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Effect of some organic, inorganic and natural compounds on removal of biogenic amine using spectrophotometric method

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Abstract

A number of common foods contain appreciable levels of amines, such as serotonin, epinephrine, tyramine, tryptamine, histamine and dopamine. These compounds are referred to collectively as pressor amines because they act as potent vasoconstrictors and thereby elevate the blood pressure. So, this work has been carried out to investigate the conditions which lead to removal of the biogenic amines through the model system. Biogenic amines; histamine and tyramine, from some foods such as tomato, strawberry, banana and mango are removed in order to prevent their allergy effect. Histamine and tyramine have been affected by ,pyrogallol, catechol, starch, ascorbic and chlorogenic acids at different levels with different conditions. Some natural additives showed an effective effect on disappearance of histamine and tyramine.

Key word: biogenic amines, histamine, tyramine, TLC, spectrophotometric method, HPLC

Introduction

Bioactive amines (BAs) are organic bases of low molecular weight which participate in normal metabolic processes in living tissues. The factors that affecting on the presence of these biogenic amines included pH, free amino acids, water activity and temperature. Polyamines, such as spermine and cadaverine, can potentiate the toxic effect of tyramine and histamine but act as precursors of carcinogenic nitrosamine. The production of amines required the availability of free amino acid and appropriate status of environmental factors such as pH and temperature. Especially, high levels of BAs were found after microbiological spoilage of foods or in fermented foods (Smith 1980, Ascar et al. 1986, Stratton et al. 1991, Cinquina et al. 2004). Some BAs such as putrescine, cadaverine, spermidine and histamine have been found to be useful as quality indices for the decomposition of fish. The histaminic poisoning, known since 1910, still raises relevant questions. The intake of histamine may cause an allergic toxicity known as "scombroid poisoning".

BAs such as histamine, tyramine, 2-phenylethylamine and tryptamine are related precursor to amino acids in food such as cheese, red wine, beer, tofu and soy sauce. It is found that the total

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amines in cheese were up to 1284.8 mg/L, wine and beers gave values from 0.5 to 27.2 mg/L[1]. Ming et al. (1994) stated that increases in BAs content of pork stored at different temperatures is temperature dependent. Szerdahely et al. (1993) found that significant increases in BAs concentrations occurred only after several weeks of cold storage at 4°C to pork, and were associated with spoilage. Secondary amines are known to form carcinogenic N-nitrosamine by reaction with nitrosating compound, storage of salted and smoked fish more than 15 days led to an increase in the histamine content than normal (Çakli and Taşkaya 1995). There are many factors that showed clear effects on histamine such as pH, free amino acids, water activity and except histamine compound temperature (Krizek et al. 2004).

Tyramine has been proposed as major mutagen precursors in soy sauce and become mutagenic after treatment with nitrite under acidic conditions (Ochiai et al. 1984). It is noticed that level of tyramine content in sardine decrease in the 1-2 months storage when marinated with 2 % acetic acid then began to increase as reported (Casal et al. 2004). Sellers et al. added sodium sulphite to meat products as sausages and noticed an increase in tyramine level accumulation (Sellers et al. 2006).

In continuation to our work previously published (Mohamed et al., 2010), the main target of this work aimed to study the effect of adding different organic, inorganic or natural additives on the removal of these biogenic amines such as (histamine and tyramine) and hence their allergic effects are studied. The different conditions affecting the inhibition of amine effects (hazard effects) are optimized. Obeyence to Beer's law is followed and the proposed methods, TLC and spectrometry are used for the determination of tyramine and histamine in pure form, and after treatments with different additives.

Materials and Methods

Solutions

Stock solutions of histamine and tyramine were prepared by dissolving 0.05 and 0.2 g, respectively, in 100 mL deionized water. Another stock solution 0.5 mg/mL of histamine and tyramine were prepared by dissolving 41.40 and 31.39 mg, respectively, in 50 mL deionized water. 0.05 g of each one of ascorbic acid, sodium sulphite, sodium metabisulphite and sodium chloride was dissolved in 10 mL deionized water. 1% solutions of glucose, gallic acid, pyrogallol, catechol, tannic acid, chlorogenic acid, starch, caffeine, curcuma, ginger, milk, rosemary, cinnamon and thyme were prepared by dissolving 0.1 g of each one in 10 mL deionized water. Solutions of citric and caffic acids were prepared by accurate weighing of 0.2 g for citric acid and 0.04-0.4 g for caffic acid and each one of them was dissolved in 10 mL deionized water.

0.2N hydrochloric acid solution was prepared by accurate dilution from the concentrated hydrochloric acid solution. Dilute solutions (0.1 and 0.02 N) were prepared by accurate dilution from the stock solution. 10% solutions of acetic acid, acetone, orange juice, lime juice and mandarin juice were prepared by dissolving 1mL of each in 9mL deionized water. All solutions were kept in dark bottles and refrigerator.

Acetate buffer solution with different pH values (pH = 4.0 and 5.2) were prepared by mixing 0.2 M solution of acetic acid and sodium acetate by ratio 41.0 : 9.0 and 10.5 : 39.5 for pH 4.0 and 5.2, respectively, then completed to 100 mL using de-ionized water. The pH of the resulting solutions were checked and readjusted. Citrate buffer solution with pH = 6.0 was prepared by mixing 9.5 mL and 41.5 mL of 0.1 M solution of citric acid and sodium citrate, respectively, then completed to 100 mL using

deionized water. Solution of 5% trichloroacetic acid was prepared by dissolving 5 g of trichloroacetic acid to 100 mL deionized water. Dansyle chloride solution was prepared by dissolving 500 mg in 100 mL acetone.

