaz-Pires et al. 2008) and several squid species stored in ice (Todarodes pacificus, Todaropsis eblanae, Illex coindetii) (Inaba *et al*., 2004; Paarup *et al.,* 2002; Vaz-Pires *et al*., 2008; Zhao *et al.*, 2007). On the other hand, putrescine alone or biogenic amine index were proposed as markers of squid decomposition (Paarup *et al.,* 2002; Prester *et al.*, 2011). For crustaceans, putrescine has been found as the best indicator of decomposition for Penaeid shrimp and Norway lobster (*Nephrops norvegicus*) stored at a wide range of temperatures. Levels of putrescine at 3 and under 7 mg kg^{-1} in shrimp and Norway lobster have been proposed as thresholds for human consumption, respectively (Benner *et al*., 2003; Prester *et al.,* 2011). The production of cadaverine followed a pattern similar to that of putrescine in several shrimp species (Benner *et al.*, 2003; Shakila *et al.*, 1995).

In respect to the poor data available in literature, in this study different limits of freshness acceptability were found both for crustaceans and cephalopods. Indeed, some authors suggest value of 30 mg/100 of TVB-N in shrimp and lobster against the obtained limit of 42 mg/100 g. Other authors suggest levels of 3 and under 7 mg kg^{-1} for putrescine in crustaceans as limits of acceptability, while the results found in our study are useless, since putrescine (as cadaverine, tyramine and histamine) was not detectable.

For cephalopods, a limit of acceptability of 42 mg/100 g of TVB-N emerges from this study, while no limits are suggested in literature. Instead, we are in agreement with some authors to propose biogenic amines as indicators of freshness, both for putrescine alone and BAI.

Tyramine and histamine contents have not been detected at any stage, in both crustaceans and cuttlefish, while spermidine was rarely found, especially at low levels in conditions of advanced decay. Instead, spermine has always been detected, both in crustaceans and in cephalopods, with values that do not show significant increases over time (means ± standard errors: 4±0.02 mg kg^{-1} in crustaceans and 19±0.06 mg kg^{-1} in cephalopods).

Finally, from this paper it emerges that BAI cannot be used as an indicator of freshness in crustaceans, as putrescine, cadaverine and histamine are always not detected, except for putrescine in conditions of advanced decay. For cuttlefish instead, a BAI of 1 can be set as a limit of acceptability.

Conclusions

In this study, high significant differences were observed between values of TVB-N and biogenic amines, in crustaceans and cuttlefish. Defrosted products show generally lower values of both TVB-N and biogenic amines than fresh ones. Biogenic ammines show generally moderate contents in cephalopods and even not detectable in crustaceans that become potentially high only in case of advanced decay. Therefore, crustaceans and cephalopods do not represent from this point of view a hazard for consumers, since the accumulation of biogenic amines in fishes is involved in nitrosamine formation, known as carcinogens (Sen *et al.,* 1985; Yurchenko and Mölder, 2006). Furthermore, biogenic amines subjected to heat may also form nitrosatable amines. Putrescine and cadaverine, which are commonly found in decomposed fish and shellfish, may generate N-nitroso compounds, nitrosopyrrolidine (NPYR) and nitrosopiperidine (NPIP), respectively (Al Bulushi *et al*., 2009; Yurchenko and Mölder, 2006).

Finally, from this work it is possible to set limits of freshness acceptability of 42 and 30 mg/100 g of TVB-V, respectively for crustaceans and cephalopods while 17 mg kg^{-1} of putrescine and 5 mg kg^{-1} of cadaverine can be set only for cephalopods.

In conclusion, considering that fish and shellfish are highly perishable and since the deterioration in the quality of shellfish is species specific and highly temperature and time dependent, further investigations are needed to find the right indicators of decomposition for crustaceans and cephalopods.

References

Abbas KA, Mohamed M, Jamilah B, Ebrahimian M, 2008. A review on correlations between fish freshness and pH during cold storage. Am J Biochem Biotechnol 4:416-21.

Al Bulushi I, Poole S, Deeth HC, Dykes GA, 2009. Biogenic amines in fish: roles in intoxication, spoilage, and nitrosamine formation – a review. Crit Rev Food Sci Nutr 49:369-77.

Amegovu AK, Sserunjogi ML, Ogwok P, Makokha V, 2012. Nucleotited degradation products, total volatile basic nitrogen, sensory and microbiological quality of Nile pearch (Lates niloticus) fillets under chilled storage. J Microbiol Biotechnol Food Sci 2:653-66.

Auerswald L, Morren C, Lopata AL, 2006, Histamine levels in seventeen species of fresh and processed South African seafood. Food Chem 98:231-9.

Benner Jr RA, Staruszkiewicz WF, Rogers PL, Otwell S, 2003. Evaluation of putrescine, cadaverine, and indole as chemical indicators of decomposition in Penaeid shrimp. J Food Sci 68:2178-85.

