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Research Article

Changes of biogenic amines in silver carp (*Hypophthalmichthys molitrix*) fillet under different packaging conditions

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ABSTRACT

A comparative study of the effects of different packaging on the formation of biogenic amines during storage of silver carp (*Hypophthalmichthys molitrix*) at 4 ºC was carried out. Silver carp fillet was stored in air, modified atmosphere packaging (MAP) and vacuum packaging (VP) for 16 days. The biogenic amine content generally increased in all treatments with increasing storage time. The concentrations of putrescine and/cadaverine in fish stored in air reached maximum levels of 132.18 and 143.4 mg 100 g^{-1} after 16 days, respectively significant differences were found ($p<0.05$) in the levels of cadaverine and putrescine among the three treatments. Spermidine and spermine levels increased slightly and did not change much throughout the storage period for all experimental conditions. The amine contents of silver carp were highest in silver carp stored in air, followed by VP and MAP. The use of MAP showed a with carbon dioxide to extension the shelf-life (approximately four days) of silver carp by inhibiting microbial growth compared to air and VP.

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***Keywords*:** Biogenic amines; Fish quality; Food packaging; Storage.

1. Introduction

Biogenic amines (BA) have been studied extensively because of their role in foodborne disease. They are low molecular weight compounds, and the most important biogenic amines occurring in food are histamine (HIS), putrescine (PUT), cadaverine (CAD), spermidine (SPD), spermine (SPM), tyramine (TYR), tryptamine (TRY), 2-phenylethylamine (PHE), and agmatine (AGM), which are produced by decarboxylation of amino acids. Among the biogenic amines, histamine is potentially hazardous and the causative agent of histamine intoxication associated with the consumption of seafood (Morrow *et al.*, 1991). The other biogenic amines, such as putrescine and cadaverine, have been reported to enhance the toxicity of histamine (Stratton *et al.*, 1991).

Seafood, including fish, is a rich source of high-quality protein, essential vitamins and healthful unsaturated fatty acids. The high content of proteins, on the other hand, represents a risk in the decomposition processes. The disintegration of proteins yields peptides and amino acids, which are susceptible to further decay, resulting in BAs, which can be widely distributed in proteinaceous foods (Kří´žek *et al.*, 2004; Kenari *et al.*, 2009; Dehghani *et al.*, 2019).

Furthermore, fish is one of the most highly perishable food products and the shelf life of such products is limited in the presence of normal air by the chemical effects of atmospheric oxygen and the growth of aerobic spoilage microorganisms

(Özogul *et al.*, 2004; Alak *et al.*, 2011).

Modification of the atmosphere within the package by decreasing the oxygen concentration, while increasing the content of carbon dioxide and/or nitrogen, has been shown to significantly prolong the shelf life of perishable food products at chill temperatures (Parry, 2012). VP is also a type of MAP system because air is removed from the package. VP is normally placed in a pack of low oxygen permeability, air is evacuated and the package sealed (Church, 1998). MAP and VP, along with refrigeration, have become increasingly popular preservation techniques, which have brought major changes in storage, distribution, and marketing of raw and processed products to meet consumer demands (Özogul *et al.*, 2004).

BAs such as putrescine, cadaverine, spermidine, spermine, histamine and tyramine are non-volatile and low-molecular weight compounds and are generally produced by microbial decarboxylation of specific amino acids (Kim *et al.*, 2009; Hwang *et al.*, 2010).

The amount and type of formed BAs is related to initial quality of the raw material, the availability of free amino acids, the presence of bacterial biogenic amine decarboxylases and the temperature (Kří´žek *et al.*, 2004). Determination of BAs in proteinaceous foodstuff is important because they can effect on human health and can be used as quality marker (Hosseini *et al.*, 2013).

BAs play an important role in the degradation pathway of amino acids and since they are produced by spoilage bacteria toward the end of shelf life, their level can represent the quality of food (Rodríguez-Méndez *et al.*, 2009).

BA formation in fish and fish products depends on the amino acid content of fish, the presence of bacterial biogenic amine decarboxylases and favorable environmental conditions. Accumulation of BAs leads to toxicological hazards. Hence, this could be used as an indicator for assessment of the quality of fish and fish products (Sivertsvik *et al.*, 2002).

The effects of MAP and VP on seafood have been reviewed extensively but little information exists regarding biogenic amine production in silver carp, a species of freshwater cyprinid fish and one the most popular fish in the Asian countries, stored in modified atmospheres and vacuum packs. Therefore, in the present investigation, a comparative study of the effect of modified atmosphere and VP on biogenic amine formation in silver carp (*H. molitrix*), with an emphasis on a potential quality index, was attempted (Farshbaf Aghajani *et al.*, 2023).

