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# Effect of Spices on the Biogenic Amine Formation and Other Quality Characteristics of Indian Mackerel During Refrigerated Storage

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## Abstract

The inhibitory effect of certain spices (viz., turmeric, pepper, cardamom, cinnamon and clove) on biogenic amine formation in Indian mackerel during refrigerated storage was studied along with sensory, microbiological and volatile amine changes. No significant difference in total plate count value was observed between control and treated samples except for turmeric ( $P < 0.05$ ). Spice treatments showed reduction in trimethylamine nitrogen, but not total volatile base nitrogen. The formation of histamine and other biogenic amines was greatly inhibited by all the spices, and the effect was greater ( $P < 0.001$ ) in the turmeric, pepper and cardamom treatments.

## Introduction

Biogenic amines are formed as a result of bacterial decarboxylation of amino acids in food (Rice et al. 1976). Some of the amines like cadaverine, putrescine and agmatine are identified as chemical quality indices of freshness (Yamanaka et al. 1986; Hollingworth et al. 1990). Cadaverine and putrescine are also known to potentiate histamine toxicity (Bjeldanes et al. 1978). Biogenic amines formed in food have good thermal stability and hence, cannot be destroyed by heat. Their inhibition would be the most practicable way to ensure food safety.

Spices and herbs possess antimicrobial activity (Shelef et al. 1980; Zaika 1988) and are safe to incorporate in foods. Recently, attention is focused on the beneficial effects of spices. In mackerel muscle extract, Wendakoon and Sakaguchi (1992) reported that clove and cinnamon effectively arrested histamine and cadaverine formation at 30°C. In India, various spices like turmeric, pepper and cardamom are generally used for processing the fish. In addition, clove and cinnamon are also included in some processed products. In the present study, the inhibitory effect of the above spices on biogenic amine formation and other quality characteristics during refrigerated storage of whole mackerel was studied.

## Materials and Methods

### ***Raw Material***

Six kilograms of fresh Indian mackerel (*Rastrelliger kanagurta*) of acceptable quality were obtained from the local fish market in Mysore, India, and divided into six 1-kg lots. The fish were beheaded, eviscerated and washed with potable water. Food grade powdered spices, viz., clove, cinnamon, cardamom, turmeric and pepper, were obtained from commercial sources.

### ***Treatment***

One lot of mackerel was stored without treatment at 5°C (control), and each of the remaining lots was treated separately with finely powdered spices (at 10% level by weight of the fish) by smearing directly on the wet surface of the eviscerated fish. The samples were packed in unsealed polythene bags and stored at 5°C for 8 d.

### ***Sampling***

Required quantities of samples were periodically drawn (after 4, 6 and 8 d) and analyzed in triplicate for sensory, total plate count, volatile amines and other non-volatile biogenic amines.

### ***Sensory Analysis***

Appearance, color, odor and texture of the samples were analyzed on a 5-point scale by a panel of six. Scores were given as follows: (excellent-5; good-4; acceptable-3; doubtful-2; unacceptable-1). Samples with a score of 3 and above were considered acceptable.

### ***Microbiological Analysis***

Ten grams of sample was homogenized with 90 ml sterile peptone water. Multiple decimal dilutions were made with the same diluent. The total plate count (TPC) was enumerated on plate count agar (Hi-Media Laboratories, Bombay, India) after 7 d incubation at 5°C following pour plate technique (Speck 1976).

### ***Volatile Amine Estimation***

All the reagents and chemicals used for the chemical analysis were of analar grade. Volatile amines, viz., trimethylamine nitrogen (TMA-N) and total volatile base-nitrogen (TVB-N), were estimated following the micro diffusion method (Conway 1962).

### ***Biogenic Amine Determination***

Extraction and derivatization were done by the method described by Rosier and Petegham (1988). A 5-g sample was homogenized and extracted with 15.0 ml hot (80-90°C) 5% trichloroacetic acid (TCA) solution. Putrescine (Put), cadaverine (Cad), histamine (His) and tyramine (Tyr) (Sigma Chemicals Co., USA) were prepared by dissolving 200-250 mg of each amine in 10 ml 5% TCA solution. A 10-fold dilution formed the working standard.

For derivatization, 1.0 ml of the sample TCA extract or working standard was mixed in a screw cap test tube with 1.0 ml phosphate buffer (pH 9.0), 2.0 ml dansyl chloride (50 mg in 10 ml acetone) and a drop of 4N sodium hydroxide solution. After thorough mixing for 10-15 seconds, the test tubes were covered with aluminium foil and incubated at 55°C for 1 h.

