

Effects of different levels of CO₂ on biochemical changes and relationships among different quality indices in Indian mackerel (*Rastrelliger kanagurta*)

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Article history

Received: 30 March 2012
Received in revised form:
28 May 2012
Accepted: 3 June 2012

Abstract

The profile of total volatile base nitrogen (TVBN), pH, biogenic amines were studied in Indian mackerel packed under different levels of CO₂. Guttled and beheaded Indian mackerel was stored in air, vacuum packaging (VP), 30% CO₂/65% N₂/5% O₂ (M30C), 60% CO₂/35%N₂/5%O₂ (M60C), 80% CO₂/15%N₂/5% O₂ (M80C) and 100% CO₂ (M100C) at 5°C for 12 days. The application of VP and MAP was effective in retarding the formation of TVBN, total biogenic amines and improve the shelf life of Indian mackerel. Cadaverine obtained the best correlation with storage time when compared with other biogenic amines. Cadaverine or cadaverine + putrescine can serve as a reliable objective freshness indicator of fish stored in different atmospheres. Among the commonly used freshness indices, TVBN was the best quality indicator correlated with histamine. VP and MAP conditions influenced the performance of quality indicators. pH was a good quality indicator of spoilage in air-stored fish except for VP and MAP packed fish. Lower value of TVBN (30 mg/100g) was suggested as an upper limit for this species of fish under MAP condition based on APC and sensory result.

Keywords

Histamine
storage
aeromonas
vacuum packaging
dominant microflora

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Introduction

Modified atmosphere packaging received much attention due to its economic and uncomplicated method to preserve the food (Speranza *et al.*, 2009). Nowadays, there is a trend toward the convenient and healthy food among the consumer. Modified atmosphere packaging provides a potential method to preserve the products with minimum processing as compared to the conventional method such as canning. This method also avoids the use of preservative. Modified atmosphere packaging has been reported to improve the quality and shelf life of fishery products successfully (Yesudhasan *et al.*, 2009; Yesudhasan *et al.*, 2010). Fish is highly perishable commodities. After the capture, fish loses its natural characteristics and starts to undergo biochemical, physical and microbiological changes. The deterioration occurs in fish result in the formation of certain compounds in the muscle. These chemical compounds can be quantified indicating the progress of spoilage.

The traditional methods to assess the freshness of fish include sensory evaluation, microbiological

and biochemical methods. The biochemical indicator has been commonly used to assess the deterioration changes includes total volatile bases nitrogen (TVBN). There is no literature available on the effect of different levels of CO₂ on the formation of TVBN in fish. Biogenic amine is one of the spoilage products produced during fish decomposition. Histamine receives much concern due to the previous history implicated in seafood poisoning. Histamine poisoning produces the symptoms including diarrhea, dizziness, headache, reddening of the face, vomiting, neck and upper chest, abdominal cramps and nausea (Zaman *et al.*, 2010; Zaman *et al.*, 2011). Several countries have established the regulatory limit to prohibit the distribution of fishery products contaminated with histamine. However, histamine intoxication is still common in seafood borne illness over the world (Chong *et al.*, 2011).

Biogenic amine has been suggested as chemical freshness indicator in fish. The suitability of biogenic amine as chemical indicator provides an alternative for objective evaluation of fish quality. Therefore, it is of interest to investigate the kinetic of biogenic

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amine in relation with other biochemical and microbiological indicator during spoilage. Indian mackerel (*Rastrelliger kanagurta*), local name known as kembong, is the important food fish in Malaysia. Indian mackerel is widespread in South East Asia and it can be processed into fish cracker and sausage. Indian mackerel belongs to *Scombridae* family which potentially contains a high concentration of histamine in muscle. Thus, the purposes of this study were to study how the different levels of CO₂ in modified atmosphere packaging affect the biochemical, sensory indices and relationships among different quality indices in histidine rich fish species.

