

Original Article

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

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ABSTRACT — This work has been carried out to investigate the conditions which lead to removal of the biogenic amines through the model system. Also, the main goal of this research work is trying to remove biogenic amines; histamine and tyramine, from some Egyptian foods such as tomato, strawberry, banana and mango 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 like glucose, spices, milk, vanillin, starch, orange juice, ascorbic and citric acids, showed an effective effect on disappearance of histamine and tyramine. By studying the effect of some additives on biogenic amines, it was found that tomato showed a decrease in histamine and tyramine concentrations by adding spices. Strawberry and banana showed a clear decrease in histamine and tyramine concentrations by treating them with ascorbic acid. Treating mango by milk led to increase of histamine level while milk with chocolate increases both histamine and tyramine concentrations.

Key words: Histamine, Tyramine, Thin layer chromatography (TLC), Spectrophotometric method, High performance liquid chromatography (HPLC)

INTRODUCTION

The biogenic amines (histamine, tyramine, cadaverine...etc.) were naturally present in some plant tissues, animals and humans and can be used as indicator of the quality or the hygiene of food. They were categorized as vasoactive or psychoactive compounds which could be formed during storage or processing of the product by thermal treatments or bacterial enzymatic decarboxylation of free amino acids (Vermeer *et al.*, 1998; Jover *et al.*, 1996a). Several toxicological problems (nausea, respiratory distress, hot flush, sweating, heart palpitations, headache, bright red rash, oral burning and hyper- or hypotensive) were resulted from the ingestion of food containing relatively high levels of biogenic amines. Histamine was physiologically active biogenic amines, found in fishes, cheeses, pickled cabbage, tomatoes and food rich by protein and fermented food. Level of histamine above

1,000 mg/kg in food was considered potentially dangerous to human health where diseases such as hypotension, bright red rash, hot flush, burning in the mouth; abdominal pain and dizziness could affect the humans. Also histamine concentration > 20 mg/100 g induced headache and Great Britain implied that the presence of > 10 mg/100 g not > 50 mg/100 g histamine in fish is indicative of its potential cause of histamine poisoning. On the other hand, tyramine was vasoactive biogenic amine present in fish, shrimp, aged cheese, wine, beer, yeast, banana, peel, potato, paprika, avocado, cabbage ...etc. The overdoses of tyramine caused hypertensive crisis, peripheral vasoconstriction, increase in cardiac output respiration and evaluate blood glucose ...etc. It had recently been shown that diet rich in amine containing foods (such as fish) combined with a high intake of nitrate could lead to an increase in N-nitrosamines formation (Vermeer *et al.*, 1998).

Biogenic amines might be used as an indicator of food spoilage. These amines were usually produced in foods by decarboxylation of amino acids as the result of the decarboxylase activity of bacteria present in the food, or that contaminate the food during transporting, handling, processing and marketing (Sabater *et al.*, 1993; Wendakoon and Sakaguchi, 1993; Eerola *et al.*, 1996; Jover *et al.*, 1996b; ten Brink *et al.*, 1990; Rawles *et al.*, 1996).

Some biogenic amines such as putrescine, cadaverine, spermidine and histamine had been found to be useful as quality indices for the decomposition of fish. The intake of histamine might cause an allergic toxicity known as "scombroid poisoning" while secondary biogenic amines could potentiate the toxicity of histamine and in addition could react with nitrite to form carcinogenic nitrosamine (Cinquina *et al.*, 2004; Taylor *et al.*, 1989; Kim *et al.*, 2000). It was found that the total amines in cheese were up to 1284.8 mg/l, wine and beers gave values from 0.5 to 27.2 mg/l (Arlorio *et al.*, 1998). Moret *et al.* (2005) made a survey on free biogenic amines contents in fresh and preserved vegetable products and the analysis was carried out using high performance liquid chromatography (HPLC) method. It was found that tyramine level was 4.9 mg/100 g in canned sauerkraut while other samples presented levels not exceeding 1.2 mg/100 g. The spinach sample showed the highest histamine content of 2.0 mg/100 g. Histamine was detected in all samples (100%), followed by spermine (93%), cadaverine (87%), tyramine (79%) and spermidine (79%). Lange and Wittmann (2002) determined biogenic amines in fish and meat products, sauerkraut, beer, dairy product, wine and further fermented foods by an enzyme sensor array. The lower detection limits were found to be 10 mg/kg for histamine and tyramine and 5 mg/kg for putrescine with linear range up to 200 mg/kg for histamine and tyramine and 100 mg/kg for putrescine.

The uncooked and ripening meat also contained statistically and significantly higher amount of tyramine and histamine than cooked meat products (Carous *et al.*, 1990). Formation of nitrosamines, which were potential carcinogens, constituted an additional toxicological risk associated to biogenic amines, especially in meat products that contain nitrite and nitrate salts as curing agents (Scanlan, 1983). The concentration of amine was not only important when considering illnesses such as histamine poisoning but also in the production of carcinogens. Also it was found that tomato, orange and banana had histamine content of 2.25, 2.25 and 2.70 mg/100 g (Ali, 1985). Levels of histamine in tomato fruits were found to be 10 mg/kg for green tomatoes and red ripe contains 2, 10, 20 and 50 mg/kg (Bolygo *et al.*, 2000). Storage of salted and

smoked fish more than 15 days led to an increase in the histamine content than normal (Çakli and Taskaya, 1995). Landete *et al.* (2004) found that histamine levels above 500-1,000 mg/kg food were considered potentially dangerous to human health.

Tarjàn and Jännossy (1978) reported that the high mean levels of tyramine for different vegetables were 84.0, 26.6, 25.0 and 67.0 mg/100 g for potato, paprika, tomato and cabbage, respectively. On the other hand, tomato and orange contained 1.35 and 1.57 mg/100 g of tyramine, respectively. Treatment of manufacturing sausage with different concentration of cloves and freezing decrease level of tyramine more than treatment with cinnamon, while thermal treatment had no high effect in decreasing it (Morsy, 2001).

So, the main target of this work aimed to determine the concentration of histamine and tyramine; as examples of biogenic amines, present in different food products available in the Egyptian markets like tomato, banana, sweet potato, ...etc. The effect of adding different organic, inorganic or natural additives on the removal of these biogenic amines and hence their allergic effects. The different conditions affecting the inhibition of amine effects (hazard effects) are optimized. Obedience to Beer's law is followed and the proposed methods, thin layer chromatography (TLC), spectrometry and HPLC are used for the determination of tyramine and histamine in pure form, and after treatments with different additives, and how to apply these results on Food Technology.