Precoated silica gel GF 254, TLC plate (20x20) obtained from E. Merck, India, was used for the fractionation following the procedure of Spinelli et al. (1974) with slight modification. Ten micro liters of the dansylated sample along with standard mixture was applied on the TLC-plate, and developed using the solvents, chloroform: triethylamine (100:25). Distinctly separated spots were sprayed with isopropanol:triethanolamine (80:20) to enhance fluorescence, and viewed under UV light at 365 nm. The different amines were quantified using the computerized UV-vis Spectrophotodensitometer (Shimadzu Corporation, Model CS-930, Japan) at the wavelength of 365 nm. From the fluorescent peak intensities, with the help of authentic standards, the quantities of the different amines present in the samples were calculated.

### ***Statistical Analysis***

All the results were average values of three determinations. Levels of significance between means were calculated using the students 't' test (Snedecor and Cochran 1980).

## **Results and Discussion**

### ***Initial Quality of the Raw Material***

Indian mackerel (*R. kanagurta*) was assessed for sensory, microbiological and chemical quality. Initially, the fresh mackerel sample had a high sensory score of 4.5 and was of good quality. The initial TPC appeared to be high ( $1.01 \times 10^6 \text{ g}^{-1}$ ), but such levels are commonly reported for tropical fishes (Shewan 1977). The volatile amines, TMA-N and TVB-N were found to be 12.73 and 120.14 mg kg<sup>-1</sup>, respectively, still within the limits of acceptability of 50 mg kg<sup>-1</sup> and 300 mg kg<sup>-1</sup>, respectively (Connell 1995). None of the biogenic amines were detected initially in the fresh mackerel.

### Sensory and Microbiological Quality Changes

Table 1 gives the data on sensory and microbiological quality changes during refrigerated storage of mackerel treated with different spices. Sensory analysis showed that the control sample remained in acceptable condition up to 4 d at 5°C, and thereafter began to develop an off smell. All the spice-treated samples had a higher sensory score compared to control even after 4 d storage. Treated samples exhibited the aroma of the respective spices, and no other deteriorative changes were noticed. Pepper and turmeric treatment had a high score of 4.25. After 6 d storage, all the samples started showing deteriorative changes and scored less than 2.5 except the turmeric- and pepper-treated samples, which remained in good condition. Storage was continued until all the samples reached unacceptable condition. These results indicated that turmeric and pepper treatments could delay spoilage of mackerel, and extend the shelf life of refrigerated mackerel by 2 d.

Microbiological analysis of the samples showed that, in control and treated samples, there was a gradual increase in TPC and  $10^6$ - $10^8$ g<sup>-1</sup> upon storage for 8 d. By the fourth day of storage, clove and turmeric treatments showed significantly lower TPC compared to control ( $P < 0.05$ ). The same trend continued in the case of turmeric treatment up to 8 d storage ( $P > 0.05$ ), whereas TPC in other treatments did not show any significant difference from that of control. Microbiological quality changes did not reflect the observed differences in sensory changes between treated and untreated samples. Except for turmeric, the other spices did not appear to be effective in inhibiting the total psychrotrophic microflora present in the samples.

### Volatile Amine Changes

Fig. 1 presents data on the changes in TMA-N and TVB-N during storage of mackerel treated with spices along with the control. TMA-N and TVB-N are considered as freshness indicators of fish (Connell 1995). In general, TMA-N and TVB-N values increased gradually upon storage in both control and treated

Table 1. Sensory and microbiological changes of refrigerated stored mackerel treated with spices.

