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DESTRUCTION OF HISTAMINE BY COOKING INGREDIENTS- AN ARTIFACT OF ANALYSIS

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Abstract: A recent article reported that histamine can be destroyed by cooking ingredients such as *Garcinia cambogia* (goraka), *Tamarindus indica* (siyambala) and *Averrhoa bilimbi* (bilin). It was further reported that the same effects are observed with some pure common organic acids normally present in food. In this paper we provide evidence that the results previously obtained was an artifact of analysis, specifically caused by the use of methanol prior to extraction of histamine for assay. Quantification of histamine was done using TLC- densitometry. Silica gel G_{60} plates (100 µg) were used as adsorbent and chromatographed using acetone : NH_4OH (sp.gr. 0.88) 80:22.5 solvent system developed in this study. The spots were visualized using ninhydrin and quantified at 500 nm using an Advantec 300 densitometer.

Key words : *Averrhoa bilimbi*, *Garcinia cambogia*, Histamine, *Tamarindus indica*.

INTRODUCTION

Histamine is an allergenic amine formed by the decarboxylation of the amino acid histidine, which is found naturally in foods, most commonly the so-called heaty blood fish[1] and cheese.[2] It is most commonly formed by the action of microorganisms.[3] The allergic reaction is manifested by a number of symptoms ranging from redness of eyes to vomiting and diarrhoea.[4,5] Histamine is stabilized by the inhibition of diamine oxidase by the anti-tuberculosis isoniazid drugs.[5,6]

A recent report[1] that histamine could be destroyed by certain cooking ingredients and common organic acids aroused interest.

Our preliminary objective was to try to elucidate the mechanism by which tartaric, lactic and acetic acids and the extracts of *Garcinia cambogia* (goraka), *Tamarindus indica* (siyambala) and *Averrhoa bilimbi* (bilin) destroy histamine.

METHODS AND MATERIALS

Plant Materials: Dried goraka and siyambala pods were purchased from the Homagama market. The siyambala was deshelled and deseeded. Ripe bilin was collected from a home garden. Extracts were prepared as described previously.[1]

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Food acids: Tartaric, acetic and lactic acid were obtained as analytical grade chemicals and added as cooking ingredients in the ratio of 1:1 with authentic histamine solution (400μg. ml $^{-1}$) to give a concentration of 50% of pure acid in the final solutions. Total volume was 2 ml.

Fish: Fresh balaya (Skipjack, *Katsuwonus pelamis*) was cut into 1-cm^3 pieces and blended. The blended fish (1g) was used in the reaction mixture.

Histamine: Analytical grade histamine was purchased in vials from Sigma and diluted to the stock solution (0.5 mg 50 ml^{-1}) from which aliquots were taken so that the reaction mixture usually contained 200 μg ml^{-1}.

Reaction Mixtures: Dried goraka and siyambala were soaked in water for a few hours before blending with water at the ratio of 1:5 of fruit : water in a blender until a fine paste was obtained. In the case of fresh bilin the ratio of fruit: water was 1 :1. These extracts were centrifuged and supernatants used as the fruit extract.

Histamine 400 μg ml^{-1} and fruit extracts were mixed so that the ratio of extract: histamine volume was (1:1).

To determine the effect of fruit extracts and food acids on level of histamine, the reaction mixture containing histamine : extract in 1:1 (Table 1) were mixed and incubated for 1 hour at ambient temperature (30-32 ^{0}C).

To blended *Katsuwonus pelamis* (1g) histamine (3 mg) was added and incubated at 37^{0}C for 24 h. with and without goraka in a total reaction volume of 3 ml. After 1-hour's incubation, 5 μl of reaction mixture was spotted on TLC silica gel G_{60} plates. A water histamine mixture of the same concentration was used as an additional control.

Quantification of histamine: This was carried out by withdrawing 10 μl aliquots, unless otherwise specified from reaction mixtures and spotting on silica gel G_{60} plates (100 μg). This was chromatographed (45 min.) using acetone : NH_4OH (sp.gr. 0.88) 80:22.5, and held for 1 hour in an air oven at 80^{0}C to expel NH_3. The spots were visualized using ninhydrin spray (1% in acetone) after heating the plates at 100^{0}C for 5 minutes.