MATERIALS AND METHODS

Sample preparation

Curcuma, ginger, rosemary, glove, thyme, milk powder (Tanbouli), fresh tomato, strawberry, banana, mango and chocolate were supplied from local markets. Tomato ketchup (Heinz), mix banana milk and mix chocolate milk (Johayna), strawberry nectar (Yahoo) and mango nectar (Faragello) were also used.

Standard preparation

To 1 ml of standard histamine or tyramine solutions were added 1 ml of each one of ascorbic acid, sodium sulphite, sodium meta bi-sulphite, 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.

Preparation of thin-layer chromatography

Suspended solution of silica gel G was prepared by dis-

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solving 3 g in 20 ml bi-distilled 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; Voight and Eitenmiller, 1977).

Spraying solution of ninhydrin derivatives were prepared by using mixture of 0.3 g ninhydrin, 100 ml n-butanol and 3.0 ml of acetic acid.

Preparation of tomato samples

Fresh tomatoes were selected, cleaned and then blended. Different treatments were carried out as follows:

- a. 5 g fresh tomato juice without any additions was used as a control sample.
- b. 100 g of tomato juice was added to 5 g of glucose then heated to 100°C for 15 min.
- c. 100 g of fresh tomato juice was mixed with 2 g of mixture of equal weights of rosemary, clove, curcuma, thyme, ginger, cassia and marjoram.
- d. Concentrated tomato juice was prepared by heating 100 g until the total soluble solids (TSS) reach 21% (concentrated tomato puree).
- e. 100 g of concentrated tomato puree was mixed with 5 g glucose then heated to 100°C for 15 min.
- f. 2 g of mixture of equal weights of rosemary, clove, curcuma, thyme, ginger, cassia and marjoram were added to 100 g of concentrated tomato puree during heating process.
- g. Tomato ketchup was made using 1 l tomato juice and 60 g sugar, 30 g sodium chloride, 20 ml of 3 % vinegar, 5 g of chill and clove, 20 g of onion and garlic, 20 g of mixture of equal weight of rosemary, clove, curcuma, thyme, ginger, cassia and marjoram.
- h. Tomato ketchup (Heinz) with ingredients: tomatoes, sugar, vinegar, salt, chili, clove, cassia, garlic, celery, onion and the TSS were 29%.

Preparation of strawberry samples

Fresh and clean strawberry fruits were used and treated as follows:

- a. Fresh strawberry fruits without any treatments were used as control samples.
- b. 50 g of strawberry was soaked in 100 ml milk powder and left for 12 hr.
- c. 50 g of strawberry fruits was soaked in 100 ml orange juice.
- d. 50 g of strawberry was soaked in 100 ml fermented milk and leaved for 12 hr.
- e. Strawberry jam was prepared by mixing 1 kg of strawberry and sugars, respectively, then 10 ml lime juice was added and the mixture was heated.

- f. Strawberry jam was prepared by mixing under heating 1 kg of strawberry and sugars, respectively, then 10 ml of citric acid (3 g citric acid dissolve in 1,000 ml water) and 1% ascorbic acid were added.
- g. 100 g of strawberry puree was mixed with 1% ascorbic acid.
- h. 100 g of strawberry puree was mixed with 1% ascorbic acid then heating was carried out at 100°C for 30 min.
- i. Strawberry nectar (Yahoo) with ingredients: 40% natural strawberry pulp, sucrose, water, citric acid, natural flavour, natural colour, natural stabilizer, 0.01% sodium benzoate and TSS not less than 12%.

Preparation of banana fruit

After peeling, different treatments of banana fruits were carried out:

- a. Banana fruits without any treatment as control sample.
- b. 50 g banana fruit soaked with 100 ml milk powder and leaved for 12 hr.
- c. 50 g banana fruit soaked with 100 ml fermented milk and leaved for 12 hr.
- d. 50 g banana fruit soaked with 100 ml orange juice and leaved for 12 hr.
- e. 50 g of banana fruit puree mixed with 1% ascorbic acid.
- f. 50 g of banana fruit puree mixed with 1% ascorbic acid and heated at 100°C for 30 min.
- g. Mix banana milk (Juhayna) with ingredients: U.H.T. cow milk, 1.5% fat, 5% sugar, 15% total solids, natural banana extract, stabilizer and 20% milk powder.

Preparation of chocolate samples

Crude black chocolate was used and treated as follows:

- a. Crude black chocolate without any treatments used as a control sample.
- b. Sowed black chocolate added to milk powder by ratio 1:1, respectively.
- c. Sowed black chocolate added to milk powder by ratio 1:2, respectively.
- d. 100 g of sowing black chocolate mixed with 5 g glucose.
- e. 100 g of sowing black chocolate mixed with 1 g ascorbic acid.
- f. 100 g of sowing black chocolate mixed with 3 g vanillic acid.
- g. 100 g of sowing black chocolate mixed with 3 g vanillic acid and 2 g of starch.

- h. Mixed chocolate milk (Juhayna) with ingredients: U.H.T. cow milk, 1.5% fat, 5% sugar, 15% total solids, natural chocolate extract, stabilizer and 20% milk powder.

Preparation of mango samples

Fresh mango fruits were treated after peeling then blended as follows:

- Mango juice without any treatments used as control sample.
- 100 g of mango juice mixed with 1 g of ascorbic acid.
- 100 g of mango juice mixed with 2 g of starch.
- 100 g of mango juice mixed with 10 g of milk powder.
- 100 g of mango juice mixed with 5 g glucose.
- Mango nectar (Faragello), its ingredients: 35% natural mango pulp, sucrose, citric acid, natural flavour, natural colour Annatto (E 160 b), water and 15.5% TSS.

All the previous treatments left at ambient temperature for 1 hr. The above mango treatments; steps a-e, repeated and sterilized at 100°C for 30 min.