2. Materials and Methods

2.1. Sample preparation

Forty-five individuals of silver carp (*H. molitrix*), representing the size range commercially

available to customers (mean weight: 950±30 g) were collected randomly from a local fish farming (Karaj, Iran) in March 2015. Selected samples were transported to the laboratory in ~1 h and during transportation, all the samples were covered by ice. The fish to ice ratio was approximately 1:3 and thickness of ice layer was ~10 cm. In the laboratory, fish were washed with distilled water and then filleted manually. Afterward, all fillet divided into three lots. One lot was packed in air, and the remaining two lots, were packed in MAP and VP by a vacuum packaging machines (Guater Control Co., Tehran, Iran). Then all the samples were kept at refrigerator (Azemayesh Co., Iran) for 16 days and at each sampling time (0, 4, 8, 12 and 16 days), three randomly chosen fillet were removed from refrigerator and their microbial and biogenic amine content were determined.

2.2. Total viable count (TVC)

At each storage interval, surface of each sample was first cleaned with alcohol and then 10 g of flesh with both white and dark muscle was taken and transferred into 90 ml 0.1% peptone water, homogenized using a Stomacher Lab-Blender (Seward type 400, London, UK) for 1 min. From this dilution, other serial decimal dilutions were prepared. In this study, TVC were determined using plate count agar (Oxide Inc. London, UK), according to the standard American public health association method (Downes & Ito, 2001) by counting the colony forming units (log 10 CFU g^{-1}) after incubating the plates at 30 °C for 48 h. Microbial analyses were performed in triplicate on three subsamples of each of the replicates.

2.3. Determination of biogenic amine (BA) content

All standard BAs and LC grade materials i.e., methanol, chloroform, butanole, diethyl ether, and n-heptanewere were obtained from Merck Co.; double distilled and deionized Millipure water (Millipore, Mississauga, Canada) was used for dilution and chromatographic separation. A Waters 1525 HPLC (Waters Co., Milford, USA) equipped with a Waters 2487 UV-detector set at 254 nm was use for the BA analyses. The column was a reversed phase C18 Waters Spherisorb ODS-2 (250 x 4.60 mm; particle diameter, 5 µm) with a Waters Spherisorb pre-column cartridge (10 mm length) packed with the same material. The mobile phase was an isocratic mixture of methanol:water (62:38 by volume) and the flow rate was 1.1 ml min^{-1} at room temperature.

From each sample, 5 g of flesh was homogenized in a Waring blender (Waring, New Hartford, CT) for 1 min. The procedure for extraction, separation, and quantification described by Paleologos *et al.* (2003) was used and explained by Moini *et al.* (2012). According to this procedure a solution of BA is obtained after sample treatment with trichloroacetic acid (6% w/v) and centrifugation (6×130 g, 20 min, 4 °C; 236 HK, Hermle, German). BA were isolated after derivatization with benzoyl chloride and surfactant mediated cloud point extraction before HPLC separation and quantification. Results are reported as μg^{-1} fish muscle.

2.4. Quality index (QI) and biogenic amines index (BAI)

Calculating of the quality index and biogenic amines index were done by the methods described by Veciana-Nogués *et al.* (1997), respectively, as follows:

Quality index= (HIS+PUT+CAD)/(1+SPM+SPD)

Biogenic amines index= (HIS + PUT + CAD + TYR)

3. Results & Discussion

3.1. Biogenic amines analysis

The Changes in biogenic amine contents in Silver carp stored in air, MAP, and VP for 16 days at 4 °C are shown in Table 1, respectively. The amount of cadaverine increased during the storage period and reached to 143.4±11.3 mg 100 g^{-1} for air storage, 122±15.1 mg 100 g^{-1} for VP and 102±17.5 mg 100 $g^{-1}$for MAP. The increase of cadaverine samples in air was higher than in samples stored in VP and MAP throughout the time of storage, putrescine was also a lot of changes, reaching the levels of 132.18±9.36 mg 100 $g^{-1}$for air storage, 82.7±5.89 mg 100 g^{-1} for VP and 76.3±2.94 mg 100 g^{-1} for MAP. Large changes in the contents of putrescine and cadaverine were observed

Table 1
Changes in biogenic amines contents (mg 100 g^{-1}) of Silver carp (*Hypophthalmichthys molitrix*) stored in vacuum, modified atmospher and air (control) condition

Day	PUT			CAD			SPM		
	MAP	VP	air	MAP	VP	air	MAP	VP	air
0	1.01±0.36	1.24±0.68	8.43±2.18	ND	4.76±0.95	13.2±4.69	9.69±1.67	8.52±0.63	9.08±1.85
4	3.14±0.89	7.39±1.41	13.6±2.36	5.96±0.97	8.35±1.76	36.5±11.41	7.68±1.79	8.79±1.86	10.4±2.36
8	11.4±2.45	18.8±3.35	46.7±4.28	16.5±4.02	41.3±5.38	62.4±9.30	8.41±1.52	8.68±1.58	12.5±1.79
12	43.9±1.36	35.2±3.61	96.6±11.01	42.7±6.25	63.4±8.68	116±14.58	12.5±2.41	13.2±2.79	16.1±1.58
16	76.3±2.94	82.7±5.89	132.2±9.36	102±17.47	122±15.10	143±11.31	13.0±1.53	12.1±2.69	15.0±1.31