All chemicals used were of the highest purity available and used as received. They included standard histamine dihydrochloride (Hist) and standard tyramine (Tyr). They were supplied from Sigma, USA. Gallic and tannic acids were supplied from Aldrich Chemical Co. Ltd., USA.

Standard preparation

To 1 mL of standard histamine or tyramine solutions were added 1 mL of each one of ascorbic acid, sodium sulphite, sodium metabisulphite, sodium chloride, acetic acid, acetone, orange juice, lime juice, mandarin juice, pyrogallol, catechol, tannic acid, chlorogenic acid, starch, curcuma, ginger, milk, rosemary, cinnamon and thyme, respectively, at temperature 30°C and left for 15 min.

Procedures

Effect of temperature. 1 mL of standard histamine or tyramine solutions was mixed well with 1 mL of glucose, gallic acid and (2 N) hydrochloric acid and the reaction mixture was left at 40°C and 100°C for 15 min.

Effect of different buffers with different pH at different temperatures: The interaction between histamine and tyramine solutions and different compounds using different buffers with different pH values at different temperatures was studied as follow:

To 1 mL standard histamine solution was added 1 mL of acetate buffer (pH = 4.0 and 5.0 and 4.0 and 5.2 for histamine and tyramine, respectively) and 1 mL of each one of glucose, ascorbic, gallic and citric acids, respectively, were added at temperatures of 40°C and 60°C and the reaction mixture was left for 15, 30 and 45 min.

1 mL of standard histamine or tyramine solutions was mixed with 1 mL citrate buffer (pH = 6.0) and 1 mL of each one of glucose, ascorbic, gallic, citric and tannic acids, pyrogallol and catechol, respectively, was added at different temperatures of 60°C and 100°C and the reaction mixture was left for 15, 30 and 45 min.

Preparation of thin-layer chromatography

Suspended solution of silica gel G was prepared by dissolving 3 g in 20 mL deionized water. A mixture solution of chloroform, methanol and ammonium hydroxide (12: 7: 1, v/v) were prepared and used as mobile phase for TLC separation (Aures et al. 1968, Voigt and Eitenmiller 1977, Halasz et al. 1994). Spraying solution of ninhydrine derivatives were prepared by using mixture of 0.3 g ninhydrine, 100 mL n-butanol and 3.0 mL of acetic acid.

Quantitative analysis of histamine and tyramine using spectrophotometric method

Histamine and tyramine were determined spectrophotometrically according to previously recommended procedures (Lovenberg and Engelmann 1971, Voigt and Eitenmiller 1977). The area spot on silicagel G layer containing the amine zone was scraped into layers and put in test tube containing 5 mL methanol (50% v/v) to elute the colour. The resultant colour was measured spectrophotometrically after filtration using ashless filter paper (Watmann no. 1) at wavelength 570 nm and the concentration of the samples was calculated from the standard curve. The amines content was calculated as µg related to standard histamine or tyramine.

Results and Discussion

Effect of some acids, organic and inorganic compounds on histamine and tyramine

The results of the reaction of histamine and tyramine with different acids, inorganic and organic compounds at 30°C, 15 min and spectrophotometric measurement at λ = 570 nm, are listed in Table 1. It is clear from this table that histamine content was disappeared by reaction with acetone, pyrogallol and catechol with the appearance of more than one spots on the TLC plate. The reaction of histamine with pyrogallol led to two spots at R_f = 0.19 and 0.40 but the reaction with catechol led to three spots at R_f = 0.22, 0.40 and 0.58 values. On the other hand, acetic and tannic acids have no effect on histamine as indicated by the R_f value given in Table 1.

It was observed also from Table 1 that the reaction of histamine with ascorbic acid, sodium sulfite and sodium metabisulfite in aqueous solution at 30°C for 15 min led to formation of one spot at the same R_f value of 0.26 like standard histamine with concentrations 42, 36 and 55 µg, respectively.

Table 1. Reaction of histamine and tyramine with different acids, inorganic and organic compounds at 30°C for 15 min using spectrophotometric method at λ = 570 nm

Reaction	Spot no.	R_f	Compound	Conc. as Hist. µg
Standard Histamine (authentic sample)	1	0.26	Histamine	35.0
St. Hist. + acetic acid	1	0.26	Histamine	35.0
St. Hist. + tannic acid	1	0.26	Histamine	34.0
St. Hist. + acetone	1	0.21	unknown	29.0
St. Hist. + pyrogallol	1 2	0.19 0.40	unknown unknown	37.5 14.5
St. Hist. + catechol	1 2 3	0.22 0.40 0.58	unknown unknown unknown	28.5 21.5 73.0
St. Hist. + ascorbic acid	1	0.26	Histamine	42.0
St. Hist. + sod. Sulphite	1	0.26	Histamine	36.0
St. Hist. + sod. meta bi-sulphite	1	0.26	Histamine	55.0
St. Hist.+ chlorogenic acid	1	0.18	unknown	27.0
St. Hist. + Starch	1	0.18	unknown	44.0
St. Hist. + NaCl	1 2	0.36 0.46	unknown unknown	42.5 53.0
				Conc. as Tyr. µg
Standard Tyramine (authentic sample)	1	0.78	Tyramine	172
St. Tyr. + acetic acid	1	0.68	unknown	30
St. Tyr. + tannic acid	1	0.66	unknown	43
St. Tyr. + acetone	1	0.71	unknown	20
St. Tyr. + pyrogallol	1 2	0.71 0.89	unknown unknown	84 30
St. Tyr. + catechol	1 2	0.74 0.94	unknown unknown	170 182
St. Tyr. + ascorbic acid	1	0.78	Tyramine	370
St. Tyr. + sod. Sulphite	1	0.78	Tyramine	200
St. Tyr. + sod. meta bi-sulphite	1	0.76	unknown	240
St. Tyr.+ chlorogenic acid	1	0.82	unknown	106
St. Tyr. + starch	1	0.42	unknown	132
St. Tyr. + NaCl	1	0.73	unknown	360

But the reaction of histamine with chlorogenic acid and starch leads to disappearing of histamine and production of one spot at R_f = 0.18 and 0.18 with concentrations 27 and 44 µg, respectively.