Fallah F, Ebrahimnezhad Y, Maheri-Sis N, Ghasemi-Sadabadi M, 2016. The effect of different levels of diet total volatile nitrogen on performance, carcass characteristics and meat total volatile nitrogen in broiler chickens. Arch Anim Breed 59:191-9.

FAO, 2016. The state of world fisheries and aquaculture, 2016. Contributing to food security and nutrition for all. FAO, Rome.

Gulsun O, Esmeray K, Serhat O, Fatih O, 2009. Sensory, microbiological and chemical assessment of the freshness of red mullet (Mullus barbatus) and gold-band goatfish (Upeneus moluccensis) during storage in ice. Food Chem 114:505-10.

Halász A, Baráth A, Simon-Sarkadi L, Holzapfel W, 1994. Biogenic amines and their production by microorganisms in food. Trends Food Sci Technol 5:42-9.

Haouet MN, 2001. Conservazione ed alterazioni chimiche dei prodotti ittici. Webzine Sanità Pubblica Veterinaria® Numero 8-9 - ottobre 2001. Available from: http://www.spvet.it/arretrati/numero_8_9/ittici.html.

Haouet MN, Altissimi MS, Blasi G, Petruzzelli A, Scuota S, Cenci T, 2006. Shelf life assessment and prediction of traditional food of Central Italy. Ital J Food Sci 19:82-6.

Hungerford JH, 2010. Scombroid poisoning: a review. Toxicon. 56:231-43.

Inaba Y, Tokishita S, Hamada-Sato N, Kobayashi T, Imada C, Yamagata H, Watanabe E, 2004. Development of agmatine sensor using the combination of putrescine oxidase and agmatinase for squid freshness. Biosens Bioelectron 20:833-40.

Lehane L, Olley J, 2000. Histamine fish poisoning revisited. Int J Food Microbiol 58:1-37.

Little TM, Hills FJ, 1978. Agricultural experimentation: design and analysis. 31. John Willey and sons, New York.

López-Caballero ML, Martínez-Alvarez O, del Carmea Gómez-Guillén M, Montero P, 2007. Quality of thawed deepwater pink shrimp (Parapenaeus longirostris) treated with melanosis-inhibiting formulations during chilled storage. Int J Food Sci Technol 42:1029-38.

Önal A, 2007. A review: current analytical methods for the determination of biogenic amines in food. Food Chem 103:1475-86.

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

Prester L, 2011. Biogenic amines in fish, fish products and shellfish: a review. Food Add Contam 28:1547-60.

Rea S, Ricciutelli M, Cecchini S, Stocchi R, Loschi AR, 2005. Caratteristiche fisico-chimiche e contenuto di amine biogene nel salame ciauscolo. Industrie Alimentari 44:38-45.

Sen NP, Tessier L, Seaman SW, Baddoo PA, 1985. Volatile and nonvolatile nitrosamines in fish and the effect of deliberate nitrosation under simulated gastric conditions. J Agric Food Chem 33:264-8.

Shakila RJ, Vasundhara TS, Rao DV, 1995. Rapid quality assessment of shrimps during storage by monitoring amines. J Food Sci Technol 32:310-4.

Smaldone G, Marrone R, Vollano L, Chirollo C, Pellicane A, Palma G, 2011. Shelf life of thawed crustaceans treated with sulphites. Ital J Food Safety 1:758.

Sykes AV, Oliveira AR, Domingues PM, Cardoso CM, Andrade JP, Nunes ML, 2009. Assessment of European cuttlefish (Sepia officinalis, L.) nutritional value and freshness under ice storage using a developed Quality Index Method (QIM) and biochemical methods. Food Sci Technol 42:424–432.

Vaz-Pires P, Seixas P, Mota M, Lapa-Guimarães J, Pickova J, Lindo A, Silva T, 2008. Sensory, microbiological, physical and chemical properties of cuttlefish (Sepia officinalis) and broadtail shortfin squid (Illex coindetii) stored in ice. Food Sci Technol 41:1655-64.

Venugopal V, 2002. Biosensors in fish production and quality control. Biosensors Bioelectronics 17:147-57.

Wu TH, Bechtel PJ, 2008. Ammonia, Dimethylamine, Trimethylamine, and Trimethylamine Oxide from raw and processed fish by-products. J Aquat Food Prod Technol 17:27-38.

Yurchenko S, Mölder U, 2006. Volatile N-nitrosamines in various fish products. Food Chem 96:325-33.

Zhai H, Yang X, Li L, Xia G, Cen J, Huang H, Hao S, 2012. Biogenic amines in commercial fish and fish products sold in southern China. Food Control 25:303-8.

Zhao QX, Xu J, Xue CH, Sheng WJ, Gao RC, Xue Y, Li ZJ, 2007. Determination of biogenic amines in squid and white prawn by high-performance liquid chromatography with postcolumn derivatization. J Agric Food Chem 55:3083-8.