The histamine spots were quantified at 500 nm using an Advantec 300 densitometer from Kaishna, Japan, using a peak area vs μg histamine standard curve (r^2= 9489).

The effect of methanol was determined by using the above procedure. However, in this case histamine 400 μg ml^{-1} was prepared in methanol.

RESULTS

Reduction of histamine in the presence of food ingredients and food acids

Histamine levels in fish (200 ppm) were reported to be destroyed to the extent of 90% by certain ingredients.[1] Experiments were carried out to check the veracity of these results, but in this case using authentic histamine.

It was observed that there was virtually no reduction in the levels of histamine in the presence of the supernatants of the food ingredients or acids (Table 1). Incubating the above mixtures for 24 h. at room temperature (30-32°C) or boiling (just above 100°C) the above solution (10 min.) or using whole blended food ingredients (instead of using only the centrifuged supernatant) also did not bring about any change in the result.

Table 1 : Histamine content in the presence of certain cooking ingredients and food acids.

Reaction Mixture	Initial Histamine ($\mu g\ ml^{-1}$)	Final Histamine ($\mu g\ ml^{-1}$)	Percentage Reduction
Goraka + H	200	190	5
Siyambala + H	200	194	3
Bilin + H	200	196	2
Water + H	200	199	<1
Acid water + H (pH=3)	200	199	<1
Acetic acid (1M) + H	200	190	5
Lactic acid (1M) + H	200	192	4
Tartaric (1M) + H	200	192	4

H= histamine (400 $\mu g\ ml^{-1}$)
Histamine: additive = 1:1
Total volume= 2ml in each case

Percentage reduction in histamine by food ingredients and food acids in the presence of methanol.

This set of experiments were carried out to check whether methanol used by the previous workers[1] for extraction procedures has any effect on the loss of histamine. The reaction was carried out in the presence of methanol (50%).

A precipitate formed immediately in the presence of methanol in the ratios used. When the resulting supernatant (after centrifugation at 2000 rpm) was spotted there was a major reduction of histamine content by the cooking ingredients and acids (Table 2). No precipitate was formed and no reduction in the level of histamine was observed in both controls [(1) absence of methanol and (2) absence of methanol and acids]. To obtain a pH of 3, HCl (1M) and water was added. (pH meter)

Table 2 : Percentage reduction in histamine by cooking ingredients and food acids in the presence of methanol.

Reaction Mixture	Initial Histamine ($\mu g\ ml^{-1}$)	Final Histamine ($\mu g\ ml^{-1}$)	Percentage Reduction
Goraka + H	200	34	83
Siyambala + H	200	50	75
Bilin + H	200	66	67
Water + H	200	199	<1
HCl + H (pH=3)	200	198	1
Acetic acid (1M) + H	200	30	85
Lactic acid (1M) + H	200	36	82
Tartaric (1M) + H	200	28	84

H= histamine (400 $\mu g\ ml^{-1}$) dissolved in methanol
Histamine: additive = 1:1
Final histamine was determined in supernatant after centrifugation
Water (1ml) was added as control
Total volume was 2ml in each case
HCl was added as an acid control, to show that pH alone was not responsible

Release of histamine formed from precipitate by the addition of HCl (pH 1.5)

There appeared to be an insoluble precipitate formed between histamine and components of the acidic cooking ingredients and acids in the presence of methanol. It was important to determine whether this precipitate would release histamine at gastric pH.

HCl (2 ml, pH 1.5) was added to the centrifuged pellet obtained from the methanolic solution and pH readjusted to 1.5. The precipitate was almost completely and immediately dissolved and gave a slightly turbid solution. Histamine was determined in the colloidal suspension without centrifugation. This gave back the histamine at pH 1.5. The recovery was high. Column 2 of table 3 gives the histamine

content calculated from the previous experiment (table 2) based on the assumption that any histamine that was not present in the methanol extract would reside in the centrifuged pellet. That is, initial histamine is equated by calculation (not determined) to be 200 μg minus histamine in the supernatant as given in Table 2.