Quality characteristics	Days of storage	Treatment with different spices					
		Control	Clove	Cinnamon	Cardamom	Pepper	Turmeric
Sensory score	0	4.50	-	-	-	-	-
	4	3.00 <sup>a</sup>	3.75 <sup>b</sup>	3.75 <sup>b</sup>	4.00 <sup>b</sup>	4.25 <sup>b</sup>	4.25 <sup>b</sup>
	6	1.25 <sup>a</sup>	1.50 <sup>a</sup>	2.25 <sup>b</sup>	2.50 <sup>b</sup>	3.75 <sup>b</sup>	3.25 <sup>b</sup>
	8	1.00 <sup>a</sup>	1.00 <sup>a</sup>	1.00 <sup>a</sup>	1.25 <sup>a</sup>	2.00 <sup>b</sup>	2.00 <sup>b</sup>
Microbiological quality TPC (count g <sup>-1</sup> )	0	1.01 x 10 <sup>6</sup>	-	-	-	-	-
	4	<sup>a</sup> 4.11 x 10 <sup>7</sup>	<sup>b</sup> 1.81 x 10 <sup>7</sup>	<sup>a</sup> 3.42 x 10 <sup>7</sup>	<sup>a</sup> 5.34 x 10 <sup>7</sup>	<sup>a</sup> 2.94 x 10 <sup>7</sup>	<sup>b</sup> 2.21 x 10 <sup>7</sup>
	6	<sup>a</sup> 1.53 x 10 <sup>8</sup>	<sup>a</sup> 7.84 x 10 <sup>7</sup>	<sup>a</sup> 8.04 x 10 <sup>7</sup>	<sup>a</sup> 9.42 x 10 <sup>7</sup>	<sup>a</sup> 8.41 x 10 <sup>7</sup>	<sup>b</sup> 8.17 x 10 <sup>7</sup>
	8	<sup>a</sup> 3.44 x 10 <sup>8</sup>	<sup>a</sup> 3.34 x 10 <sup>8</sup>	<sup>a</sup> 5.27 x 10 <sup>8</sup>	<sup>a</sup> 3.10 x 10 <sup>8</sup>	<sup>a</sup> 2.17 x 10 <sup>8</sup>	<sup>b</sup> 9.25 x 10 <sup>7</sup>

Figures with same superscripts are not significant at 5% level of significance.

samples. After 4 d storage, there was no significant difference in the TMA values of control and treated samples. By the sixth day, only clove, cinnamon and turmeric treatments showed significantly lower TMA-N values ( $P < 0.05$ ) than control. Upon further storage, all the treatments exhibited significantly lower TMA-N ( $P < 0.05$ ) values. The spices tested were partially effective in inhibiting bacteria associated with trimethylamine formation.

On the other hand, TVB-N values did not show any significant difference between treated and control samples throughout the storage period, except for clove treatment ( $P < 0.05$ ). The spices except clove did not seem to be effective against microorganisms associated with the total volatile base production in mackerel stored at 5°C.

### Separation of Biogenic Amines by TLC-Densitometry

The qualitative separation pattern of the biogenic amines as determined by TLC-densitometry is given in Fig. 2. By this method, different amines could be separated and quantified accurately. The coefficient of variation of this method was less than 3%, and the linear regression coefficient was 0.998. The minimum detectable level was 1 mg kg<sup>-1</sup> of the applied sample. Biogenic amines, viz., Put, Cad, His and Tyr, were found to be the important amines present in mackerel. Experiments indicated that some other basic nitrogenous constituents like ammonia, amino acids and aliphatic amines were also getting danylated and fractionated on TLC plates but none of these constituents interfered with amine resolution as they had much lower R<sub>f</sub> values. Only ammonia had a slightly higher R<sub>f</sub> value but still below that of Put spot. Though ammonia was detected in all the samples, because of its volatile nature, quantification was not carried out. The scanning pattern (Fig. 2) clearly shows the effect of

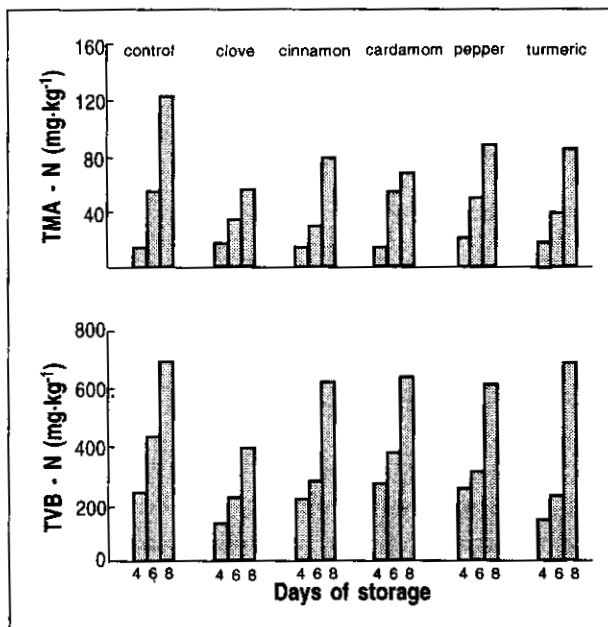


Fig. 1. Changes in TMA-N and TVB-N content during refrigerated storage of spice-treated and untreated mackerel.

various spices in arresting biogenic amine formation in mackerel, stored for 6 d at 5°C along with the fresh mackerel sample (initial). All the four amines were present in very high concentrations in the control sample though they were absent in fresh mackerel; whereas in the treated samples their concentration was much lower. In the pepper and turmeric treatments, amine concentration was found to be lowest, and the samples remained acceptable.