In addition the reaction of histamine with sodium chloride led to disappearance of histamine and formation of two spots at R_f values of 0.36 and 0.46 with concentrations 42.5 and 53 μg as histamine, respectively.

The reaction of tyramine compound with acetic, tannic, chlorogenic and ascorbic acids, acetone, pyrogallol, catechol, sodium sulfite, sodium meta bi-sulfite, starch and sodium chloride led to disappearance of tyramine and formation of another compounds with ascorbic and chlorogenic acids, sodium sulfite, sodium meta bi-sulfite, starch and sodium chloride (Table 1) and change in the tyramine concentrations was found to be in the range from 20 – 370 μg.

The effect of glucose, gallic and hydrochloric acids on histamine and tyramine was studied at 40 and 100°C with stirring for 15 min and spectrophotometric measurements at 570 nm. The data are given in Table 2. It was found that histamine concentration was decreased from 35 to 28.2 μg by the reaction with glucose while the R_f value does not affected. But the reaction of histamine with gallic and hydrochloric acids led to disappearance of histamine compound and formation of unknown compounds with R_f values at 0.22 and 0.36, respectively. By increasing the temperature to 100°C, it was observed from the data in Table 2 that histamine was disappeared by the reaction with hydrochloric acid (2N) and a low decrease in histamine concentration by the reaction with gallic acid. It is clear also that glucose still has no effect on histamine. The addition of citric and caffic acids or caffeine to histamine led to disappearance of histamine.

Table 2. Reaction of histamine and tyramine with glucose, gallic and hydrochloric acids at 40°C and 100°C for 15 min and histamine with caffeine and caffic and citric acids using spectrophotometric method at $\lambda = 570$ nm

	Reaction	Spot no.	R_f	Compound	Conc. as Hist. or Tyr. μg
	Standard Histamine (authentic sample)		0.26 0.78	Histamine Tyramine	35.0 172
At T = 40°C and for 15 min	St. Hist. + glucose St. Hist. + gallic St. Hist. + 2N HCl	1 1 1	0.26 0.22 0.36	Histamine unknown Unknown	28.5 34.0 15.0
At T = 40°C and for 15 min	St. Tyr. + glucose St. Tyr. + gallic St. Tyr. + 2N HCl	1 1 1	0.76 0.78 0.80	unknown Tyramine Unknown	30 148 130
At T= 100°C and for 15 min	St. Hist. + glucose St. Hist. + gallic St. Hist. + 2N HCl	1 1 1	0.26 0.26 0.36	Histamine Histamine Unknown	55.5 31.5 16.0
At T = 100°C and for 15 min	St. Tyr.+ glucose St. Tyr. + gallic St. Tyr. + 2N HCl	1 1 1 2	0.83 0.76 0.36 0.78	Unknown Unknown Unknown Tyramine	46 120 140 38
Test used only for histamine	St. Hist. + Citric + Caffic acid (0.2 mg)	1	0.18	Unknown	38.0
	St. Hist. + Citric + Caffic acid (0.02 mg)	1	0.16	Unknown	31.0
	St. Hist. + Citric + Caffeine	1	0.18	Unknown	49.0

From Table 2, it was found that tyramine disappeared by reaction with glucose and 2N hydrochloric acid at 40°C for 15 min but gallic acid led to decrease of tyramine concentration to 148 μg. The reaction of tyramine with glucose and gallic acid at 100°C led to disappearance the

amine compound and formation unknown compounds but by reaction with hydrochloric acid (2N) at 100°C the tyramine concentration decreased to 38 µg as given in Table 2.

Effect of using natural additives

The effect of adding different natural compounds on histamine and tyramine elimination is illustrated in Table 3 and Fig. 1 and 2. It is obvious from Table 3 that curcuma, ginger, milk, rosemary, cinnamon and thyme in aqueous solution led to elimination of histamine and tyramine compounds and formation of unknown compounds. Also, from Table 3 it was found that addition of lime juice to histamine solution led to disappearance of histamine compound and formation of two compounds that reacted with ninhydrine and give R_f values at 0.15 and 0.45, respectively. While the addition of orange juice led to disappearance of histamine compound and formation of three compounds that reacted with ninhydrine and give TLC spots with R_f = 0.2, 0.4 and 0:52, respectively. Also mandarin juice add to the aqueous solution of histamine led to formation of three unknown compounds at R_f = 0.25, 0.43 and 0.37 whereas the original amine was disappeared.

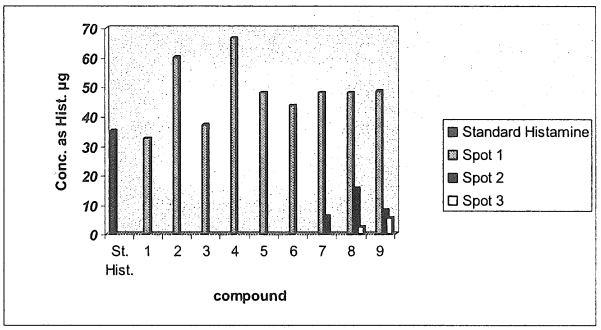

Figure 1. Effect of addition of natural compounds on histamine using spectrophotometric method. 1: St. Hist. + curcuma, 2: St. Hist. + ginger, 3: St. Hist. + milk, 4: St. Hist. + rosemary, 5: St. Hist. + cinnamon, 6: St. Hist. + thyme, 7: St. Hist. + lime, 8: St. Hist. + orange and 9: St. Hist. + mandarin.