Table 3 : Recovery of histamine from centrifugate (Table 2) at pH 1.5

Reaction Mixture	Initial Histamine* ($\mu g\ ml^{-1}$)	Recovered Histamine ($\mu g\ ml^{-1}$)	Percentage Recovery
From Goraka	166	144	89
From Siyambala	150	134	92
From Bilin	133	122	94
From Acetic acid	170	148	89
From Lactic acid	164	142	89
From Tartaric	172	148	88

HCl was added to the precipitate formed as shown in table 2
* calculated from table 2
Total volume = 2ml

Effect of fish on the histamine - goraka (*Garcinia cambogia*) reaction

Gunarathne *et al.*[1] used a more complex system (containing fish) in their study. The next set of experiments was carried out to determine if fish (skipjack) contributed a yet unknown factor which influences a reaction between histamine and the cooking ingredients.

Table 4 : The concentration of histamine in the presence and in the absence of goraka in a complex of skipjack containing medium.

Reaction Mixture	Histamine ($\mu g\ 5\mu l^{-1}$)	Percentage Recovery
Skipjack + water (1:2)	Not detectable	Not applicable
Skipjack + goraka + histamine (1:1:1)	4.52±0.229	90.4
Skipjack + water + histamine (1:1:1)	4.49±0.100	90.0
Water + histamine (2:1)	5.00	100

Blended skipjack (1g) was added to the ingredients in column 1 so that the total volume was 3 ml.
Histamine added= 3mg
n=4

Results indicated there was no effect, even by the most potent cooking ingredient (goraka), on the reduction of histamine in the presence of the blended fish (Table 4).

The controls using goraka or water showed no significant difference in free histamine, indicating that the small decline is not significant.

DISCUSSION

Histamine is reported to be stable to irradiation, O_2 and N_2O [6] and also to plant and certain animal deaminases.[7] It is however susceptible to human histaminases and diamine oxidases.[4] Boiling in water at 100 ^{0} C at neutral pH does not destroy histamine and coconut milk at pH 7.1 was ineffective in destruction of histamine.[1] Therefore its destruction by the acidic cooking ingredients[1] was interesting.

Results clearly show that histamine is not destroyed but is converted into an insoluble complex when methanol is present. This methanol had been added as an analytical reagent for spectrofluorometry used in the earlier study.[1]

A plausible reason for what we have observed is that at pH= 3, NH_2 groups of histamine exist as NH_3^+. On the other hand, the organic acids will have some COO^- (from pKa values)

Therefore it is not surprising that the two components could complex. The complex is soluble in aqueous medium (Table1), but clearly a precipitate forms in the presence of methanol. Therefore it is postulated that histamine forms a complex with an acid constituent that is insoluble in methanol. This accounts for the sediment formed in methanol and absence of histamine in supernatant which would have misled the previous workers to interpret it as the destruction of histamine.[1] Another disadvantage of using methanol for extraction of histamine is that other amines which are known to be formed in fish[8] would also be extracted and interfere with quantification of histamine. The quantitative technique used in this study is simple and did not suffer from the above defect.

In this study, synthetic (exogenous) histamine was used instead of endogenous (fish). Histamine has no chiral center so it cannot pose a problem, as there are no optical steriomers. Further, endogenous histamine would be accompanied by other amines that could interfere with the result.

The only argument against is that there is a chance that a fish constituent plays a role in the destruction of histamine by the cooking ingredients and acids. The results in (table 4) using *Katsuwonus pelamis* rules out this possibility, the reduction (10%) in histamine can be accounted for by (perhaps) adsorption.

The fact that coconut milk does not destroy histamine[1] is in line with our conclusions that no destruction occurs. Coconut milk (pH 7) did not contribute to the formation of a methanol insoluble complex with histamine and this is not surprising since it was neutral in reactivity.

Studies have shown that the effect of goraka extracts is the inhibition of histamine formation and not its destruction.[8] .

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