Sample preparation

For spectrophotometric method

The method of Lovenberg and Engelmann (1971) which described by Voight and Eitenmiller (1977) was used for the extraction of amines in foods. The procedure involved:

10 g of sample homogenized with 20 ml 0.1 M hydrochloric acid in a centrifuge tube. The tube was then centrifuged at 4°C for 10 min at 4,000 rpm and aqueous layer decanted to provide Lovenberg's crude extract. The aqueous phase was transferred to a second centrifuge tube, adjusted to pH = 10 with sodium carbonate and then saturated with excess sodium chloride (~ 5.0 g). 15 ml of n-Butanol was added and the mixture was agitated on a vortex mixer four times over a 10 min period. After centrifuging at 4,000 rpm for 15 min, the n-butanol layer was decanted for assay.

For HPLC method

The method of Mietz and Karmas (1977) was used for extraction of biogenic amines in foods.

Ground sample (50 g) was extracted with 5% trichloroacetic acid (TCA) (3 x 75 ml) using a warring blender. Each blended mixture was centrifuged and the clear extracts were combined. The volume was adjusted to 250 ml with TCA (5%) solution. The equivalence of 2 g of

samples as the TCA extract (10 ml was made alkaline by adding 1 ml 50% sodium hydroxide) and then extracted with n-butanol: chloroform mixture (1:1, v/v) (3 x 5 ml). The combined organic phase after addition of an equal amount of n-butanol (15 ml) was extracted with several portion of 0.02 M HCl (1 ml each), and the aqueous extract was dried using current air.

Quantitative analysis of histamine and tyramine

Using spectrophotometric method

Histamine and tyramine were determined spectrophotometrically according to previously recommended procedures (Voight and Eitenmiller, 1977; Lovenberg and Engelmann, 1971).

The area spot on silica gel G layer containing the amine zone was scraped into layers and put into test tubes containing 5 ml methanol (50%, v/v) to elute the colour. The resultant colour was measured spectrophotometrically after filtration using ashless filter paper (Wattmann no. 1) at wavelength 570 nm and the concentrations of the samples were calculated from the standard curve. The amines contents were calculated as µg related to standard histamine or tyramine solutions.

Using high performance liquid chromatography

HPLC method was used for the quantitative estimation of biogenic amines. The determination was carried out using Hypersil BDS-C18 column at 45°C with UV-Vis detection at 254 nm. The mobile phase used was solvent A (acetonitrile: 0.02 M acetic acid (1:9, v/v)) and solvent B (0.02 M acetic acid: acetonitrile: methanol (2:9:9, v/v)). The gradient program used was 60% solvent B in solvent A to 100% solvent B using linear program over 30 min period and 1 ml/min constant flow rate. 10 µl of standard solution (as derivative) or sample were injected into HPLC apparatus.

RESULTS AND DISCUSSION

Chromatography is a powerful separation technique that finds application to all branches of science. Now, chromatography not only set as a separation technique but also used for qualitative analysis. The wealth of chromatography, has led to the development of the techniques and equipment for HPLC which allows separation and measurement to be made in a matter of minutes with a high efficiency. So HPLC is considered as one of the most popular and powerful chromatographic technique. In this paper, HPLC method was utilized for the separation and quantitative determination of histamine and tyramine in

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different natural fruits. Figs. 1A and B shows the HPLC chromatograms of histamine and tyramine, respectively. The retention times were found to be 1.530 and 10.275 min for histamine and tyramine, respectively.

Effect of some natural additives on the histamine content of fruit

The effect of adding some natural additives such as glucose, spices, milk, vanillin, starch, orange juice, ascorbic and citric acids on the histamine or tyramine present in different natural fruits like tomato, strawberry, banana, chocolate and mango is studied in order to eliminate their allergic effect on the human body.

Treatment and processing on tomato

The data obtained are listed in Table 1. The concentration of histamine was found to be 38.50 mg/100 g on fresh matter (on fresh weight biogenic amines (Fw)). This concentration was decreased to 3.50 mg/100 g by processing to tomato puree and to 7.00 mg/100 g fresh matter by adding 2% spices to 100 g concentrated tomato puree. The treatment of tomato juice by 5% glucose and 2% spices led to a decrease in histamine concentration to 31.50 and 26.00 mg/100 g (Fw). Also the treatment of concentrated tomato puree by 5% glucose showed a disappearance of histamine and formation of another compound with $R_f = 0.40$. The treatment of ketchup by spices and vinegar showed histamine with concentration amounted to 37.90 mg/100 g (Fw).

It was observed that tomato and its products were free of tyramine, but other amine compounds may exist with $R_f = 0.56$ in concentrated tomato puree. The addition of glucose or species to tomato juice and concentrated tomato puree leads to a remarkable disappearance of the tyramine and other amines. It was found that ketchup product (Heinz) did not contain histamine, so this product is free of histamine in comparison with the fresh toma-

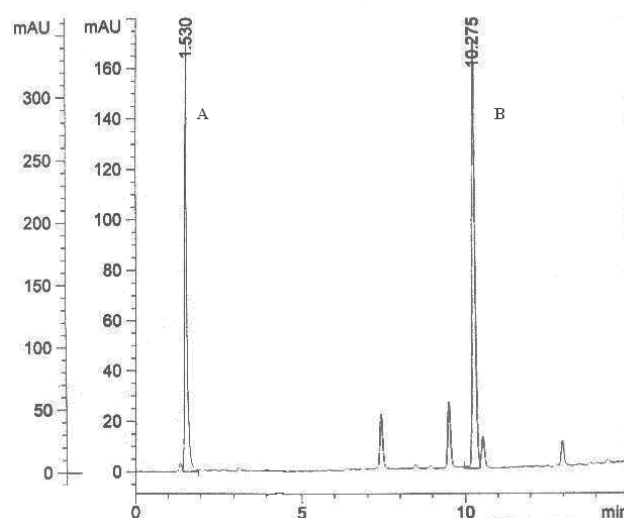


Fig. 1. Retention time of standard histamine (A) and tyramine (B) using HPLC.

