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The Effect of Cooking Ingredients on Histamine in Fish

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ABSTRACT. *Histamine is produced in fish during microbial spoilage. Histamine is allergic to humans. The need, therefore, arises to find methods of destruction of histamine in fish during processing or cooking. Histamine in fish was estimated by extracting with methanol and quantification by TLC followed by confirmation by fluorometry. Effect of cooking ingredients on histamine content of fish was examined by soaking in or cooking with extracts or pieces of the acidic fruits 'goraka' (Garcinia cambogia), 'siyambala' (Tamarindus indica) and 'biling' (Averrhoa bilimbi), coconut milk and in pure tartaric, acetic and lactic acids which are the main acidic ingredients in plant extracts. The effect of pH on growth and histamine production by the bacteria in synthetic media was tested at 37°C.*

Cooking of fish in 'goraka', 'siyambala' and 'biling' fruit extracts destroyed histamine by 92, 78 and 68%, whereas soaking destroyed histamine by 77, 50 and 43% in the same extracts, respectively. Cooking with the fruit pieces destroyed histamine by 80, 71 and 53% while soaking with the fruit pieces destroyed histamine by 63, 41 and 38%, respectively. Pure tartaric, acetic and lactic acids with a final pH of 4.2, 4.1 and 5.0 and final concentration of 50%, destroyed histamine by 90, 90 and 80%, respectively, during cooking.

Soaking or cooking in coconut milk did not destroy the histamine in fish. Although the histamine producing bacteria were able to grow at a pH range 5 to 8, the histamine production was highest at pH 7. At pH 7, 318±21 µg/ml of histamine was produced in Niven's medium whereas no histamine was

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observed at pH 4 and pH 9. Coconut milk having a pH of 7.1 did not destroy histamine. Neutral pH of 7 appears to allow the highest histamine production by bacteria in fish. This suggests an inhibitory effect by the acids on the histamine producing bacteria in addition to destruction of histamine during cooking.

INTRODUCTION

Histamine is produced in fish due to microbial activity. Consumption of fish contaminated with histamine is reported to cause allergic reactions such as redness in eyes, burning and tingling sensations around mouth, oedema, itching, vomiting and diarrhoea (Uragoda and Kottegoda, 1977; Kottegoda., 1984). Among different culinary preparations of fish, addition of plant materials of acidic nature is common, specially with fish of tuna family which is susceptible to high histamine contamination. Commonly added plant materials include 'goraka' (*Garcinia cambogia*), 'siyambala' (*Tamarindus indica*) 'biling' (*Averrhoa bilimbi*), chilies (*Capsicum* sp), garlic (*Alium sativum*), pepper (*Piper nigrum*) and coconut milk, an aqueous extract of coconut kernel (*Cocos nucifera*). The aim of this study was to examine the effect of 'goraka', 'siyambala', 'biling' and coconut milk on histamine in fish, as these additives are believed to reduce the heaty effects of fish. Presence of histamine is believed to show a strong relationship with the heaty effects of red-blooded fish (Uragoda and Kottegoda, 1977).

MATERIALS AND METHODS

Fresh skipjack (*Katsuwonus pelamis*) and tuna (*Thunnus albacares*) samples previously analyzed and found to contain above 100 ppm of histamine were used. The fish was cut into approximately 3 cm cubes for the experiments.

Preparation of extracts

Dried 'goraka', fresh 'biling' and 'siyambala' pods without shells were purchased from market. 'Goraka' and deseeded 'siyambala' were soaked in water for 5 h. 'Goraka', 'siyambala' and 'biling' were separately blended with

water at the ratio of 1 : 10 of fruit : water in a Waring blender for 5 min at low speed. The extracts were filtered using a Buchner funnel and the filtrate was used to cook fish.

Fruit pieces

'Goraka', 'biling' and deseeded 'siyambala' were washed, drained and cut approximately to 1 cm cubes.

Food acids

Pure tartaric, acetic and lactic acid were added as cooking ingredients to give a final concentration of 50% of pure acid in the final solution which gave final pH values of 4.1, 4.2 and 5.0, respectively, in the fish preparation. The acid concentrations used were comparable with the acids available in the fruits extracted or added.

Preparation of coconut milk

Scraped coconut was blended in a Waring blender for 5 min at low speed at a ratio of 1 : 2 of coconut : water (w/w).