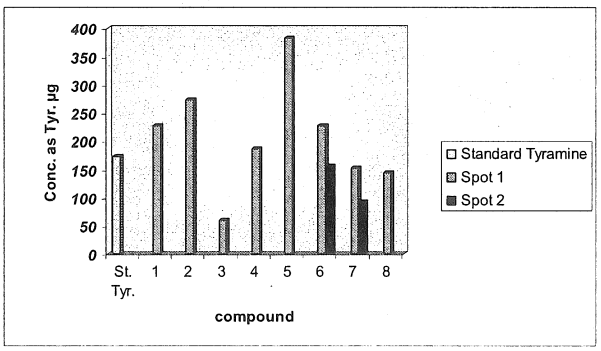

Figure 2. Effect of using natural compounds on tyramine using spectrophotometric method. 1: St. Tyr. + curcuma, 2: St. Tyr. + ginger, 3: St. Tyr. + milk, 4: St. Tyr. + rosemary, 5: St. Tyr. + cinnamon, 6: St. Tyr. + thyme, 7: St. Tyr. + lime and 8: St. Tyr. + orange.

By using of some natural juice, it was observed that lime and orange juice had good role on elimination of the tyramine compound and as a result formation of unknown compounds.

Table 3. Effect of addition of natural compounds on histamine and tyramine using spectrophotometric method

Reaction	Spot no.	R_f	Compound	Conc. as Hist. µg
Standard Histamine (authentic sample)	1	0.26	Histamine	35
St. Hist. + curcuma	1	0.22	Unknown	32.5
St. Hist. + ginger	1	0.19	Unknown	60
St. Hist. + milk	1	0.18	Unknown	37
St. Hist. + rosemary	1	0.18	Unknown	66.5
St. Hist. + cinnamon	1	0.19	Unknown	48
St. Hist. + thyme	1	0.19	Unknown	43.5
St. Hist. + lime	1	0.15	Unknown	48
	2	0.45	Unknown	6
St. Hist. + orange	1	0.20	Unknown	48
	2	0.40	Unknown	15.5
	3	0.52	Unknown	2.5
St. Hist. + mandarin	1	0.26	Histamine	48.5
	2	0.43	Unknown	8
	3	0.57	Unknown	5.5
				Conc. as Tyr. µg
Standard Tyramine (authentic sample)	1	0.78	Tyramine	172
St. Tyr. + curcuma	1	0.35	Unknown	277
St. Tyr. + ginger	1	0.36	Unknown	274
St. Tyr. + milk	1	0.85	Unknown	60
St. Tyr. + rosemary	1	0.35	Unknown	186
St. Tyr. + cinnamon	1	0.34	Unknown	383
St. Tyr. + thyme	1	0.25	Unknown	228
	2	0.40	Unknown	186
St. Tyr. + lime	1	0.65	Unknown	152
	2	0.85	Unknown	156
St. Tyr. + orange	1	0.61	Unknown	144
	2	0.83	Unknown	92

Effect of using glucose

The effect of adding glucose on the elimination of histamine is illustrated in Table 4. The reaction of histamine with glucose for 15, 30 and 45 min at 40°C and 60°C and pH 4.0 and 5.20 using acetate buffer led to disappearance of histamine compound and formation of unknown compounds as indicated by the change in the R_f values. At 60°C and 100°C the reaction of histamine with glucose at pH 6.0 (citrate buffer) for 15, 30 and 45 min led to disappearance of the histamine and formation of another compounds having the R_f values listed in Table 4. After 45 min, a decrease in histamine concentration to 17.5 µg was observed.

At pH 4.0 (acetate buffer) it was found that glucose had good effect on decreasing tyramine concentration at temperature 40°C from 172 µg to 80, 70 and 64 µg after 15, 30 and 45 min, respectively (Table 4). But at 60°C, disappearance of tyramine was observed. At pH 5.2 (acetate buffer) a decrease in tyramine concentration at temperature 40°C from 172 µg to 52 and 50 µg after 15 and 30 min. is found and disappearance of tyramine after 45 min. beside formation of unknown compound as shown in Table 4 is also observed. At the same pH 5.2 and temperature 60°C, tyramine was disappeared after 15, 30 and 45 min and unknown compounds were formed. Tyramine compound was disappeared during its reaction with glucose at 60°C and 100°C for 15,

30 and 45 min. in citrate buffer of pH= 6.0 and new compounds were formed with different R_f values as shown in Table 4.

Table 4. Effect of glucose on histamine and tyramine at different buffers with different pHs at different temperatures and times using spectrophotometric method