Table 1. Some physicochemical composition of tomato products

	pH value	Amines content on fresh weight biogenic amines (mg/100 g) by TLC			Amines content on fresh weight biogenic amines (mg/100 g) by HPLC		
		Histamine	Tyramine	Unknown	Histamine	Tyramine	Unknown
a- Fresh tomato	4.50	38.50	0.00	0.00	42.80	4.20	0.00
b	4.30	31.50	0.00	0.00	0.00	0.00	0.00
c	4.40	26.00	0.00	0.00	27.90	0.00	0.00
d	3.90	3.50	0.00	98.00	0.00	0.00	0.00
e	3.80	0.00	0.00	15.75	0.00	0.00	0.00
f	3.90	7.00	0.00	0.00	0.00	0.00	0.00
g	3.90	12.25	0.00	0.00	37.90	0.00	0.00
h	3.50	0.00	0.00	567.0 35.00	0.00	0.00	0.00

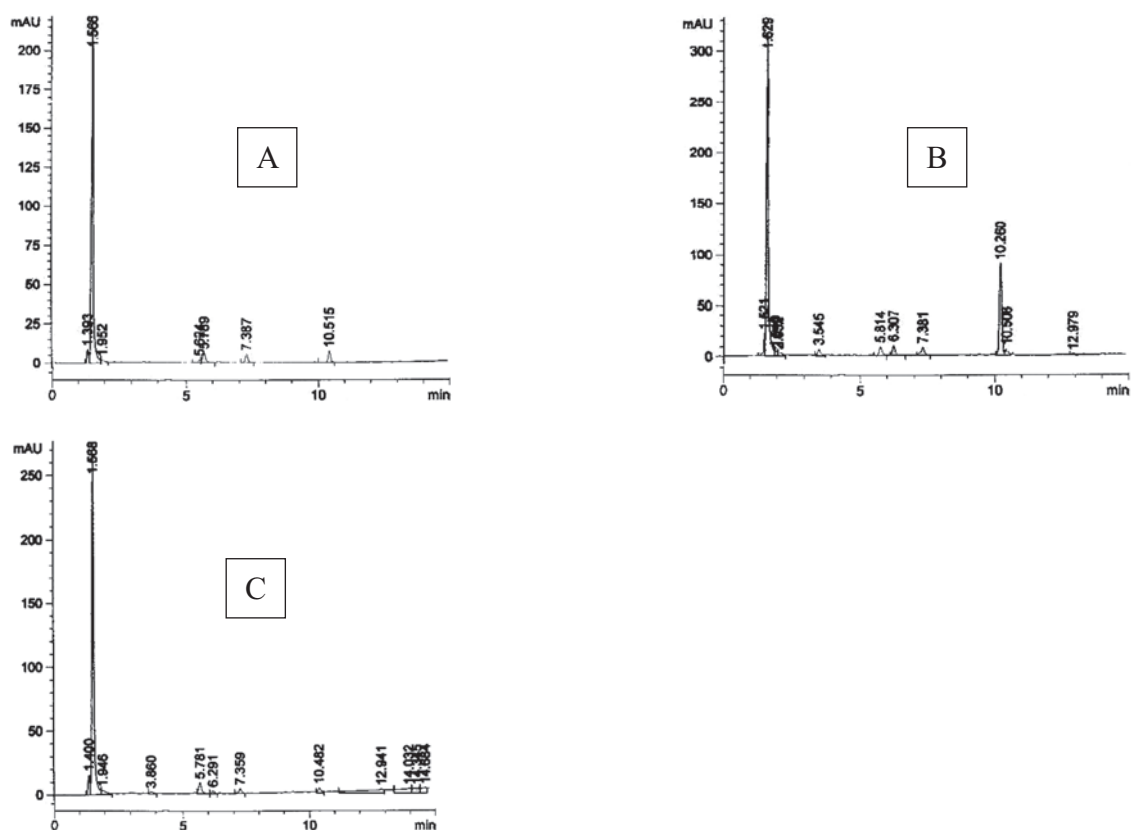


Fig. 2. Fractionation of amino compounds in fresh tomato (A), tomato juice + 2% spices (B) and in ketchup and acetic acid (C) using HPLC.

Table 2. Some physicochemical composition of strawberry products

	pH value	Amines content on fresh weight biogenic amines (mg/100 g) by TLC			Amines content on fresh weight biogenic amines (mg/100 g) by HPLC		
		Histamine	Tyramine	Unknown	Histamine	Tyramine	Unknown
a- Fresh strawberry	3.50	15.13	0.00	74.25	26.10	0.19	0.00
b	4.30	0.00	0.00	8.44	0.00	0.00	0.00
c	3.50	16.88	0.00	0.00	0.00	0.00	0.00
d	4.00	0.00	0.00	0.00	0.00	0.00	0.00
e	3.40	0.00	0.00	0.00	0.00	0.00	0.00
f	2.70	0.00	0.00	0.00	0.00	0.00	0.00
g	3.50	0.00	0.00	0.00	36.40	0.60	0.00
h	3.30	0.00	94.50	0.00	0.00	0.00	0.00
i	2.80	0.00	0.00	247.5 45.02	0.00	0.00	0.00

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to (38.50 mg/100 g fresh matter), while unknown compound was formed with concentration of 567 mg/100 g fresh matter in ketchup product (Heinz).

The measurement of histamine and tyramine using HPLC technique showed relatively more concentration than TLC measurements as shown in Table 1 and Fig. 2. This small increase in concentration may be due to the high accuracy of HPLC determination in comparison with TLC and also due to the loss of solvent in the TLC method. So, the histamine contents were found to be 42.80, 27.90 and 37.90 mg/100 g (Fw) of fresh tomato, tomato juice containing spices and ketchup with spices and vinegar, respectively. Tyramine was found to be 4.20 mg/100 g (Fw) in fresh tomato and disappeared in the other treatments.

Treatment and processing on strawberry

Table 2 shows the effect of treating strawberry with different natural additives. It is obvious from the results that the fresh strawberry samples contain histamine with concentration of 15.13 mg/100 g fresh matter. This con-

centration was increased to 16.88 mg/100 g fresh matter of strawberry immersed in orange juice for 12 hr (over night). It was also found that histamine was disappeared during the following treatments: immersing the strawberry (50 g) in milk (100 ml), yoghurt (100 g), processing to jam, jam containing 1% and 0.3% ascorbic and citric acids, respectively, puree of strawberry containing 1% ascorbic acid and puree of strawberry containing 1% ascorbic acid treated for 30 min at 100°C (Table 2).

