

Detection of Some Biogenic Amines content in Thai Sauces

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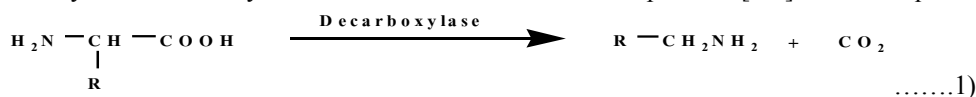
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Abstract: There are many toxic substances present in food, biogenic amine compounds are the one in those toxic substances. An important biogenic amines found in food are histamine, putrescine, cadaverine and phenylethylamine. The aim of this work was to screening test the histamine, cadaverine and putrescine in seasoning sauces made from fish, soybean, oyster and mushroom that sold in Thailand by TLC method. Then, the present biogenic amine was analysed its content by Spectrofluorometry technique. The result from TLC screening test showed only histamine in 32 sample sauce products. Soy sauce products contained highest histamine content approximately 17.76-25.33 mg/kg, the mushroom vegetarian sauce contained the lowest histamine approximately 8.02- 8.15mg/kg. The result from monitoring toxic substances as biogenic amine found only histamine in all sauces that sold in Thailand, which its content not exceed the recommendation values.

Keywords: Biogenic amines, histamine, putrescine, cadaverine, Thai sauces

I. Introduction

Biogenic amines (BAs) are nitrogenous low molecular weight organic compounds, which have been derived from enzymatic decarboxylation of some free amino acids and proteins [1-3] as in the equation 1)



The Biogenic amines can be classified into 3 groups according to their structures such as aromatic amines, aliphatic diamines and heterocyclic amine [4]. These biogenic amines involve in neurotransmitters system such as acetylcholine, catecholamines, and serotonin[5]. The formation of biogenic amines in food relate to the decarboxylation reaction of amino acids by microorganism[6]. The important biogenic amines that found in food are histamine, tyramine, putrescine, cadaverine and phenylethylamine. In this work, histamine, cadaverine and putrescine are scoped. Histamine has the IUPAC name as 2-(1*H*-imidazol-4-yl)ethanamine and its structural formular shows as in Fig.1

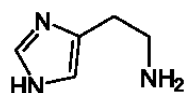
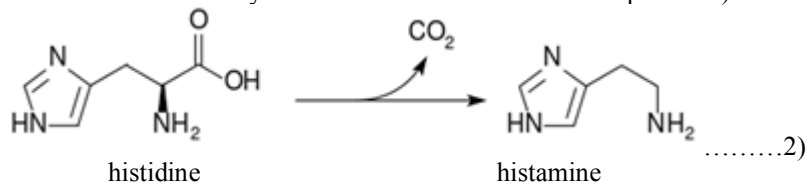


Figure 1. The structural formular of histamine [7]

Histamine are products from the decarboxylation of histidine as showed in equation 2).



It has been implicated as the causative agent in outbreaks of food poisoning where intoxication results from the ingestion of foods containing excessive amounts of histamine[8].

Putrescine is also one of the most common biogenic amine. Its IUPAC name is butane-1,4-diamine as in Fig.2

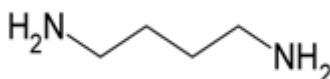
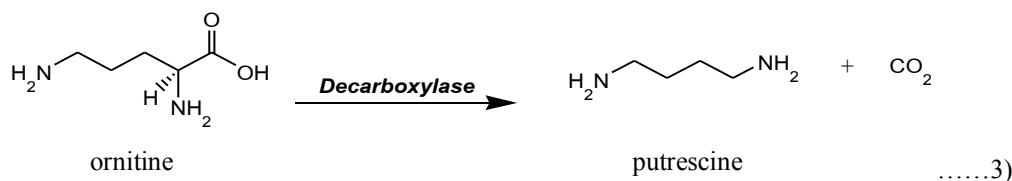


Figure 2. The structural formular of putrescine [9]

Putrescine also forms by the decarboxylation of ornitine as equation 3).

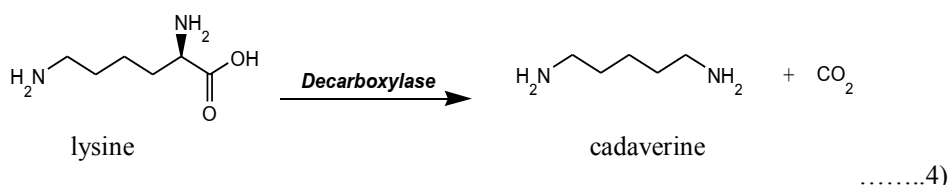


Putrescine is also found in fermented products. Lactic acid bacteria especially lactobacilli and staphylococci able to produce putrescine and cadaverine[10- 13].

Cadaverine is the last one that also be the popular biogenic amine compound, it has an IUPAC name as Pentane-1,5-diamine[14] as in fig.3. It was changed from the decarboxylation of lysine amino acid as in equation 4)



Figure 3. The structural formular of cadaverine[14]



Both putrescine and cadaverine can be formed during the food storage[15-18].The biogenic amines in food effect on the health of the consumers. Some consumer suffered from allergic reactions and showed many symptoms such as difficulty in breathing, itching, rash skin, vomiting, fever and hypertension[5]. There are many food reports referred the high levels of biogenic amines in some food such as fish, fish products and fermented foods[5],[19-21]

Seasoning sauces such as fish sauces , soy sauces and oyster sauces are very important for Thai cuisine. There are many works referred about the biogenic amines in fish sauces not only in Thailand but also in other country [22].Especially ,histamine was controlled in fish sauce at the level of 40 mg/100g fish sauce[23]. The other amine such as tyramine was also found in moromi during soy sauce fermentation process[24]. The latest research work report that tyramine and histamine could be detected in Japanese soy sauce in trace amount level[25].However, there was a few report literature about the above three biogenic amines in other type of seasoning sauces such as oyster sauces, mushroom sauces. The objective of this work was to analyse the histamine ,cadaverine and putrescein in seasoning sauces which made of fish , soybean, oyster and mushroom that sold in Thailand market.The qualitative analysis was preliminary performed to screen biogenic amines by Thin layer Chromatography.Then the content of biogenic amines were analysed by Spectrofluorometric technique.