Compound + Glucose		T = 40°C			T = 60°C			T = 100°C		
		15 min	30 min	45 min	15 min	30 min	45 min	15 min	30 min	45 min
Acetate buffer (pH = 4)	Rf	0.3	0.32	0.34	0.36	0.36	0.34	0.00	0.00	0.00
	Compound	Unk.	Unk.	Unk.	Unk.	Unk.	Unk.	------	------	------
	Concn. As Hist (μg)	53	65.5	101.5	39.5	74.5	26.0	0.0	0.0	0.0
Acetate buffer (pH =5.2)	Rf	0.33	0.33	0.37	0.33	0.30	0.30	0.00	0.00	0.00
	Compound	Unk.	Unk.	Unk.	Unk.	Unk.	Unk.	------	------	------
	Concn. As Hist (μg)	43.5	41.0	48.0	21.5	21.5	13.5	0.0	0.0	0.0
Citrate buffer (pH = 6)	Rf	0.00	0.00	0.00	0.15	0.19	0.26	0.20	0.21	0.23
	Compound	------	------	-----	Unk.	Unk.	Hist.	Unk.	Unk.	Unk.
	Concn. As Hist (μg)	0.0	0.0	0.0	12.0	15.0	17.5	10.5	16.0	17.5
Acetate buffer (pH = 4)	Rf	0.78	0.78	0.78	0.83	0.85	0.91	0.00	0.00	0.00
	Compound	Tyr.	Tyr.	Tyr.	Unk.	Unk.	Unk.	------	------	------
	Concn. As Tyr. (μg)	80.0	70.0	64.0	148.0	86.0	215.0	0.0	0.0	0.0
Acetate buffer (pH = 5.2)	Rf	0.78	0.78	0.80	0.82	0.80	0.83	0.00	0.00	0.00
	Compound	Tyr.	Tyr.	Tyr.	Unk.	Unk.	Unk.	------	------	------
	Concn. As Tyr. (μg)	52.0	50.0	144.0	263.0	136.5	278.0	0.0	0.0	0.0
Acetate buffer (pH = 6)	Rf	0.00	0.00	0.00	0.76	0.96	0.75	0.85	0.83	0.81
	Compound	-----	-----	-----	Unk.	Unk.	Unk.	Unk.	Unk.	Unk.
	Concn. As Hist (μg)	0.00	0.00	0.00	88.0	42.0	156.0	150.0	122.0	68.0

Effect of using ascorbic acid

The reaction of histamine with ascorbic acid at temperatures 40°C and 60°C and pH values of 4.0 and 5.2 for 15, 30 and 45 min are also studied and the data are given in Table 5. It is obvious from the data that histamine compound is disappeared and new unknown compounds are formed. Also the same reaction has been done at pH 6.0 and at different temperatures of 60°C and 100°C for different time intervals such as 15, 30 and 45 min. These reactions led to disappearance of histamine content as shown in Table 5.

Table 5 shows the effect of adding ascorbic acid on tyramine. The reaction of tyramine with ascorbic acid at pH 4.0 using acetate buffer at 40°C showed a clear decrease in tyramine concentration from 172 μg to 144, 60 and 46 μg at different time intervals of 15, 30 and 45 min, respectively. But after 60°C tyramine disappeared and new compounds were formed. The effect of ascorbic acid on tyramine at 40°C and pH 5.2 (acetate buffer) led to a decrease of tyramine concentration to 110 μg after 15 min and formation of unknown compounds after 30 and 45 min (Table 5). The same reaction at 60°C and pH 6.0 (citrate buffer) led to decrease of tyramine concentration to 114 and 20 μg after 15 and 30 min, respectively, and disappearance of tyramine after 45 min but at 100°C tyramine was disappeared during different time intervals and unknown compounds were formed (Table 5).

Effect of using gallic and citric acids

The reaction of histamine and gallic acid at pH 4.0 and 6.0 showed clear disappearance of histamine at 40 and 60°C for different times except at temperature 60°C for 15 min showed a little decrease in histamine content to 30 μg. Also at pH 6.0 using citrate buffer, the results showed a disappearance of histamine and formation of new compound (Table 6).

Table 5. Effect of ascorbic acid on histamine and tyramine at different buffers with different pH at different temperatures and time using spectrophotometric method

Compound + Ascorbic acid		T = 40°C			T = 60°C			T = 100°C		
		15 min	30 min	45 min	15 min	30 min	45 min	15 min	30 min	45 min
Acetate buffer (pH = 4)	Rf	0.30	0.30	0.35	0.32	0.33	0.35	0.00	0.00	0.00
	Compound	Unk.	Unk.	Unk.	Unk.	Unk.	Unk.	------	------	------
	Concn. as Hist (µg)	25.5	32.0	35.0	28.0	28.0	43.0	0.0	0.0	0.0
Acetate buffer (pH =5.2)	Rf	0.22	0.22	0.19	0.27	0.24	0.24	0.00	0.00	0.00
	Compound	Unk.	Unk.	Unk.	Unk.	Unk.	Unk.	------	------	------
	Concn. as Hist (µg)	25.0	27.0	23.5	31.0	27.5	25.0	0.0	0.0	0.0
Citrate buffer (pH = 6)	Rf	0.00	0.00	0.00	0.33	0.33	0.28	0.31	0.31	0.30
	Compound	------	------	-----	Unk.	Unk.	Unk.	Unk.	Unk.	Unk.
	Concn. as Hist (µg)	0.0	0.0	0.0	64.0	52.0	38.0	44.5	42.5	42.0
Acetate buffer (pH = 4)	Rf	0.78	0.78	0.78	0.83	0.85	0.91	0.00	0.00	0.00
	Compound	Tyr.	Tyr.	Tyr.	Unk.	Unk.	Unk.	------	------	------
	Concn. as Tyr. (µg)	144.0	60.0	46.0	92.0	76.0	114.0	0.0	0.0	0.0
Acetate buffer (pH = 5.2)	Rf	0.78	0.82	0.82	0.82	0.85	0.78	0.00	0.00	0.00
	Compound	Tyr.	Unk.	Unk.	Unk.	Unk.	Tyr.	------	------	------
	Concn. as Tyr. (µg)	110.0	76.0	132.0	102.0	170.0	430.0	0.0	0.0	0.0
Acetate buffer (pH = 6)	Rf	0.00	0.00	0.00	0.78	0.78	0.80	0.72	0.75	0.76
	Compound	-----	-----	-----	Tyr.	Tyr.	Unk.	Unk.	Unk.	Unk.
	Concn. as Hist (µg)	0.00	0.00	0.00	114.0	20.0	213.5	497	341.0	230.0