Fresh strawberry was found to contain 74.25 mg/100 g fresh matter of unknown amine compound ($R_f = 0.62$) but strawberry puree containing 1% ascorbic acid (heated at 100°C for 15 min) was found to contain tyramine with $R_f = 0.78$ and concentration of 94.50 mg/100 g (Fw). All other treatments of strawberry (Table 2) showed disappearance of tyramine and any other amines that may exist or formed during the treatment. So, milk, orange juice, yoghurt and sterilization (jam) with addition of lime juice and ascorbic acid led to removing the tyramine and any other amine compounds. Strawberry juice (Yahoo) was free from histamine which may be related to the steri-

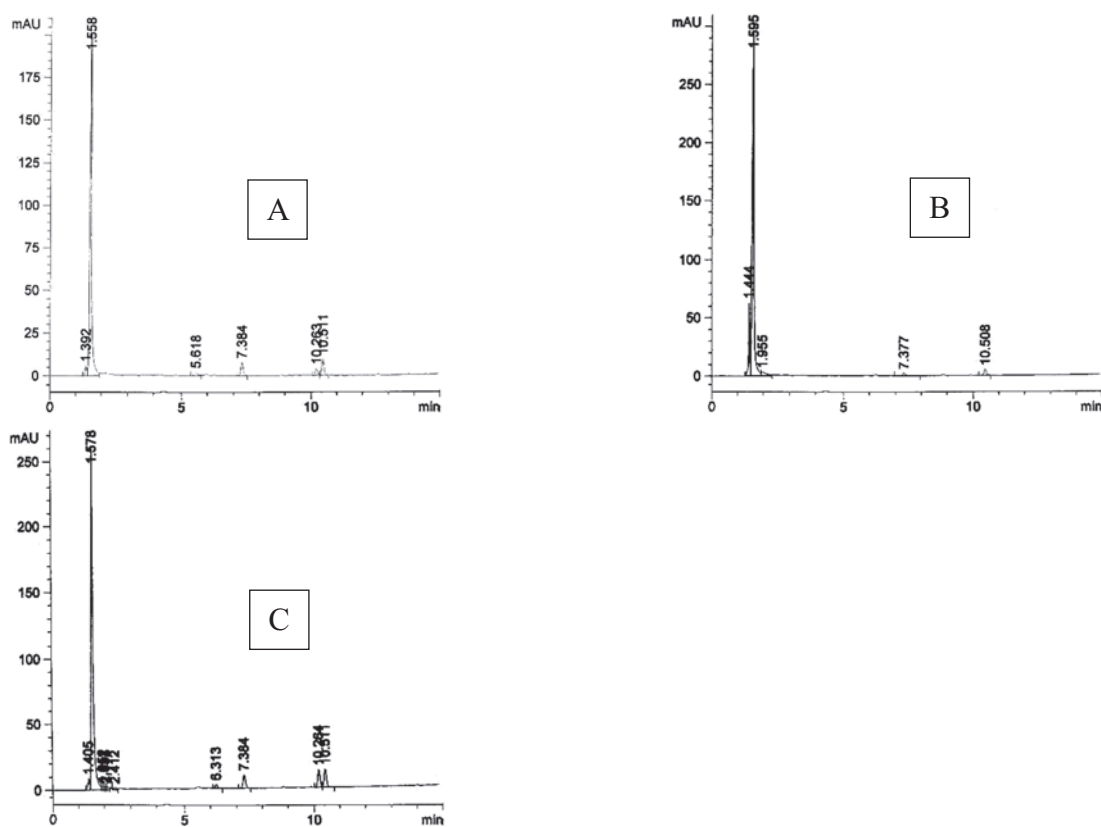


Fig. 3. Fractionation of amino compounds in fresh strawberry (A), strawberry + 100 ml milk (B) and strawberry puree + 1% ascorbic acid (C) using HPLC.

lization operation but contain 247.5 mg/100 g (Fw) of unknown amine compounds.

The HPLC chromatograms of fresh strawberry or those of

strawberry with 100 ml milk or 1% ascorbic acid are given in Figs. 3A-C. It is obvious from Table 2 that the histamine content obtained from HPLC method, of fresh strawberry,

Table 3. Some physicochemical composition of banana products

	pH value	Amines content on fresh weight biogenic amines (mg/100 g) by TLC			Amines content on fresh weight biogenic amines (mg/100 g) by HPLC		
		Histamine	Tyramine	Unknown	Histamine	Tyramine	Unknown
a-Fresh banana	5.20	9.63	0.00	0.00	33.10	0.00	0.00
b	5.60	2.75	0.00	0.00	0.00	0.00	0.00
c	4.70	0.00	0.00	4.13	30.00	0.40	0.00
d	4.40	8.25	0.00	0.00	0.00	0.00	0.00
e	4.40	13.75	0.00	0.00	32.10	0.00	0.00
f	4.30	0.00	0.00	22.00 115.5	0.00	0.00	0.00
g	6.80	0.00	195.0	0.00	0.00	0.00	0.00