Materials and Methods

Sample

Indian mackerel (*R. kanagurta*) were obtained from Beserah, Kuantan, East Coast of Malaysia in the month of September. The fish was immediately beheaded, gutted and washed upon landing and transported to laboratory in ice-cooler box. The fish arrived at laboratory after 4 hours of capture. The weight of the fish ranged from 90 to 130 gram. Three beheaded and gutted fish were randomly put into a bag and divided into six lots. The bag was consisted of nylon, polyethylene and high density polyethylene layers (0.09 mm thick, dimension 220×300 mm). The transmission rate of the bag were 1.55, 0.465, 6.15 cm³/m²/day atm for O₂, N₂, CO₂ and 15 g/m²/day atm for H₂O (38 °C), respectively. The fish was vacuum-packed using vacuum packer model DZQ400A. The sample was subjected to storage with six different atmospheres including control (C), vacuum-packaging (VP), 30% CO₂+65% N₂+5% O₂ (M30C), 60% CO₂+35%N₂+5%O₂ (M60C), 80% CO₂+15%N₂+5% O₂ (M80C) and 100% CO₂ (M100C) using gas mixer model MAP mix 9001 ME (Ringsted, Denmark). The ratio of gas/samples in all bags was 2:1 (v/w). The packaging headspace composition was confirmed using Illinois Instruments model 6600 headspace analyzer (USA). All treatments were stored in a chiller (5±1°C). Triplicate muscle samples (each sample consisted of pooled muscle sample of three fish) were taken for biochemical analysis every 3 days during storage at chilling temperature. Duplicate muscle samples (each sample consisted of pooled muscle sample of three fish) were taken for microbiological analysis.

Headspace analysis

The measurement of packaging headspace composition was conducted every sampling day using Illinois Instruments model 6600 headspace analyzer (USA). A volume of 3 ml of gas was collected with

gas tight syringe to confirm the concentration of CO₂ and O₂.

pH measurement

pH determination was carried out by mixing homogenized Indian mackerel muscle with water in the ratio of 1:2. The measurement was carried out using pH meter (Mettler Toledo, Schwerzenbach, Switzerland). Calibration of pH meter was performed using buffer solutions (pH 4 and pH 7). Triplicate muscle samples were taken for pH analysis.

Determination of total volatile basic nitrogen

Total volatile basic nitrogen (TVBN) was analyzed according to the procedure of Antonacopoulos and Vyncke (1989). Ten gram of fish muscle was mixed with 90 ml distilled water for 1 min with homogeniser. An amount of 2 g of Magnesium Oxide and 2 drops of silicone anti form emulsion were added. The vessel was inserted into distillation unit and distilled with a steam flow of approximately 10 ml/min. Distillation time was exactly 12 minutes. The volatiles bases were absorbed in 10 ml 3 % boric acid with 3 drops of methyl red, filled up to approximately 100 ml with distilled water. The distillate was titrated with 0.1 N HCl until reaching the neutral point. Total volatile basic nitrogen was expressed as milligrams per 100 g muscle sample.

Preparation of standard amines solution

The concentration of working standard solution of 1.0 mg ml⁻¹ for each amine was prepared. Putrescine dihydrochloride (45.75 mg), cadaverine dihydrochloride (42.85 mg), spermidine trihydrochloride (43.85 mg), spermine tetrahydrochloride (43.0 mg), histamine dihydrochloride (41.4mg) and tyramine hydrochloride (31.7 mg) were dissolved in 25 ml distilled water as a working solution. All the standards were purchased from Sigma (St. Louis, MO, USA). The standard amine solution was analysed by High Performance Liquid Chromatography (HPLC). The mean peak areas of the chromatogram from duplicate standards were calculated.

Extraction and derivatization of amines from fish sample

Five gram of ground fish sample were weighed and transferred to 50 ml centrifuge tube. The fish muscle was homogenized with 20 ml 6% trichloroacetic acid (TCA) for 3 min. The homogenate was centrifuged at 8000g, 4°C for 10 min and filtered using Whatman No. 2 filter paper. The filtrate was transferred and made up to 50 ml using a volumetric flask. Benzoyl chloride was used to derivatize the extract with procedure

proposed by Hwang *et al.* (1997). One milliliter of extract or mixed amines standard solution was added with 1 ml of sodium hydroxide (2M) and 10 μ l of benzoyl chloride. The mixture was homogenized by vortex mixer and then incubated at 30°C for 40 min. Two milliliters of saturated NaCl solution were added to stop the benzylation process and 3 ml of diethyl ether was used to extract the solution. The solution was centrifuged at 10000 rpm and 4°C for 10 minutes. Later, the organic layer was put into a 5 ml vial and dried with nitrogen gas. The residue was dissolved in 1 ml of acetonitrile and filtered with 0.2 μ m nylon filter. Aliquots of 20 μ l were injected for HPLC analysis.