Continue of table 1

Day	HIS			SPD			TYR		
	MAP	VP	air	MAP	VP	air	MAP	VP	air
0	ND	ND	ND	5.36±1.79	5.67±0.98	7.11±0.69	ND	ND	0.31±0.02
4	ND	ND	0.39±0.06	9.14±1.08	8.41±1.41	7.05±0.65	ND	ND	0.85±0.31
8	ND	ND	0.58±0.19	10.3±2.41	11.9±2.05	12.0±2.68	ND	ND	0.94±0.27
12	ND	0.41±0.095	0.54±0.08	9.08±2.53	11.5±203	12.6±2.23	0.63±0.09	1.05±0.63	1.62±0.79
16	0.37±0.06	0.49±0.074	0.71±0.15	11.3±1.07	12.1±1.38	12.8±2.50	1.05±0.41	1.94±0.41	2.01±0.31

throughout the storage period of silver carp in MAP, VP and in air storage.

The concentration of putresine increased during the storage period of Silver carp held in air, VP and MAP, reaching maximum levels of the days 16. Significant differences were found ($p<0.05$) in the levels of cadaverine and putresine among the three treatments. Ababouch *et al.* (1996) found that cadaverine and putresine accumulated rapidly, reaching levels of 235 mg 100 g^{-1} and 30 mg 100 g^{-1}, respectively, after 24 h of storage at ambient temperature. In this experiment, these amine contents of silver carp were highest in silver carp stored in air, followed by VP and MAP. These findings were similar to work on herring stored in air and MAP at 2±2 °C. It was found that the concentrations of these amines in herring held at 2±2 °C increased more rapidly than in herring stored in MAP (Özogul *et al.*, 2002; Özogul *et al.*, 2004). Spermidine and spermine levels increased slightly and did not change much throughout the storage period for the three different conditions. Significant differences were observed in spermine and spermidine contents among the treatments ($p<0.05$). However, lower values were obtained for silver carp stored in MAP (Table 1). This is in agreement with findings for herring stored in MAP (Özogul *et al.*, 2002). Ababouch *et al.* (1996) reported that ice storage inhibited the formation of these two amines in sardines. However, at ambient temperature, spermine and spermidine levels reached 5 and 6 mg 100 g^{-1} after 24 h, respectively. The amount of histamine increased during the storage period and reached 0.71 ± 0.15 mg 100 g^{-1} for air storage, 0.49 ±

Table 2
Quality and biogenic amine indices of Silver carp (*Hypophthalmichthys molitrix*) stored in vacuum, modified atmosphere and air (control)

Day	QI			BAI		
	MAP	VP	air	MAP	VP	air
0	0.06±0.01	0.40±0.01	1.26±0.11	1.01±0.47	6.01±1.03	22.0±2.61
4	0.51±0.04	0.87±0.47	2.74±0.43	9.10±1.04	15.8±3.11	51.4±6.42
8	1.41±0.31	2.79±0.65	4.30±0.86	27.9±2.21	60.1±8.19	111±11.81
12	3.83±0.17	3.16±0.49	7.17±1.03	87.2±6.41	99.8±13.4	215±19.51
16	7.07±1.04	8.16±1.05	9.60±1.21	180±8.68	208±9.79	278±16.05

0.074 mg 100 g^{-1} for VP increased during the storage period and reached 0.71 ± 0.15 mg 100 g^{-1} for air storage, 0.49 ± 0.074 mg 100 g^{-1} for VP and 0.37 ± 0.06 mg 100 g^{-1} for MAP. Lowest histamine was obtained from silver cap stored under MAP, indicating that the presence of the CO2 in the pack inhibited the growth of microorganisms, which resulted in preventing of spoilage and extending of shelf life of the fish. Sato *et al.* (1994) reported that the histamine content of mackerel, stored at 5 ˚C, increased rapidly when the number of bacteria reached above 106 cells g^{-1}. Histamine-forming bacterial species and strains differ considerably in amounts of histamine formation, and the type of spoilage bacteria present depends on an aquatic environment (López-Sabater *et al.*, 1994; Silva *et al.*, 1998; Alikhani Chamgordani *et al.*, 2024; Farshbaf Aghajani *et al.*, 2024).