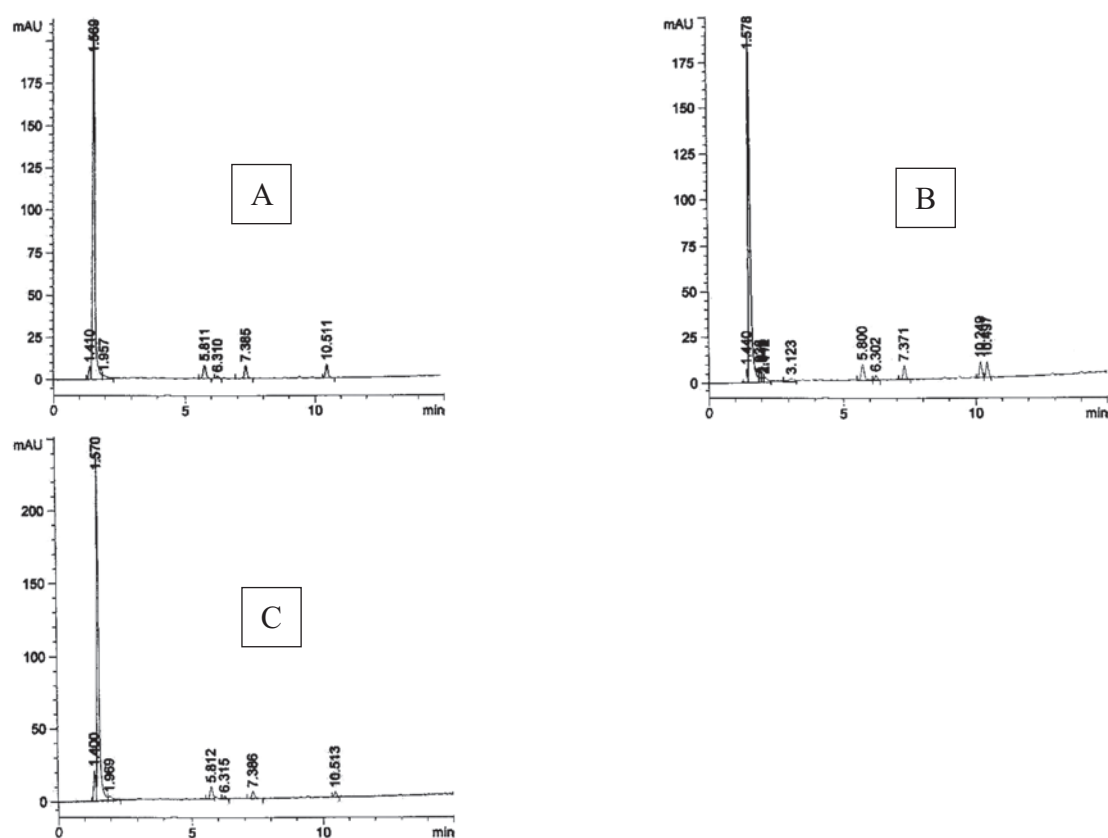
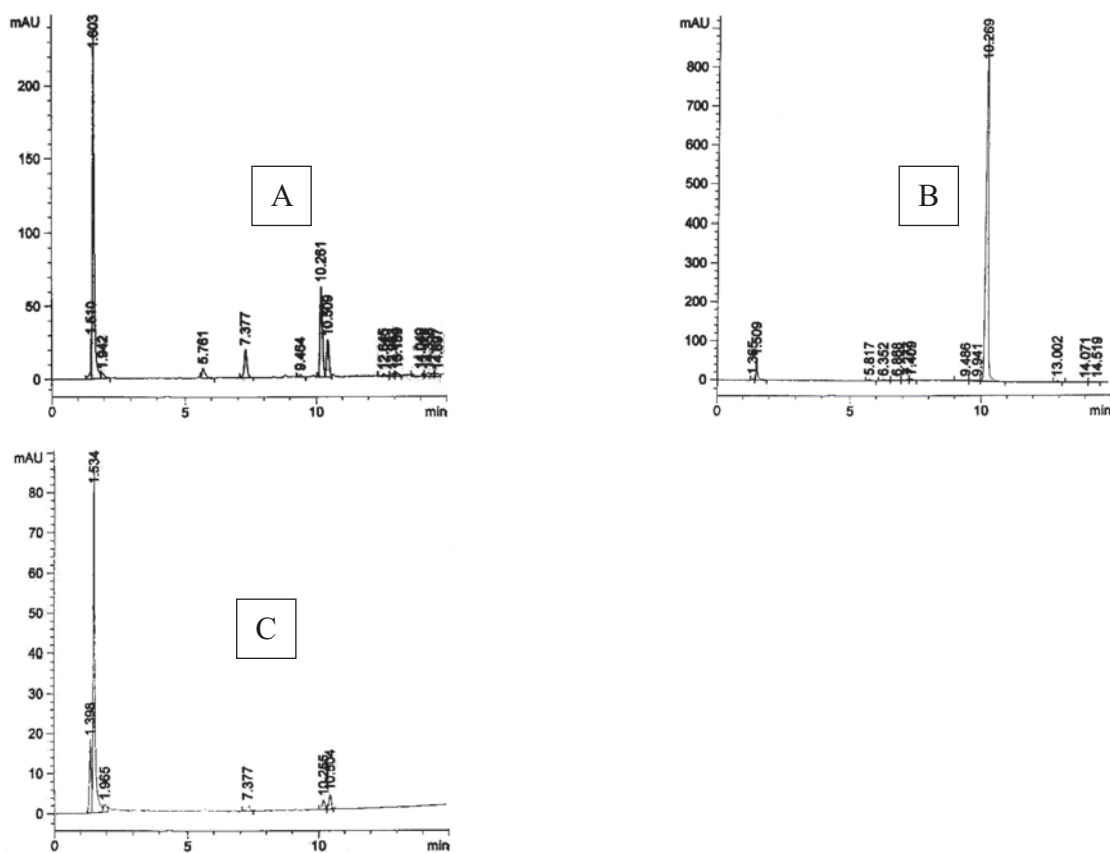


Fig. 4. Fractionation of amino compounds in fresh banana (A), banana soaked in yoghurt overnight (B) and banana puree + 1% ascorbic acid (C) using HPLC.

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Table 4. Some physicochemical composition of chocolate products

	pH value	Amines content on fresh weight biogenic amines (mg/100 g) by TLC			Amines content on fresh weight biogenic amines (mg/100 g) by HPLC		
		Histamine	Tyramine	Unknown	Histamine	Tyramine	Unknown
a- Fresh chocolate	8.10	20.13	15.00	0.00	20.10	2.80	0.00
b	7.60	5.00	75.0	0.00	0.00	0.00	0.00
c	7.80	7.50	40.0	0.00	0.00	0.00	0.00
d	8.40	0.00	0.00	0.00	12.20	81.40	
e	6.70	20.00	0.00	90.00	0.00	0.00	0.00
f	8.20	0.00	90.0	0.00	0.00	0.00	0.00
g	8.10	0.00	0.00	62.50	0.40	0.10	
h	7.00	0.00	285.0	78.75	0.00	0.00	0.00

**Fig. 5.** Fractionation of amino compounds in fresh chocolate (A), chocolate + 5% glucose (B) and chocolate + vanillin + starch (C) using HPLC.

strawberry containing milk and strawberry puree containing ascorbic acid were 26.10, 0.00 and 36.40 mg/100 g (Fw) which indicate that ascorbic acid led to an increase in the histamine content. In addition, the HPLC results listed in Table 2 showed that the tyramine content was 0.19, 0.00 and 0.60, respectively.

Treatment and processing on banana

From Table 3, it was noticed that fresh banana contained 9.63 mg/100 g fresh matter of histamine and by dipping it for 12 hr (over night) in milk, a decrease in histamine concentration to 2.75 mg/100 g (Fw) was observed. This decrease may be related to the decrease in the optical density which resulted from the low pH value of the resulting solution (pH = 4.4). The dipping of banana in orange juice led to an increase in the histamine concentration to 8.25 mg/100 g (Fw). The treatment of banana by immersing it in yoghurt for 12 hr (overnight), addition of 1% ascorbic acid to banana puree and the same treatment as the last addition with heating for 30 min at 100°C

led to disappearing the histamine compounds and formation of unknown compounds with R_f values of 22.00 and 115.5 mg/100 g (Fw).