Separation of biogenic amines by HPLC

Biogenic amines were determined by Agilent technologies 1200 series chromatographic system (Waldron, Germany) comprising of vacuum degasser, auto sampler, quaternary pump, and diode array detector. The separations were performed on C18 SunfireTM (150 mm \times 4.6 mm, 5.0 μ m) column (Waters Corporation, Massachusetts, USA). The absorbance of the sample was determined at 254 nm. The mobile phase was pumped at 0.8 ml min⁻¹ flow rate and consisted of acetonitrile–water solution in a gradient elution program according to Chong *et al.* (2012).

Microbiological analysis

Twenty five gram of fish flesh from each replicate of pooled muscle sample of three fish was transferred aseptically to 225 mL sterile peptone water (0.1% peptone water and 0.85% NaCl). The mixture was blended in a sterile stomacher bags (Bagmixer 400, Interscience, France) for 1 minute. The homogenate was serially diluted with 9 ml peptone water. Dilutions of 100 μ l were spread on aerobic plate count agar (APC) containing 0.5% NaCl. The plates were incubated for 2 days at 37 °C. After the plates were incubated, the bacterial colonies were counted and expressed in log colony forming unit CFU g⁻¹.

Sensory evaluation

The sensory evaluation was conducted by 30 untrained panelists. The evaluation was performed for the appearance, texture, odour and overall acceptability of Indian mackerel by 9 point hedonic scale (Maqsood and Benjakul, 2010). 9, like extremely; 8, like very much; 7, like moderately; 6, like slightly; 5, neither like nor dislike; 4, dislike slightly; 3, dislike moderately; 2, dislike very much; 1, dislike extremely. The rating was converted to score with 9 equivalent to the highest quality and 5

being the acceptable limit of freshness. The fish was rejected when the score lower than 5.

Shelf life evaluation

Shelf life of *Rastrellinger kanagartha* was evaluated based on biochemical, microbiological and sensory method. Shelf life of fish was determined when the indices exceeded recommended upper limit. These upper limits were 50 ppm of histamine (FDA, 1996), 35 ppm of total volatile base nitrogen (EU, 1995) and 7 log CFU g⁻¹ of aerobic plate count (ICMSF, 1986). For sensory evaluation, the fish was rejected when the score lower than 5 (Maqsood and Benjakul, 2010).

Results and Discussions

Headspace analysis

Changes of CO₂ levels in MAP packaging are given in Figure 1. After day 3 of storage, the concentrations of CO₂ in MAP decreased drastically. The greater decrease was observed in M80C and M100C. After day 3 of storage, the levels of CO₂ in MAP with M30C and M60C increased slightly toward to the end of storage. At the same period of time, the levels of CO₂ in M80C and M100C decreased at a slower rate. After reaching the minimum concentration, the CO₂ level for all MAP increased slowly toward to the end of storage. The O₂ levels were completely depleted after day 6 of storage until to the end of the study.

The decrease of CO₂ level in MAP indicated CO₂ dissolved into the liquid phase of Indian mackerel. This result was similar to the findings reported by Sivertsvik *et al.* (2003) in Atlantic salmon. In the present study, the slight increase of CO₂ was observed during the later stage of storage and it could be due to microbial metabolism. The level of O₂ depleted probably as a consequence of consumption by aerobic bacteria inside the package. Torrieri *et al.* (2006) observed that CO₂ increased from 0 \pm 2% to 17 \pm 3% and O₂ decreased from 20.8% to 0.2 \pm 0.3% over the storage period. The percentage of CO₂ was reduced by 46%, 33%, 27%, 3% in MAP with M100C, M80C, M60C and M30C. Assuming that the diminished level of CO₂ dissolved completely in tissue liquid, the initial CO₂ concentration in the gas phase had a strong influence on the concentration of dissolved CO₂. This result agrees with the findings by Devlieghere *et al.* (1998). The other factors such as lower storage temperature and higher gas/product ratio would increase the dissolution of CO₂ into product thereafter increasing the effect of MAP.