Cooking of fish

In all experiments cooking of fish (100 g) in a liquid phase of 100 ml was carried out for 10 min at 100°C. The fish was stirred to prevent scaling and to allow uniform cooking.

Detection of histamine

Histamine in fish (5 g) was detected by blending with methanol (35 ml) in a Waring mini-blender for 2 min at low speed and maintaining the extract at 60°C for 15 min to enhance extraction. The extract was made up to 50 ml with methanol and filtered. The filtrate was used to spot (20 μl) on TLC plates pre-coated with silica gel G 60 and activated at 105°C for 30 min. Histamine standards of 50, 100, 200, 300, 400, and 500 μg/g (ppm) were

spotted (20 μl) on the same plate and the plates were allowed to develop in an equilibrated tank with chloroform : methanol : conc. ammonia (2 : 2 : 1). The developed plate was visualized using ninhydrin spray and the colour intensities of the unknown extracts and the standards were compared for semi-quantitative determination of histamine (Lieber and Taylor, 1978; Baranowski, 1985; Gunaratne and Samarajeewa, 1994). The semi-quantitative results obtained from the TLC method were confirmed by the following fluorometric method.

The methanol extract (1 ml) was purified in a Dowex ion exchange resin (50-100 mesh) column of 1 cm diameter and 8 cm height using 30 ml distilled water. The purified extract was made up to a final volume of 50 ml. The resulting extract (5 ml) was mixed with 10 ml of 0.1N hydrochloric acid, 3 ml of 1N sodium hydroxide, 1 ml of 0.1% o-phthalic dicarboxaldehyde and 3 ml of 3.57N phosphoric acid and fluorescence measured using the Perkin-Elmer model No. 203 fluorimeter with excitation at 350 nm and emission at 444 nm (AOAC, 1990).

Effect of pH on histamine producing bacteria

Histamine producing bacteria maintained on Nutrient agar slants were activated in trypticase soy broth supplemented with histidine (TSBH) for 24 h at (25±2)°C. Activated bacteria were inoculated on to Niven's agar plates and broths at pH 5.3 (Niver *et al.*, 1981) and incubated at 37°C for 7 days. Growth of colonies was observed on agar plates and the histamine concentrations were estimated in the Niven s broth.

RESULTS AND DISCUSSION

Effect of fruit extracts and pieces

The 10% aqueous extracts of 'goraka', 'siyambala' and 'biling' destroyed histamine by 92, 78 and 68% after cooking at 100°C for 10 min. The percentage destructions on soaking for 30 min in the same solutions were only 77 and 50 and 43%, respectively. Cooking with pieces of 'goraka', 'siyambala' and 'biling' destroyed histamine by 80, 71 and 53% and soaking in water with same fruit pieces destroyed histamine by only 63, 41 and 38%, respectively (Table 1).

Table 1. Effect of extracts or pieces of acidic fruits, pure food acids, coconut milk and water on histamine destruction in fish.

Ingredients	No. of Samples	Initial histamine (ppm)	After soaking		After cooking	
			Final histamine (ppm)	% destruction	Final histamine (ppm)	% destruction
Extracts						
'goraka'	15	147±39	34±7	77±3	12±3	92±1
'siyambala'	11	151±31	75±15	50±1	33±7	78±1
'biling'	11	172±37	98±20	43±2	56±12	68±1
Fruits						
'goraka'	11	167±34	62±12	63±2	34±8	80±2
'siyambala'	11	163±36	96±22	41±2	47±11	71±1
'biling'	11	175±28	108±17	38±1	82±15	53±2
Pure acids						
tartaric	11	156±26	50±9	68±1	16±3	90±1
acetic	11	167±36	67±14	60±1	17±3	90±1
lactic	11	178±37	85±17	52±1	36±8	80±1
Coconut milk	11	173±37	173±36	0	171±36	1±1
Water	14	179±33	173±33	1±1	177±32	1±1

Above results indicate the probable presence of a component in the fruits added, capable of destroying histamine in fish during cooking. Boiling or cooking in water at 100°C alone did not destroy histamine (Lancet, 1979). The destruction of histamine by fruit extracts or fruit pieces in water is probably associated with the acidic nature of the fruits or specific anions of the acids in fruits which may interact chemically with the histamine. The increased destruction by extracts than by the pieces of fruits indicate the active ingredient to be a water soluble compound.