### Quantitative Changes in Biogenic Amines

Quantification of the biogenic amines was done by scanning densitometry, and depicted in Fig. 3. None of the amines were found in detectable amounts in fresh mackerel (0 d). Only after 4-d storage were biogenic amines found in measureable quantities. Put and Cad were noticed in all the samples after 4 d, whereas His and Tyr were not detected in some spice-treated samples. Among the four amines, Put concentration was lowest at all stages of spoilage. Upon storage, in both control and spice-treated samples, there was a gradual increase in Put concentration from day 4-8. A significant difference in Put values was observed between treated and control samples ( $P < 0.001$ ) from 6-d storage onwards.

In the control sample, on day 4 of storage, Cad concentration ( $53 \text{ mg kg}^{-1}$ ) was higher than the other amines. In treated samples, Cad concentration was much lower than that in control ( $P < 0.01$ ) except for the clove treatment. After 6-d storage, all the spice-treated samples showed a significant difference in Cad concentration ( $< 70 \text{ mg kg}^{-1}$ ,  $P < 0.001$ ) compared to control ( $210 \text{ mg kg}^{-1}$ ). Turmeric, pepper and cardamom treatments were found to be more effective than the other treatments. A similar trend was observed on further storage. When the samples attained the minimum acceptable score of 3.0, Cad concentration in both treated and untreated samples was about  $50 \text{ mg kg}^{-1}$  or less.

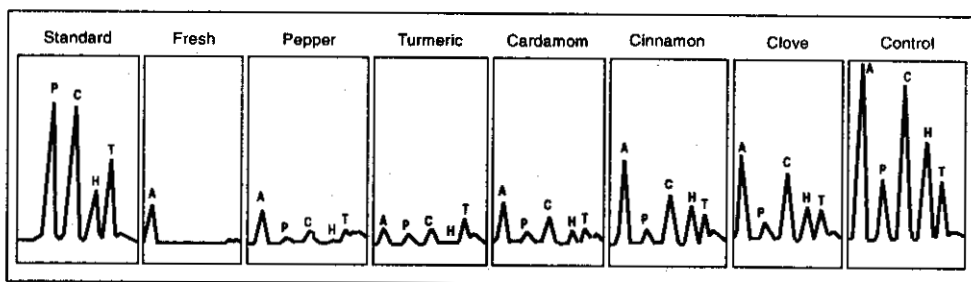


Fig. 2. TLC-densitometric scanning pattern of biogenic amines in fresh, spice-treated and untreated mackerel after 6-d storage at 5°C.

A - Ammonia; P - Putrescine; C - Cadaverine; H - Histamine; T - Tyramine

A different trend was observed with respect to histamine (His) formation in the samples. No His was detected in the pepper and turmeric treatments up to 6-d storage at 5°C, whereas in control and other spice treatments, His was detected after 4-d storage. During 4-6 d storage, the control had a sharp increase in His concentration exceeding the hazard action level of  $200 \text{ mg kg}^{-1}$ ,