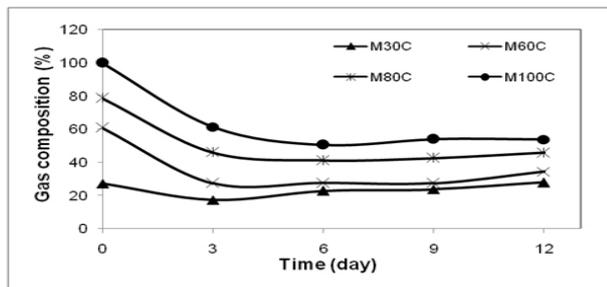


Figure 1. Changes of CO_2 composition in MAP with 30% CO_2 /65% N_2 /5% O_2 (M30C), 60% CO_2 /35% N_2 /5% O_2 (M60C), 80% CO_2 /15% N_2 /5% O_2 (M80C) and 100% CO_2 (M100C) during chilling storage

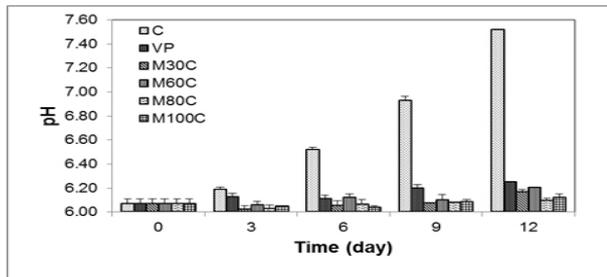


Figure 2. Changes in pH of *R. kanagurta* packaged in air (C), vacuum packaging (VP) and modified atmosphere packaging (MAP) during chilling storage

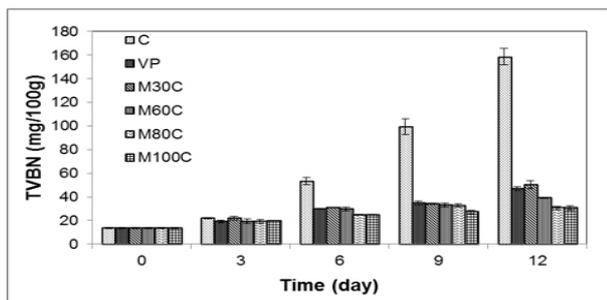


Figure 3. Changes in TVBN of *R. kanagurta* packaged in air (C), vacuum packaging (VP) and modified atmosphere packaging (MAP) during chilling storage

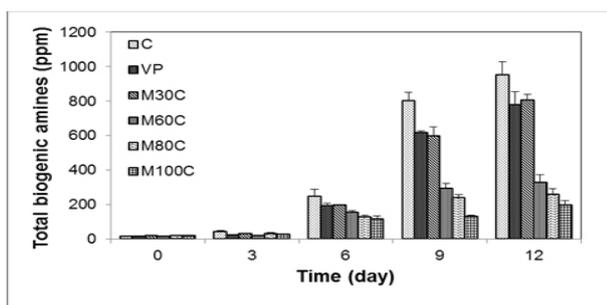


Figure 4. Changes in total biogenic amines of *R. kanagurta* packaged in air (C), vacuum packaging (VP) and modified atmosphere packaging (MAP) during chilling storage

pH

Changes of pH in Indian mackerel packaged with different atmospheres are shown in Figure 2. In the beginning of study, the pH was determined as 6.07 in fish muscle. pH in air-stored fish was much higher than those found in other treatments throughout the

storage period. The pH increased slowly in the fish packaged with VP and MAP over the storage trial. Similar results were obtained by Pastoriza *et al.* (1998), Stamatis and Arkoudelos (2007) and Torrieri *et al.* (2006).

At the end of storage, the pH of fish kept in air and VP was significantly higher than that kept in MAP. In the fish packaged under MAP, the pH decreased very slightly throughout the experimental period. The lower pH in fish stored in MAP was probably due to carbonic acid produced from dissolution of CO_2 into tissue liquid of Indian mackerel (Ordonex *et al.*, 2000). pH increased rapidly between day 9 to 12 corresponded to the increase of TVBN in the present study. The rise of pH in fish stored in air was probably due to the formation of basic substances such as trimethylamine, ammonia and other volatile bases from microbial growth during fish spoilage. The inhibition of the growth of aerobic spoilage bacteria under VP and MAP resulted in lower pH in the sample, as a consequence of lower production of the trimethylamine and volatile bases.

pH of fish kept in air increased as the storage time progressed, but the fish packaged in VP and MAP changed only slightly for the entire experimental period. It indicated that pH is not a good quality indicator of spoilage under VP and MAP conditions. Devlieghere *et al.* (1998) reported that CO_2 solubility increased with an increase in pH. Low pH will direct the equilibrium reaction toward the CO_2 instead of formation of a hydrogen and bicarbonate ion (Devlieghere *et al.*, 1998).