Effect of food acids

Pure tartaric, acetic and lactic acid at a final concentration of 50% and pH of 4.1, 4.2 and 5.0 destroyed 90, 90 and 80% histamine, respectively, when fish pieces were cooked at 100°C for 10 min, whereas, only 68, 60 and 52% histamine was destroyed when soaked for 30 min in the same acids. The destruction of histamine by the acids provide further evidence on the role of an acidic component present in the fruits used on destruction of histamine during cooking. The percentage destruction of histamine decreased with the decrease in acidity as indicated by increased pH (Figure 1).

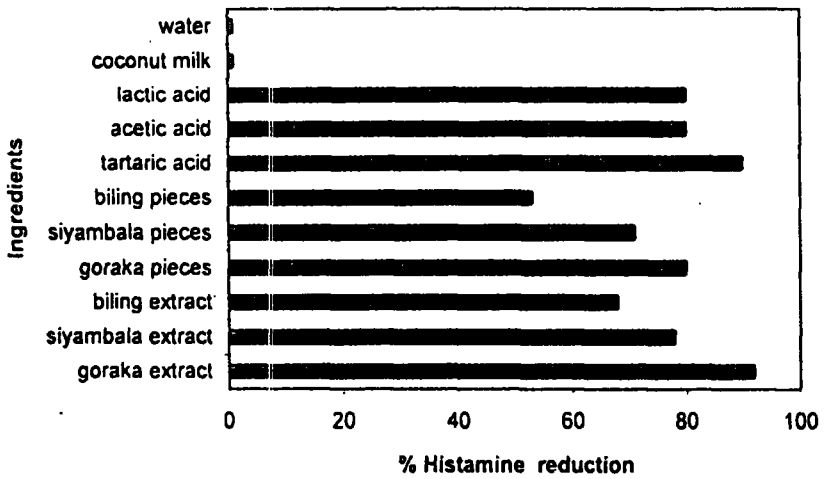

Figure 1. Effect of cooking with acidic fruits, pure food acids, coconut milk and water on histamine in fish.

Effect of coconut milk

Coconut milk at pH of 7.1 was ineffective in destruction of histamine (Table 1). Boiling in water at 100°C at neutral pH did not destroy histamine, either.

Effect of pH on histamine producing bacteria

Histamine producing bacteria were able to grow and produce histamine at a pH range of 5 to 8, in Niven's broth. The maximum histamine production occurred at pH 7 (Figure 2). The low histamine observed in Niven's medium at low pH may either be due to inhibitory effects of the added acids on bacteria or destruction of histamine by the acids as soon as histamine is produced.

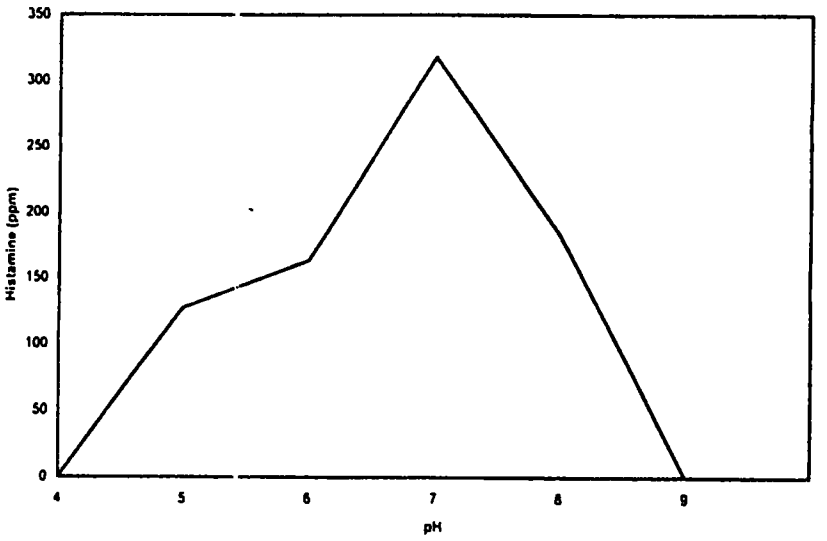

Figure 2. Effects of pH on histamine production by bacteria on Niven's medium.

CONCLUSIONS

'Goraka' was capable of destroying histamine in fish when used as an extract or pieces during cooking, while 'siyambala' and 'biling' extracts or pieces were less effective. Coconut milk was ineffective in destroying histamine in fish. Histamine producing activity of the bacteria was maximum at pH 7 and was low at low pH. No histamine was produced at pH 4.

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