The banana product (Mix) was found to be free from histamine which may be related to the addition of milk and sterilization operation but contained tyramine with concentration of 195 mg/100 g (Fw). Amines content of banana were also studied using HPLC technique. The results are given in Table 3 and represented graphically in Figs. 4A-C. Fresh banana was found to contain 33.1 mg/100 g (Fw) of histamine. This concentration was changed to 30.0 and 32.10 mg/100 g (Fw) when banana was soaked in yoghurt overnight and banana puree containing ascorbic acid. Fresh banana was found to be free from tyramine while banana soaked with yoghurt overnight has a tyramine content of 0.40 mg/100 g (Fw).

Treatments and processing on chocolate

The results of adding different natural additives on chocolate are listed in Table 4. It was found that the raw

Table 5. Some physicochemical composition of mango products

	pH value	Amines content on fresh weight biogenic amines (mg/100 g) by TLC			Amines content on fresh weight biogenic amines (mg/100 g) by HPLC		
		Histamine	Tyramine	Unknown	Histamine	Tyramine	Unknown
a-Fresh mango	4.30	0.00	0.00	52.88 165.0	0.00	0.00	0.00
b	4.20	0.00	0.00	104.5 16.50	0.00	0.00	0.00
c	4.30	0.00	0.00	2.75 451.0	32.20	0.00	0.00
d	5.40	13.75	0.00	253.0	12.30	0.00	0.00
e	4.40	4.12	0.00	121.0	0.00	0.00	0.00
f	3.50	0.00	0.00	43.13	0.00	0.00	0.00
Treatments under sterilization conditions							
Fresh mango	4.30	0.00	0.00	0.00	0.00	0.00	0.00
a	4.20	10.23	0.00	0.00	29.64	10.18	0.00
b	4.30	0.00	0.00	0.00	0.00	0.00	0.00
c	5.40	0.00	0.00	0.00	0.00	0.00	0.00
d	4.40	0.00	0.00	0.00	0.00	1.49	0.00

Removal of biogenic amines using inorganic and natural additives

chocolate contained 20.13 mg/100 g (Fw) of histamine. By adding milk powder to chocolate in two ratios (1:1) and (1:2) led to lowering the concentration of histamine to 5.00 and 7.50 mg/100 g (Fw). The addition of glucose (5%) to chocolate led to disappearing of histamine content. Meanwhile, the addition of ascorbic acid (1%) to chocolate showed a low decrease in histamine concentration. In a similar manner, vanillin (5%) and starch led to disappearing of histamine content in chocolate (Table 4).

The tyramine content in raw chocolate was 15.0 mg/100 g (Fw) which increased by mixing with milk in the ratio (1:1) and (1:2) to 75 and 40 mg/100 g (Fw), respectively. The mixing of chocolate with glucose and ascorbic acid led to disappearing of tyramine compound but the use of vanillin did not have any effect on tyramine content of chocolate. The addition of starch to chocolate led to disappearing of tyramine and formation of other compound with $R_f = 0.86$.

Chocolate (Mix) did not contain histamine compound,

but another compound was formed at $R_f = 0.23$ with concentration of 78.75 mg/100 g (Fw) but it has tyramine with concentration of 285.0 mg/100 g (Fw). By using HPLC for measuring amines contents in chocolate products (Figs. 5A-C), it was found that fresh chocolate contained 20.10 mg/100 g (Fw) histamine and 2.80 mg/100 g (Fw) of tyramine. Upon treating chocolate with glucose, a decrease in histamine and an increase in tyramine concentrations to 12.20 and 81.40 mg/100 g (Fw), respectively, were observed. But the chocolate treated by vanillin and starch showed a clear decrease in histamine and tyramine to 0.40 and 0.10 mg/100 g (Fw), respectively.

Treatments and processing on mango

It is obvious from the data listed in Table 5 that fresh mango fruit did not contain histamine or tyramine compounds.

The treatment of mango pulp by starch (1%) and milk powder (10%) showed disappearance of histamine and