Total volatile basic nitrogen (TVBN)

Results of TVBN in muscle of Indian mackerel are demonstrated in Figure 3. At the beginning of study, TVBN of 13.59 mg/100 g was detected in Indian mackerel indicating the freshness of fish. TVBN increased significantly in all treatments with an increase of storage time. Similar to pH, TVBN in air-stored fish increased much more rapidly throughout storage as compared with other packaging. Concentration of TVBN did not differ much among VP and MAP. On day 6 of storage, the fish kept in air was determined as 53.54 mg/100g exceeding the upper limit set by European Union, and other treatments were still below the upper limit of 35 mg/100g muscle. There was a large increase of TVBN between day 9 to 12 which was similar to the pattern of pH. The concentrations of TVBN increased by approximately 11 fold in fish stored in air, 2.7 fold in M30C, 2.5 fold in VP, 1.9 fold in M60C, 1.3 fold in M80C and 1.2 fold in M100C as compared to the initial concentration. TVBN in air-stored

Table 1. Pearson correlation coefficient for different freshness indices and biogenic amines in *R. kanagurta* stored in air at chilled temperature (5°C) for 12 days

	Time	pH	TVBN	APC	Sensory	Cad+Put	Cad	His	Put	Tyr	Spm	Spd
Time	1											
pH	.973**	1										
TVBN	.966**	.99**	1									
APC	.944**	.84**	.827**	1								
Sensory	.994**	.94**	.932**	.973**	1							
Cad+Put	.966**	.98**	.989**	.838**	-.937**	1						
Cad	.963**	.94**	.950**	.875**	-.948**	.98**	1					
His	.958**	.96**	.970**	.839**	-.936**	.98**	.986**	1				
Put	.931**	.99**	.994**	.762**	-.886**	.97**	.925**	.951**	1			
Tyr	.881**	.88**	.894**	.760**	-.858**	.94**	.974**	.966**	.878**	1		
Spm	.617**	.45**	.432**	.776**	.693**	.46**	-.557**	-.547**	-.330**	-.491**	1	
Spd	.834**	.88**	.903**	.652**	-.785**	.94**	.934**	.933**	.915**	.966**	-.262**	1

Cad, cadaverine, His, histamine, Put, putrescine, Tyr, tyramine, Spm, spermine, Spd, spermidine
Pearson's correlation, ** significant at P<0.01, * significant at P<0.05

Table 2. Pearson correlation coefficient for different freshness indices and biogenic amines in *R. kanagurta* packaged with VP and M30C at chilled temperature (5°C) for 12 days

	Time	pH	TVBN	APC	Sensory	Cad+Put	Cad	His	Put	Tyr	Spm	Spd
Time	1											
pH	.719*	1										
TVBN	.983**	.700*	1									
APC	.883**	.455**	.838**	1								
Sensory	.989**	.677**	.959**	.929**	1							
Cad+Put	.988**	.711**	.966**	.859**	-.983**	1						
Cad	.990**	.691**	.968**	.872**	-.985**	.998**	1					
His	.933**	.774**	.904**	.688**	-.933**	.930**	.930**	1				
Put	.962**	.757**	.941**	.797**	-.985**	.974**	.922**	.922**	1			
Tyr	.918**	.729**	.917**	.654**	-.902**	.903**	.978**	.880**	.880**	1		
Spm	-.267**	-.719**	-.220**	-.188**	.284**	-.254**	-.217**	-.249**	-.362**	-.158**	1	
Spd	.791**	.650**	.796**	.453**	-.719**	.794**	.789**	.915**	.794**	.954**	-.074**	1

Cad, cadaverine, His, histamine, Put, putrescine, Tyr, tyramine, Spm, spermine, Spd, spermidine
Pearson's correlation, ** significant at P<0.01, * significant at P<0.05

Table 3. Pearson correlation coefficient for different freshness indices and biogenic amines in MAP-packaged *R. kanagurta* with M80C and M100C at chilled temperature (5°C) for 12 days