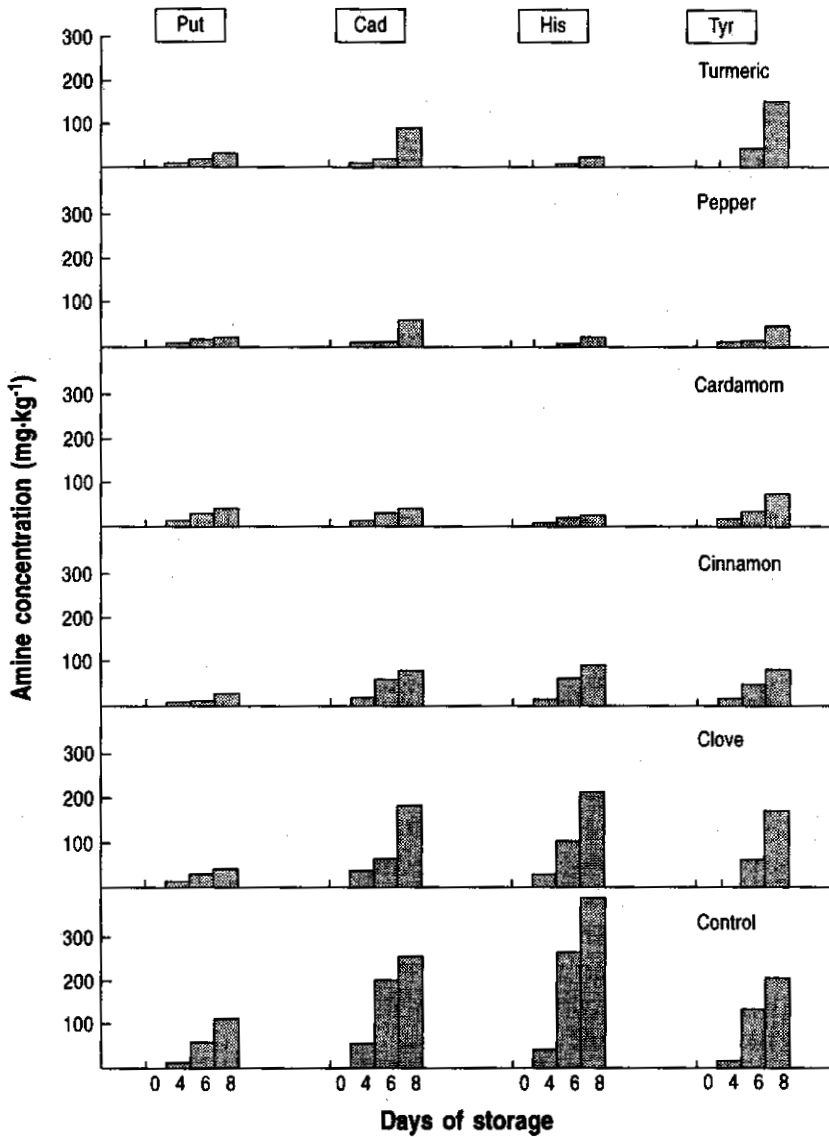


Fig. 3. Biogenic amine changes during refrigerated storage of spice-treated and untreated mackerel.

and in the clove treatment, the level exceeded the defect action level of 100 mg kg<sup>-1</sup> prescribed by European Economic Community (Ababouch 1991). The cinnamon treatment showed a slightly higher His concentration. In turmeric- and pepper-treated samples, only after 6-d storage, His formation was noted in smaller amounts (<13 mg kg<sup>-1</sup>). All spice treatments had significant inhibitory effect ( $P < 0.001$ ) on His formation in mackerel, but the significance level was lower ( $P < 0.05$ ) in the clove treatment. Pepper, turmeric and cardamom were found to be the most effective among the spices tested.



Tyramine (Tyr) concentration increased upon storage in control and reached 210 mg kg<sup>-1</sup> after 8 d. Tyr was not detected in turmeric- and clove-treated samples after 4-d storage. The spice treatment showed significantly lower Tyr values ( $P < 0.001$ ) compared to control after 6-d storage, but the clove and cinnamon treatments had slightly higher values ( $P > 0.01$ ). After 8 d, the pepper treatment recorded the lowest Tyr value (54 mg kg<sup>-1</sup>) compared to other treatments. These results indicate that the inhibitory effect of spices on biogenic amine formation was greater in the pepper, turmeric and cardamom treatments. Earlier, Wendakoon and Sakaguchi (1992) reported that clove and cinnamon were effective in inhibiting biogenic amine formation by *Enterobacter aerogenes* and *Morganella morganii* grown in mackerel muscle extract at 30°C. However, the inhibitory effect of the spices could be different against different microorganisms, and it may also be temperature-dependent. Our studies showed clove and cinnamon were not as effective as the other spices, like turmeric, pepper and cardamom, in arresting biogenic amine formation in whole mackerel stored at 5°C.

### Conclusion

Based on the study, it can be concluded that the treatment with spices could delay spoilage of mackerel for about 2 d under refrigerated condition. The total microflora did not alter much as a result of the spice treatments, except for turmeric. On the other hand, spices reduced TMA-N formation. Pepper, turmeric and cardamom selectively inhibited all the biogenic amines and histamine in particular. Spices are of great importance to the food industry. The inhibitory ability of certain spices to arrest toxic amine formation can be used beneficially and hence, their application on fish can be strongly recommended. Studies are being continued to examine their inhibitory effect on the enzyme associated with decarboxylation, and also the mechanism of inhibition of amine formation by turmeric and pepper.

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