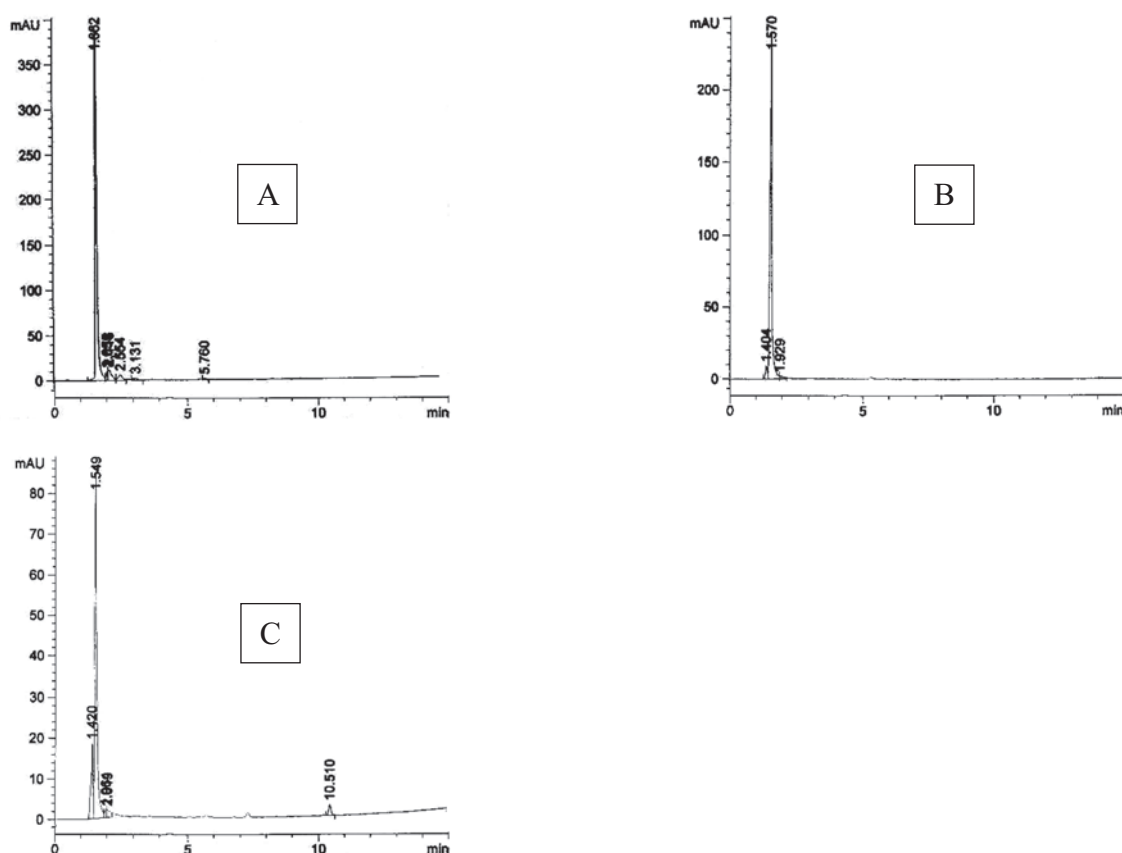


Fig. 6. Fractionation of amino compounds in fresh mango (A), mango + 1% starch (B) and mango + 10 % milk (C) using HPLC.

tyramine and formation of other compounds as given in Table 5. The raw mango and mango treated with ascorbic acid and glucose were found to be free from tyramine. Starch addition to mango pulp and addition of milk (10%) led to disappearing of tyramine and formation of unknown amine with different R_f values.

The sterilized mango pulp and its treatments did not show any amine content which may be related to the maillard reaction between the amine and sugar. Mango juice (Faragellow) was found to be free from histamine or tyramine which may be related to sterilization operation and another compound found at $R_f = 0.24$.

By measuring the histamine and tyramine of fresh mango and its products using HPLC technique, it was found that fresh mango did not contain histamine or tyramine as given in Table 5 and Figs. 6A-C and 7A-C. The same results are obtained on treating mango with starch. The mango treated with milk showed histamine with concentration of 12.30 mg/100 g (Fw) and was free from tyramine.

In conclusion, from the previous results obtained, the

following conclusions are coming out to the light:

- * It is recommended to treat concentrated tomato puree with glucose at 100°C for 15 min and spices during processing as they have great effect on decreasing histamine and tyramine content in tomato.
- * Treating strawberry with milk, yoghurt, lime juice, ascorbic and citric acids are the best treatments which led to disappearance of histamine and tyramine compounds.
- * Using ascorbic acid at 100°C for 30 min is better than 30°C for 15 min during processing with strawberry.
- * Milk, yoghurt, orange juice and ascorbic acid at both 30 and 100°C are recommended to be used with banana as they are considered as a good eliminator for histamine and tyramine.
- * Treating chocolate with milk, glucose and ascorbic acid is not recommended to be used as they increase both of histamine and tyramine contents, while vanillin and starch gave very good results.
- * Milk and glucose are not recommended to be used

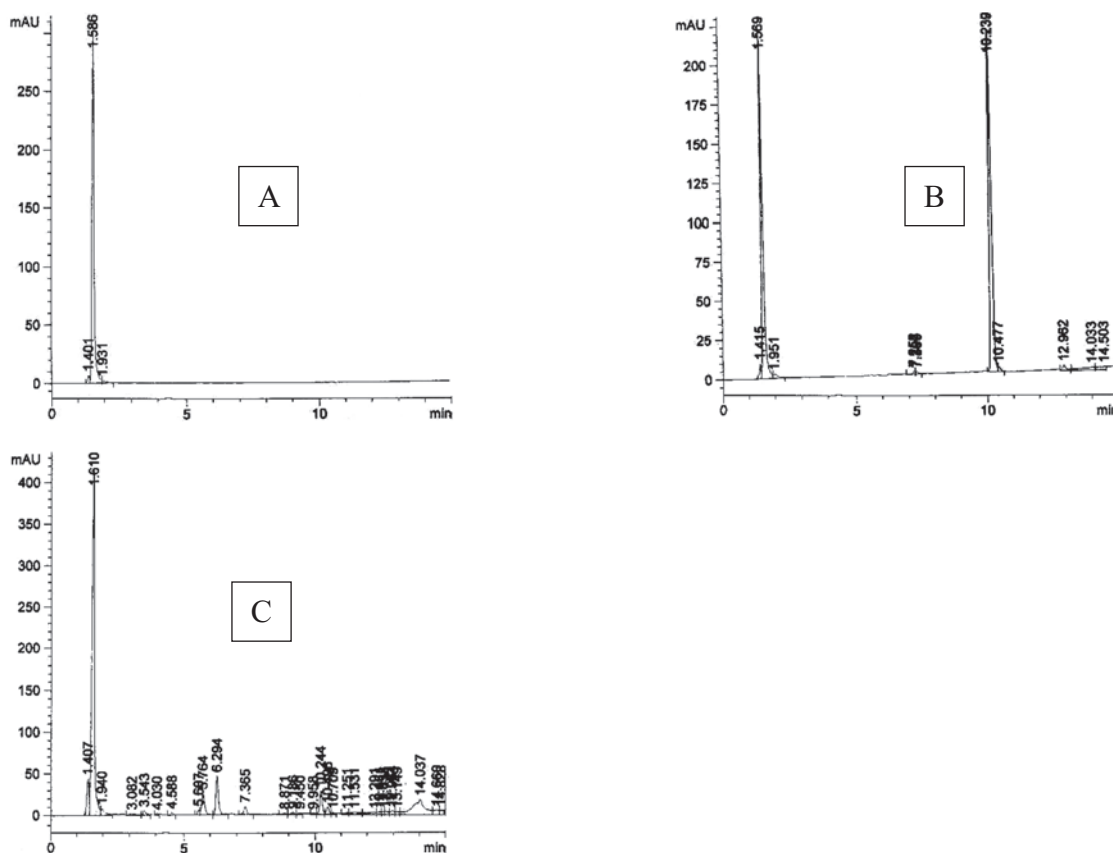


Fig. 7. Fractionation of amino compounds fresh sterilized mango (A), mango + 1% ascorbic acid under sterilized conditions (B) and mango + glucose under sterilized condition (C) using HPLC.

Removal of biogenic amines using inorganic and natural additives

during treating of mango while ascorbic acid and starch are considered as the best additives during processing operation.

- * It is recommended to sterilize food during process as it has a great effect on decreasing histamine and tyramine concentrations.

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