

Impact of Lactic Acid on Formation of Biogenic Amines in Common Carp

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ABSTRACT

Background and Objective: Raw fish is a highly perishable product that deteriorates due to chemical changes and microbial growth. Biogenic amines (BAs) are generated in course of bacterial spoilage in fish that may lead to BA toxicity, particularly in adverse storage conditions. The purpose of this study was to prevent the BAs toxicity caused by common carp (*Cyprinus carpio*).

Methods: Live Common carps were purchased from a local market. The samples were first divided into control and lactic acid treatment groups, and then stored at 4 °C. the concentrations of three BAs were determined (histamine, putrescine and cadaverine) with high performance liquid chromatography equipped with UV detector.

Results: Cadaverine and putrescine had the highest concentrations in control group (400.42 and 293.90 mg/kg, respectively). Histamine remained undetectable until the middle of storage period. Histamine and cadaverine concentrations in the lactic acid group were significantly lower compared to that of control group ($p < 0.05$).

Conclusion: The formation of histamine and cadaverine is effectively controlled by lactic acid treatment, although such effect was not observed on putrescine in the entire storage period.

Keywords: Biogenic Amines, Toxicity, Carps, Histamine.

INTRODUCTION

Common carp (*Cyprinus carpio*) is an economically important fish species that is mostly cultured in Asia, Europe, Australia and South America. It is favored for its fast growth rate, easy cultivation and high feed efficiency (1). Raw fish is a highly perishable product that deteriorates due to chemical changes and microbial growth. These changes affect fish proteins resulting in formation of peptides and amino acids, which can be further converted to biogenic amines (BAs) under the decarboxylase activity of naturally present microorganisms (2).

Generally, low concentrations of BAs in food and beverages (practically under 100 mg/kg) do not pose a significant risk for human health. Human intestinal tract has a detoxifying system consisting of monoaminooxidase, diaminooxidase, and histamine methyltransferase. However, higher amounts of BAs (generally above 100 mg/kg) may induce undesirable psychoactive and vasoactive effects including hypotension or hypertension, headache, nausea and breathing problems. Histamine (HIS) may cause the latter mentioned undesirable effects directly. Since putrescine (PUT) and cadaverine (CAD) can inhibit the detoxifying enzymatic system, they can act as potentiators of HIS's effects (2-5).

Fish and fish products are consumed insufficiently in Iran, which could lead to BA toxicity. Thus, it is essential to seek new ways to prevent the formation of BAs in fish. Organic acids are generally recognized as safe additives, but may produce adverse sensory changes. However, the dilute solutions of organic acids (1–3%) are generally without effect on the desirable sensory properties (6). The bactericidal effect of organic acids (e.g. lactic acid) is due to metabolic inhibition and reduction of pH below the optimal range for microbial growth. Therefore, it could be useful for prevention of BAs formation and inhibition of BAs toxicity.

The aim of this study was to evaluate the effects of lactic acid (as a food additive) treatment on the formation of three BAs (HIS, PUT and CAD).

MATERIAL AND METHODS

Live common carps with mean weight of 1 ± 0.1 kg were purchased from a local seafood market in the vicinity of Ahvaz, Khuzestan Province. The fishes were stunned,

decapitated, gutted and filleted (120 g average weight/slice) using market facilities. The fillets were then transported to laboratory in polystyrene boxes filled with ice. The fish fillets were washed carefully with cold water, and divided into two groups. The first group was left untreated (control group) while the second group was treated with 1.5% lactic acid solution (Merck, Germany) for 30 min. The samples were packed in polyethylene containers and stored in refrigerator at 4 °C for 18 days until analysis. The samples were analyzed after 0, 3, 6, 9, 12, 15, and 18 days of storage (3 samples per day).

Evaluation of BA formation was carried out according to the procedure previously described by Dawood et al. (7). Briefly, 10 g of each sample were homogenized with 75 ml of trichloroacetic acid (TCA, 5 g/100 ml) in a blender for 2 min. After centrifugation at 2000 g for 10 min, the supernatant was filtered through Whatman filter No. 1. The filtrate was made up to 100 ml with 5% TCA. Two ml of the solution were transferred to glass tubes (25 ml capacity); ; one ml of NaOH (2 N, Merck, Germany) and 10 µl benzoyl chloride (Merck, Germany) were added to the tubes and the tubes were immersed in 30 °C water. After adding 2 ml of saturated NaCl and 3 ml diethyl ether, the tubes were centrifuged at 3000 g for 10 min. Organic supernatant was transferred into another glass tube and placed in oven at 70 °C. The extracts were evaporated to dryness in a current of air. The residue was dissolved in 200 µl of methanol (HPLC grade) and filtered through millipore filter (0.45 µm pore size). Then, 20 µl aliquots were injected for HPLC analysis. Quantification of BAs was carried out using an HPLC system with UV-vis detection (Shimadzu, Japan). The mobile phase was an isocratic mixture of methanol: water (70: 30 by volume), with flow rate of 1 ml/min at room temperature. The peaks were detected at 254 nm.