	Time	pH	TVBN	APC	Sensory	Cad+Put	Cad	His	Put	Tyr	Spm	Spd
Time	1											
pH	.680*	1										
TVBN	.961**	.552**	1									
APC	.907**	.380**	.946**	1								
Sensory	.987**	-.586**	-.968**	-.954**	1							
Cad+Put	.968**	.676**	.966**	.889**	-.966**	1						
Cad	.971**	.665**	.965**	.904**	-.975**	.998**	1					
His	.841**	.581**	.884**	.752**	-.834**	.914**	.891**	1				
Put	.819**	.670**	.843**	.663**	-.774**	.882**	.850**	.963**	1			
Tyr	.883**	.749**	.871**	.722**	-.845**	.936**	.914**	.943**	.974**	1		
Spm	-.604**	-.816**	-.430**	-.334**	.552**	-.613**	-.611**	-.538**	-.548**	-.636**	1	
Spd	-.807**	-.564**	-.851**	-.847**	.848**	-.864**	-.884**	-.691**	-.608**	-.701**	.420**	1

Cad, cadaverine, His, histamine, Put, putrescine, Tyr, tyramine, Spm, spermine, Spd, spermidine
Pearson's correlation, ** significant at P<0.01, * significant at P<0.05

Indian mackerel was significantly higher than that stored in VP and M30C. M60C, M80C and M100C were significantly lower than M30C. No significant difference was found among M60C and M80C at the end of storage.

In the present study, the concentration of TVBN in

fish packaged with MAP was lower than that found in VP throughout the experimental period. This result is similar to that reported by Erkan *et al.* (2007), Ozogul *et al.* (2004) and Pantazi *et al.* (2008). The effect of MAP in reducing the concentration of TVBN was in agreement with the findings in chub mackerel (Erkan *et al.*, 2007) and swordfish (Pantazi *et al.*, 2008).

TVBN is the substance produced during microbial spoilage of food and is frequently served as a quality indicator for seafood products. According to TVBN acceptable limit set by European Union, the suitability for consumption of Indian mackerel were as 4-5 days of storage in air, 9 days of storage in VP and M30C, 9-10 days in M60C. The TVBN concentration of the fish kept in M80C and M100C never exceeded 35 mg/100g at the end of storage. In contrast, Goulas and Kontominas (2007) reported that the TVBN content of chub mackerel exceeded this limit after 10-11 d, 14-15 d, 16-17 d and 19-20 d stored under air, VP, 50%CO₂/30%N₂/20%O₂ and 70%CO₂/30%N₂, respectively, at 2°C.

In the present study, aerobic plate count achieved 7 log CFU g⁻¹ after 6 days of storage in fish kept in VP, M30C, M60C and M80C. This result suggests that the value of 35 mg/100 might be higher as acceptable limit under MAP condition. Based on APC and sensory result, a value of 30 mg/100g was suggested as acceptable limit for Indian mackerel.

Total biogenic amines

Changes of total biogenic amines in muscle of Indian mackerel stored in air, VP and MAP are presented in Figure 4. The use of different levels of CO₂ had a profound effect in the pattern of total biogenic amines. As storage time progressed, total biogenic amines in all treatments increased in a dose-dependent manner toward the end of storage. All the CO₂ treatments had a significant effect on inhibition of total biogenic amines formation except for M30C. When aerobic plate count reached 7 log CFU g⁻¹ at day 6 of storage, large increase of total biogenic amines was observed except for M100C. The concentration of total biogenic amines of fish stored in air was the highest throughout the storage period. The fish kept in M100C had the lowest value of total biogenic amines, but it did not differ significantly compared to fish stored in M60C and M80C during the entire storage period. There was no significant difference was found between M30C and VP over the entire storage period. This result confirms that the inhibitory effect increases as CO₂ level increases. It also indicated that total biogenic amine is closely related to the growth of total spoilage microflora in fish.