The formation of high concentrations of histamine in fish products can be fairly rapid and depends on the number of microorganisms present (Ababouch *et al.*, 1991; Pacheco-Aguilar *et al.*, 1998). Tyramine concentration increased during the storage period, reaching the levels of 2.01 mg 100 g^{-1} for silver carp in air, 1.94 mg 100 g^{-1} for VP and 1.05 mg 100 g^{-1} for MAP at 16 days. Tyramine was not detected for silver carp in VP and MAP until eight days. There are no significant differences within the three storage conditions ($p>0.05$) (Dehghani *et al.*, 2018).

Table 2 shows biogenic amine quality and indices for silver carp stored in air, VP and MAP. There was an increase in the two indices with storag However, the biogenic amine index gave values twice as high as the quality index. Mietz and Karmas (1977) proposed the value of 10 as the limit of fish acceptability for the quality index (QI). This value was reached in samples after 16 days of storage for air, indicating that the quality index correlated well with the microbiological index of silver carp stored in air, MAP, and VP. The biogenic amine index (BAI) proposed by Veciana-Nogués *et al.*, (1997) also correlated withsensory acceptability of silver carp in air, MAP, and VP since it increased with storage time. However, more studies on BAI are needed to set a limit of fish acceptability in MAP and VP (Hosseini *et al.*, 2009; Moini *et al.*, 2009; Bahmani et al., 2011; Dehghani et al., 2019).

3.2. Total viable count (TVC)

Figure 1 shows total viable counts in silver carp stored in air, in VP and in MAP at 4 °C. Bacteria grew most quickly in silver carp stored in air, followed by those in VP and the lowest counts were with MAP where the log phase was apparently extended. One of the major mechanisms of MAP and VP techniques is to change the level of oxygen in a food environment so as to have an effect on the growth of different air exhibited higher biogenic amine values than

in VP and MAP storage. Groups of microorganisms. Consequently, fish in the use of MAP with carbon dioxide has been shown to extend the shelf-life (approximately four days) of silver carp by inhibiting microbial growth the levels of biogenic amines, also showed total count. It can also be concluded that the biogenic amine index gave values twice as high as the quality index.

Conclusion is that the lowest concentrations of biogenic amines were obtained from silver carp stored in MAP because CO2 inhibits the formation of biogenic amines. Also to sum up, the maximum levels of histamine and tyramine were less than the EU acceptable level and the recommendation, therefore all samples were considered safe.

The microbial quality of fish flesh depends both on aquaculture and on sanitary conditions in the slaughterhouse. When fish are gutted, bacteria from gills, and especially from the gut, can contaminate edible muscles. Study of bacterial contamination of the carp flesh was complementary to biogenic amine determination. The removal of O2 is more important than the inclusion of high CO2 content in the pack, due to oxidation of fat. Aerobic microorganisms are generally sensitive to CO2/N2; therefore, MAP delays the spoilage of fish. Huss (1972) indicated that CO2 decreases with an increase in temperature (Daniels *et al.*, 1985).

Pastoriza *et al.* (1996) reported that significant differences ($p<0.05$) were found between control (air) and MAP-stored samples in terms bacterial counts. In the present study, significant differences ($p<0.05$) were observed between samples kept in air and in MAP. When the aerobic plate count reaches 10^6 CFU g^{-1} (colony forming units) per gram or milliliter in a food product, it is assumed to be at, or near, spoilage level. El Marrakchi *et al.* (1990) reported that initial TVC of iced sardines was 3.16×102 CFU g^{-1}, reaching the limit counts of 106-107 CFU g^{-1} at day 9, while the counts exceeded these limits within 24 h at ambient temperature. In this study, the limit of acceptability (10^6 CFU g^{-1}) in terms of total viable count was 4 days for silver carp stored in air, 8 days for VP, and 12 days for MAP. The result obtained from evaluation, after VP and MAP treatment, showed a longer shelf life when compared with microbiological assessment.

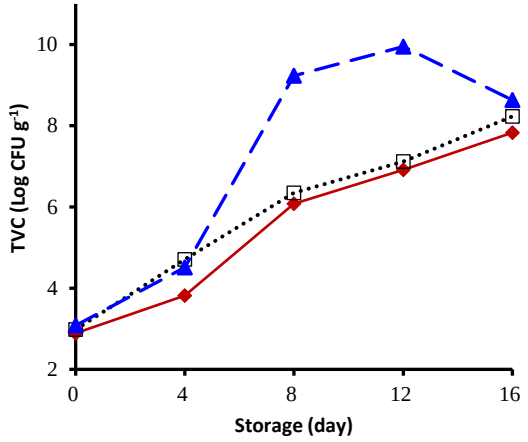

Figure 1
Total viable counts (CFU g^{-1}) in Silver carp (*Hypophthalmichthys molitrix*) stored in air (▲), in VP (□) and in MAP (♦) at 4 ˚C. Each point shown is the mean value of three determinations for each sampling. Standard deviation was not shown.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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