From Table 6, it was found that the reaction of histamine with citric acid at different temperatures (40 and 60°C) and different pH values (4.0 and 5.2) led to the disappearance of the histamine compound and formation of unknown compounds except the reaction at 40°C for 15 min and pH value 4.0 which showed a decrease of histamine concentration to 11.5 µg. The same results were obtained at pH 6.0 using citrate buffer for different temperatures (60°C and 100°C). The effect of gallic acid on the elimination of tyramine is carried out as given in Table 7. It is obvious from the results that at pH= 4.0, the reaction of tyramine with gallic acid at 40°C and 60°C led to disappearance of tyramine compound and formation of unknown compounds during all time intervals when the reaction was carried out at pH 5.2 (acetate buffer) and temperature 40°C led to a decrease in tyramine concentration to 107 µg after 15 min and formation of unknown compounds after 30 and 45 min. Also at 60°C disappearance of tyramine and formation of unknown compounds (Table 7) was observed.

The use of citrate buffer of pH 6.0 at 60°C and 100°C led to disappearance of tyramine and formation of other compounds during its reaction with gallic acid. The data resulted from reaction of tyramine with citric acid at different pH values of 4, 5.2 and 6 at different temperatures 40°C, 60°C and 100°C for different time intervals 15, 30 and 45 min are listed in Table 7. The results obtained showed disappearance of tyramine and formation of unknown compounds as indicated by the change of R_f value of standard tyramine at 0.78 to 0.67, 0.56, 0.61, 0.53, 0.65, 0.70, 0.76, 0.75 and 0.72.

Effect of using tannic acid, pyrogallol and catechol

The reaction of histamine with tannic acid (Table 8) in citrate buffer (pH 6.0) at different temperatures (60°C and 100°C) for different time intervals (15, 30 and 45 min.) led to disappearance of histamine content and formation of unknown compounds as indicated by the change in the R_f values. The reaction carried out at 100°C and left for 30 min led to decrease of histamine concentration only to 26 µg.

Table 6. Effect of gallic and citric acids on histamine at different buffers with different pH at different temperatures and times using spectrophotometric method

Histamine + Gallic acid		T = 40°C			T = 60°C			T = 100°C		
		15 min	**30 min**	**45 min**	**15 min**	**30 min**	**45 min**	**15 min**	**30 min**	**45 min**
Acetate buffer (pH = 4)	Rf	0.29	0.31	0.28	0.26	0.29	0.33	0.00	0.00	0.00
	Compound	Unk.	Unk.	Unk.	Hist.	Unk.	Unk.	------	------	------
	Concn. as Hist. (µg)	23.0	20.5	21.5	30.0	43.0	27.0	0.0	0.0	0.0
Acetate buffer (pH=5.2)	Rf	0.19	0.19	0.19	0.17	0.19	0.21	0.00	0.00	0.00
	Compound	Unk.	Unk.	Unk.	Unk.	Unk.	Unk.	------	------	------
	Concn. as Hist. (µg)	36.0	36.0	32.0	56.0	38.5	30.0	0.0	0.0	0.0
Citrate buffer (pH = 6)	Rf	0.00	0.00	0.00	0.21	0.19	0.18	0.21	0.17	0.17
	Compound	------	------	-----	Unk.	Unk.	Unk.	Unk.	Unk.	Unk.
	Concn. as Hist. (µg)	0.0	0.0	0.0	23.0	30.5	40.5	60.1	60.0	61.5
Histamine + Citric acid										
Acetate buffer (pH= 4)	Rf	0.26	0.23	0.22	0.22	0.20	0.23	0.00	0.00	0.00
	Compound	Hist..	Unk.	Unk.	Unk.	Unk.	Unk.	------	------	------
	Concn. as Hist. (µg)	11.5	15.0	20.0	13.0	14.0	26.0	0.0	0.0	0.0
Acetate buffer (pH = 5.2)	Rf	0.21	0.23	0.23	0.24	0.20	0.22	0.00	0.00	0.00
	Compound	Unk.	Unk.	Unk.	Unk.	Unk.	Unk.	------	------	------
	Concn. as Hist. (µg)	38.5	19.5	42.5	47.0	27.5	32.5	0.0	0.0	0.0
Acetate buffer (pH = 6)	Rf	0.00	0.00	0.00	0.15	0.12	0.12	0.18	0.18	0.16
	Compound	-----	-----	-----	Unk.	Unk.	Unk.	Unk.	Unk.	Unk.
	Concn. as Hist (µg)	0.00	0.00	0.00	19.5	27.0	21.5	27.0	21.5	20.5

Table 7. Effect of gallic and citric acids on tyramine at different buffers with different pH at different temperatures and times using spectrophotometric method

Tyramine + Gallic acid		T = 40°C			T = 60°C			T = 100°C		
		15 min	**30 min**	**45 min**	**15 min**	**30 min**	**45 min**	**15 min**	**30 min**	**45 min**
Acetate buffer (pH = 4)	Rf	0.76	0.74	0.71	0.70	0.74	0.80			
	Compound	Unk.	Unk.	Unk.	Unk.	Unk.	Unk.			
	Concn. as Tyr. (µg)	394	203	88	195.4	579	611			
Acetate buffer (pH =5.2)	Rf	0.78	0.73	0.73	0.66	0.68	0.73			
	Compound	Tyr.	Unk.	Unk.	Unk.	Unk.	Unk.			
	Concn. as Tyr. (µg)	107	225	135	82	271	179			
Citrate buffer (pH = 6)	Rf				0.74	0.72	0.71	0.73	0.71	0.83
	Compound				Unk.	Unk.	Unk.	Unk.	Unk.	Unk.
	Concn. as Tyr. (µg)				1452	1337	2408	713	430	314
Tyramine + Citric acid										
Acetate buffer (pH = 4)	Rf	0.67	0.56	0.61	0.53	0.65	070			
	Compound	Unk.	Unk.	Unk.	Unk.	Unk.	Unk.			
	Concn. as Tyr. (µg)	167	73	155	92	204	178			
Acetate buffer (pH = 5.2)	Rf	0.76	0.75	0.70	0.72	0.75	0.75			
	Compound	Unk.	Unk.	Unk.	Unk.	Unk.	Unk.			
	Concn. as Tyr. (µg)	80	152	210	402	284	196			
Acetate buffer (pH = 6)	Rf				0.70	0.70	0.70	0.75	0.72	0.72
	Compound				Unk.	Unk.	Unk.	Unk.	Unk.	Unk.
	Concn. as Tyr. (µg)				260	136	50	255	68	182