Table 4. Shelf life of *R. kanagurta* based on different freshness indices and biogenic amines in different modified atmosphere packaging

Indices	Shelf life (day)					
	Air	VP	M30C	M60C	M80C	M100C
TVBN ¹	4-5	9	9	9-10	>12	>12
TVBN ²	3-4	6	6	6	8	11
APC	5	6	6	6	6	9
Sensory	5	7-8	7-8	9	10-11	10-11
Histamine	5	5-6	5-6	6	7	>12
Cad	6-7	7	7	7	7-8	10
Cad+Put	6	6	6	7	7-8	10

Relationship between freshness indices and biogenic amines in Indian mackerel stored under different MAP condition

Correlation between freshness indices and biogenic amines stored under different atmospheres is given in Table 1, 2 and 3. Correlation between different freshness indices and biogenic amines with time were studied in assessing the changes of fish quality stored under MAP conditions. Correlation was compared among 3 groups including the fish stored in air, low level of CO₂ (VP and M30C) and high level of CO₂ (M80C and M100C).

Among the freshness indices, there was a low correlation for pH ($r=0.680$, $P<0.05$) in fish stored under high level of CO₂ when compared with that found in low level of CO₂ ($r=0.719$, $P<0.05$) and normal air ($r=0.973$, $P<0.05$). The other freshness indices showed a good correlation in fish stored under different atmospheres. The Pearson's correlation coefficient ranged from 0.961 to 0.983 for TVBN, 0.883 to 0.944 for APC, -0.907 to -0.994 for sensory evaluation under different atmospheres. It indicated that pH was not a good quality indicator under high level of CO₂.

Among the biogenic amines, cadaverine obtained the best correlation with storage time when compared with other biogenic amines. The Pearson's correlation coefficient of cadaverine ranged from 0.963 to 0.990 under different atmospheres. The correlation ranged from 0.841 to 0.958 for histamine, 0.819 to 0.962 for putrescine, 0.883 to 0.918 for tyramine and 0.791 to 0.834 for spermidine. There was no correlation was found in spermine.

Among the different commonly used freshness indices, TVBN was the best indicator correlated with histamine, recording the r value of 0.970 in air storage, 0.904 in low level of CO₂ and 0.884 in high level of CO₂. It was followed by sensory evaluation ($r = -0.834$ to -0.976), APC ($r = 0.688$ to 0.839) and pH ($r = 0.581$ to 0.968).

Among the biogenic amines, histamine obtained the best correlation with tyramine ($r=0.96$), followed by putrescine ($r=0.95$) and cadaverine ($r=0.95$). Spermine and spermidine showed no correlation with histamine.

Shelf life of Indian mackerel stored at different atmospheres at chilled temperature

Shelf life of Indian mackerel stored in air, VP and MAP is shown in Table 4. For the fish kept in air, there was a good correlation among APC, TVBN, sensory analysis and histamine concentration. In air storage, Indian mackerel was acceptable for 4 to 5 days according to TVBN, microbiological, sensory evaluation and histamine limit whereas 6 days of shelf life was obtained based on cadaverine and cadaverine+putrescine.

However, in the fish stored under VP and MAP, the correlation among the different indices was lower as compared to air storage. Shelf life of Indian mackerel was 6 days under VP and MAP except for M100C according to the limit of APC. This result was similar to that obtained with histamine analysis except for M80C and M100C. Shelf life of fish was longer in terms of TVBN and sensory analysis. Shelf life was extended to 9 to 12 days based on TVBN limit and 7 to 11 days based on sensory analysis for the fish kept in VP and MAP over the storage period. Shelf life predicted by CAD and CAD+PUT was similar to APC and histamine limit. TVBN and histamine concentration in fish packaged in M100C never exceeded the upper limit throughout the storage.

Based on Pearson's correlation analysis, TVBN correlated well with histamine, APC was also a good marker for histamine accumulation in Indian mackerel. Based on the result in the present study, the upper limit of 35 mg/100g was considered high for storage under VP and MAP. The value of 30 mg/100g was suggested for the fish kept under VP and MAP conditions. It is interesting to notice that TVBN is parallel with APC and histamine level when the limit of 30 mg/100g is used (Table 4). The acceptable shelf life of 6 days was observed for VP, M30C and M60C, 7 to 8 days for M80C and 11 to 12 days for M100C.

Conclusion

The present results show that MAP reduces the formation of TVBN, total biogenic amines and improved the total acceptability of fish. The total biogenic amines responded in a dose dependent manner as a function of CO₂ level. The results of the present study demonstrate that MAP is a potential alternative in controlling total biogenic amines and preserving fishery products.

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