The data was analyzed using SPSS 21 and repeated measure ANOVA. P-values < 0.05 were considered as statistically significant.

RESULTS

Table 1 show the formation of BAs in common carp in the two groups. The highest concentration of BAs was related to CAD in the control group (400.42 mg/kg).

Table 1- Changes in concentrations of BAs in common carp (mg/kg muscle)

BAs	Groups	Days						
		0	3	6	9	12	15	18
HIM (mg/kg)	Control ^a	ND	ND	0.49	0.14	ND	0.35	2.99
	Lactic acid ^b	ND	ND	ND	0.05	ND	0.040	0.82
CAD (mg/kg)	Control ^a	64.03	48.04	101.58	76.49	127.26	400.42	307.73
	Lactic acid ^b	ND	ND	ND	ND	ND	ND	ND
PUT (mg/kg)	Control ^a	21.93	ND	16.32	23.81	26.98	39.52	84.21
	Lactic acid ^b	13.65	ND	34.76	149.91	100.34	293.90	23.33

The control and lactic acid treatment groups were stored at 4 °C for 18 days.

^{ab} for each BA, different superscript letters show significant difference between groups during the study period ($P < 0.05$).

HIM was undetectable until day 9. Final concentration of HIM at the end of storage period was 2.99 and 0.82 mg/kg in the control and lactic acid groups, respectively.

CAD was not detected in the lactic acid group on the first day of storage, while concentration of 64.03 mg/kg was found in the control group. CAD concentration increased to 307.73 mg/kg at the end of storage period in the control group and remained undetectable in the lactic acid group. In this study, the amount of PUT in the control group was ranging between 21.93 and 84.21 mg/kg. The concentration of PUT in the treatment group was 13.65 mg/kg on the first day (day 0), but later increased to 293.90 mg/kg on day 15. However, the CAD concentration decreased by 23.33 mg/kg on the last day of storage.

DISCUSSION

PUT, CAD and HIS can act as indicators of spoilage in fish. HIS is the causative agent for fish poisoning, while PUT and CAD potentiate the toxicity of histamine (8). Results of this study showed low total BAs concentrations at day 0 of storage, which is similar to results obtained by Restuccia et al. (9), Hu et al. (10) and Wang et al. (11).

The highest concentration of HIM was observed in the control group. this concentration in the treatment group was significantly lower than that in the control group ($P < 0.05$). Lactic acid treatment inhibits the growth of bacteria with histidine

decarboxylase activity. HIS is the main BA involved in human food poisoning that can be a major threat to public health. According to the results of this study, application of lactic acid could be useful in prevention of histamine toxicity and food-borne poisoning.

The CAD content in the control group was significantly higher than that in the lactic acid treatment group ($p < 0.05$). Moreover, CAD concentration in the treatment group remained below the limit of detection. It was demonstrated that lactic acid can significantly reduce the formation of CAD in carp roe (12). Although CAD was not detected in the lactic acid group, PUT was found in all samples (except on day 3) because it is a substance present in all living cells. The level of PUT generally exceeds 15 mg/kg in low quality samples (12). In this study, the PUT level in the control group was higher than the mentioned limit in the entire study, while PUT concentration in the lactic acid group remained below 15 mg/kg for only three days. However, the PUT content was much higher in the lactic acid group on days 6, 9, 12 and 15. Min et al. reported that HIM, PUT and CAD concentrations reduce in beef inoculated with *Enterobacter cloacae* after one day of treatment with lactic acid (13). Sirocchi et al. (14) investigated the effect of essential oils on BAs formation in meat stored at 4 °C. They found that the essential oils have potential inhibitory effects on HIS, PUT and CAD accumulation, which increases the shelf life of fresh meat and preserves its important

nutrients. In the present study, PUT and CAD concentrations were higher than HIM concentrations in common carp muscle. Krizek et al. found that PUT and CAD are dominant BAs in carp flesh (15, 16).

BA formation is temperature-dependent (17), and declines at low temperatures (18) through inhibition of microbial growth and reduction of enzyme activity (19, 20). There is a higher chance of BA formation in South of Iran due to the hot climate and adverse storage conditions, which could pose a major threat to human health.

CONCLUSION

Lactic acid treatment of common carp at 4

°C prolongs its shelf life up to 3-5 days and reduces HIM and CAD formation. The results also showed that lactic acid only affects PUT content until day three. It is suggested to use lactic acid along with other preservatives such as essential oils to reduce BA concentrations more efficiently.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest regarding this manuscript.

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