The reaction of histamine with pyrogallol and catechol at pH 6.0 and different temperatures (60°C and 100°C) for different stirring times (15, 30 and 45 min) led to disappearance of histamine compound and formation of unknown compounds as indicated by the change in the R_f values given in Table 8 and Fig. 3.

Table 8. Effect of tannic acid, pyrogallol and catechol on histamine and tyramine using citrate buffer (pH = 6) at different temperatures and times using spectrophotometric method

		T = 60°C			T = 100°C		
		15min	**30 min**	**45 min**	**15 min**	**30 min**	**45 min**
Histamine + tannic acid	Rf	0.15	0.15	0.14	0.33	0.26	0.26
	Compound	Unk.	Unk.	Unk.	Unk.	Unk.	Unk.
	Concn. As His. (μg)	23.5	21.5	16.5	33	33	26
Histamine + pyrogallol	Rf	0.16	0.23	0.20	0.15	0.20	0.21
	Compound	Ukn.	Unk.	Unk.	Unk.	Unk.	Unk.
	Concn. As His. (μg)	25	30.5	26.5	28.5	9	16.5
Histamine + catechol	Rf	0.22	0.22	0.26	0.22	0.18	0.20
	Compound	Unk.	Unk.	Unk.	Unk.	Unk.	Unk.
	Concn. As His. (μg)	25.5	25	29.5	31.5	23.5	41.5
Tyramine + tannic acid	Rf	0.78	0.75	0.72	0.79	0.78	078
	Compound	Tyr.	Unk.	Unk.	Unk.	Tyr.	Tyr.
	Concn. As Tyr. (μg)	68	318	368	234	88	64
Tyramine + pyrogallol	Rf	0.75	0.78	0.78	0.76	0.80	0.80
	Compound	Unk.	Tyr.	Tyr.	Unk.	Unk.	Unk.
	Concn. As Tyr. (μg)	114	172	92	434	288	662
Tyramine + catechol	Rf	0.70	0.70	0.68	0.80	0.76	0.75
	Compound	Unk.	Unk.	Unk.	Unk.	Unk.	Unk.
	Concn. As Tyr. (μg)	152	174	230	508	174	106

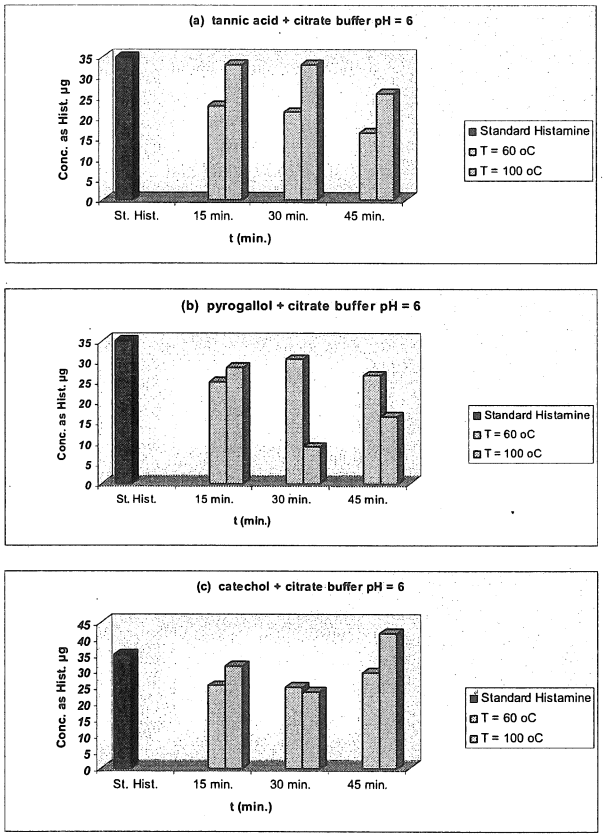

Figure 3. Effect of (a) tannic acid, (b) pyrogallol and (c) catechol on histamine using citrate buffer pH = 6 at different temperatures and times using spectrophotometric method.

By reaction of tyramine with tannic acid at pH 6.0 using citrate buffer and temperature 60°C, it was found that tyramine concentrations decreased after 15 min to 68 μg then disappeared after 30 and 45 min. When the previous reaction was carried out at 100°C it was found that tyramine

concentration decreased to 88 and 64 µg after 30 and 45 min (Table 8).On studying the effect of organic compounds such as pyrogallol and catechol, it was found that the reaction of pyrogallol with tyramine at pH 6.0 (citrate buffer) and temperature 60°C led to a decrease in the tyramine concentration after 45 min to 92 µg but by carrying out this reaction at 100°C it was found that tyramine was disappeared after 15, 30 and 45 min and unknown compounds were formed (Table 8). When catechol was reacted with tyramine at pH 6.0 (citrate buffer) and temperature 60°C and 100°C, it led to disappearance of tyramine beside formation of unknown compounds (Table 8).

Conclusion

Since the biogenic amines have some toxic effects on the health which lead to nausea, respiratory distress, hot flush, sweating, heart palpitations, headache, bright red flush, oral burning and hyper- or hypotensive. Estimation of BA is important not only from the point of view of their toxicity, but also because they can be used as indicators of the degree of freshness or spoilage of food (Arlorio et al., 1998). The factors affecting on the presence of these biogenic amines included pH, free amino acids, water activity and temperature. So we work on a model system which carried out to study the effect of natural, organic and inorganic compounds on biogenic amines such as histamine and tyramine.

Effect of model system on biogenic amines

Histamine and tyramine contents were disappeared by reaction with acetone, pyrogallol and catechol at 30°C for 15 min. The reaction of histamine with ascorbic acid, sodium sulfite and sodium metabisulfite in aqueous solution at 30°C for 15 min led to formation of one spot at the same R_f value of 0.26 like standard histamine with concentrations of 42, 36 and 55 µg, respectively. The reaction of tyramine with ascorbic and chlorogenic acids, sodium sulfite, sodium metabisulfite, starch and sodium chloride led to disappearance of tyramine and formation of another compounds. The reaction of histamine and tyramine with chlorogenic acid and starch led to disappearance of both of them. Also the reaction of tyramine with glucose and 2N hydrochloric acid at 40°C for 15 min led to disappearance of tyramine while at 100°C for 15 min, tyramine disappeared with gallic acid. Increasing the temperature to 100°C led to the disappearance of histamine by the reaction with hydrochloric acid (2N) and a low decrease in histamine concentration was resulted from the reaction of histamine with gallic acid. The addition of citric and caffic acids or caffeine to histamine led to disappearing it. Curcuma, ginger, milk, rosemary, cinnamon and thyme in aqueous solution led to elimination of histamine and tyramine compound. Addition of lime and orange juices to histamine and tyramine solution led to disappearance of both of them. The same results were obtained with histamine when treated with mandarin juice.

References

Arlorio, M., Coisson, J.D. and Martelli, A. (1998) Ion-pair HPLC determination of biogenic amines and precursor aminoacids. Application of a method based on simultaneous use of heptanesulphonate and octylamine to some foods. *Chromatographia* 48: 763-769.

Askar, A. and Treptow, H. (1986). Biogenic Amines in Lebensmitteln. Vorkommen, Bedeutung und Bestimmung, Eugen Ulmer GmbH and Co., Stuttgart, Germany.

Aures, D., Fleming, R. and Hakanson, R. (1968) Separation and detection of biogenic amines by thin layer chromatography. *J. Chromatogr.* 33: 480-493.

Cinquina, A.L., Cali, A., Longo, F., de-Santis, L., Severoni, A. and Abballe, F. (2004) Determination of biogenic amine in fish tissues by ion-exchange chromatography with conductivity detection. *J. Chromatogr. A,* 1032: 73-77.

Çakli, S. and Taskaya, L. (1995) Süt ürünlerindeki biyojen aminler. *Su Ürüleri Dergisi* 12: 375-387.

Casal, S., Mendes, E., Alves, M.R., Alves, R.C., Beatriz, M., Oliveira, P.P. and Ferreira M.A. (2004) Free and conjugated biogenic amines in green and roasted coffee beans. *J. Agric. Food Chem.* 52: 6188-6192.

Halasz, A., Barath, A., Simon-Sarkad, L. And Holzapfel, W. (1994). Biogenic amines and their production by microorganisms in food. *Trends Food Sci Tech.* 5: 42-49.

Krizek, M., Vacha, F., Vorlova, L., Lukasova, J. and Cupakova, S. (2004). Biogenic amines in vacuum-packed and non-vacuum-packed flesh of carp (*Cyprinus carpio*) stored at different temperatures. *Food Chem.* 88: 185-191.

Lovenberg, W. and Engelmann, K. Suppl., 1, Ed. D. Glick. Interscience publishers, New York, 1971.

Ming, C.C., Liang, C. L. and Gow, C.Y. (1994) Biogenic amines in pork. *J. of the Chinese Agric. Chem. Soc.* 32: 47-60.

Mohamed, G.G., El-Hameed, A.A.K., El-Din, A.M.M.N., Lara, A.M.M. (2010). High performance liquid chromatography, thin layer chromatography and spectrophotometric studies on the removal of biogenic amines from some Egyptian foods using organic, inorganic and natural compounds. *J. Toxicol. Sci.* 35: 175-187.

Ochiai, M., Wakabayashi, K., Nagao, M., Sugimura, T., Ochiai, M., Wakabayashi, K., Nagao, M., and Sugimura, T. (1984). Tyramine is a major mutagen precursor in soy sauce, being convertible to a mutagen by nitrite. *Gann.* 75: 1-3.

Sellers, B.J.M., Staggs, C.G. and Bogle, M.L. (2006). Tyramine in foods and monoamine oxidase inhibitor drugs: A crossroad where medicine, nutrition, pharmacy, and food industry converge. *J. Food Comp. Anal.* 19: S58-S65.

Smith, T. A. (1980). Amines in food. *Food Chem.* 6: 169-200.

Stratton, J. E., Hutkins, R.W. and Taylors, S.L. (1991). Biogenic amines in cheese and other fermented foods: a review. *J. Food Prot.* 54: 460-470.

Szerdahely, E.N., Freudenreich, P. and Fischer, K. (1993). Biogenic amines in prk. *Fleischwirtschaft* 73: 789-790.

Voight, M.N. and Eitenmiller, R.R. (1977). An evaluation of extraction and thin layer chromatographic procedures for the quantification of biogenic amines in food. *Lebensm. Wiss. Technol.* 10: 263-267.

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