II. Materials And Method

2.1.Materials

2.1.1 Chemicals

Histamine and putrescine standard were AR grade that purchased from Fluka.Cadaverine standard (AR grade) was purchased from Sigma Aldrich. Methanol(HPLC grade), acetonitrile (HPLC grade) and chloroform (AR grade) were purchased from Merck.Ammonia solution (AR grade), acetic acid (AR grade) and trichloroacetic acid (AR grade) were purchased from Sigma Aldrich. Ninhydrin and KI/I₂ (AR grade) was bought from Merck. *o*-Phthaldialdehyde(OPA)(AR grade) was purchased from Acros. Mercaptoethanol(AR grade) was purchased from Merck.

TLC plate was ready coated silica plate (Silica gel: DC-Fertigfolien ALUGERAM® SIL G/UV254) size 2 x 8 cm.). Microcapillary tube was VERTICAL® Drummond microcap 5.0 µL, 100/PK.

2.1.2 Samples

The sauces samples such as true fish sauces (5 samples), mixed fish sauces(5 samples),soy sauce (5 samples),sweet soy sauces(4 samples), black soy sauces(4 samples),oyster sauces(6 samples) and mushroom vegetarian sauces(3 samples), all above sample sauces were purchased from Big C supermarket and Lotus supermarket in Bangkok,Thailand.

2.2 Experimental method

2.2.1 Qualitative Analysis Study

2.2.1.1 Study on the optimization Thin Layer Chromatography systems.

The standard solution of histamine, cadaverine and putrescine were prepared 1 ppm and spotted on ready coated silica plate using microcapillary. The spotted plate was developed chromatogram in 2 systems as system 1 was methanol : NH₃ : CHCl₃ and system 2 was methanol : NH₃.

The system 1 was prepared as 4 subclass systems by variation of each component solvent for 4 systems as the following

- S1.1 methanol : NH₃ : CHCl₃ = 50 : 40 : 10 v/v,
- S1.2 methanol : NH₃ : CHCl₃ = 55 : 35 : 10 v/v
- S1.3 methanol : NH₃ : CHCl₃ = 60 : 30 : 10 v/v
- S1.4 methanol : NH₃ : CHCl₃ = 60 : 35 : 5 v/v.

The system 2 (methanol : ammonia) was also prepared as 8 subclass systems by variation of each component solvent for 8 systems as the following

- S2.1 methanol : NH₃ = 95 : 5 v/v
- S2.2 methanol : NH₃ = 90 : 10 v/v
- S2.3 methanol : NH₃ = 85 : 15 v/v,
- S2.4 methanol : NH₃ = 80 : 20 v/v
- S2.5 methanol : NH₃ = 75 : 25 v/v,
- S2.6 methanol : NH₃ = 70 : 30 v/v
- S2.7 methanol : NH₃ = 50 : 50 v/v
- S2.8 methanol : NH₃ = 5 : 95 v/v.

All of the spots on the chromatogram were detected by 2 methods such as incubated in the iodine vapor tank and spraying with ninhydrin reagent.

2.2.1.2 Preparation of Analyte Samples

All seasoning sauce samples such as true fish sauces, mixed fish sauces, soy sauces, sweet soy sauces, black soy sauces, oyster sauces and mushroom vegetarian sauces were weighed each 3.00 g and dissolved in 2.5 ml of 10 % TCA and 2.5 ml of 1 M CH₃COOH. The sample solutions were mixed together with vortex mixer and ultrasonic treatment for 5 minutes and filtered through filtered paper (Whatman paper no.1.) The sample filtrates were stored at 4 °C, until analysis as in part 2.2.2

2.2.2 Quantitative Analysis by Spectrofluorometry

The extracted filtrate sauces samples from 2.2.1.2 were analysed by measurement the fluorescence intensity using method modified from AOAC [26]. The 5 µl of 8.0x10⁻⁵ M standard histamine, cadaverine and putrescine were reacted with 5 µl of 8.0x10⁻⁵ M *o*-phthalaldehyde in NaOH, 1 ml of methanol and 5 µl of 2-mercaptoethanol. Those reaction tubes were placed in the dark cabin laboratory locker for 3 minutes. The fluorescent intensity of the derivative samples were scanned and measured at emission wavelength of each amine standard by Spectrofluorometer (Jasco FP6200). The biogenic amine contents in all samples were calculated by comparison with standard calibration curves.

III. Results And Discussion

The results from part 2.1.1 that studied in the 2 developing solvent systems (system 1 and system 2) showed that in the system 1 which was prepared 4 subclass systems by variation of each solvent ; methanol : NH₃ : CHCl₃ as 50:40:10 v/v, 55:35:10 v/v, 60:30:10 v/v and 60:35:5 v/v and the system 2 (methanol : ammonia) was also prepared as 8 subclass systems by variation of each solvent ; methanol : NH₃ as 95:5 v/v, 90:10 v/v, 85:15 v/v, 80:20 v/v, 75:25 v/v, 70:30 v/v, 50:50 v/v and 5:95 v/v showed that the best system for optimization TLC analysis method was system 2.5 methanol : ammonia with the ratio of 75:25 v/v as in Fig. 4.

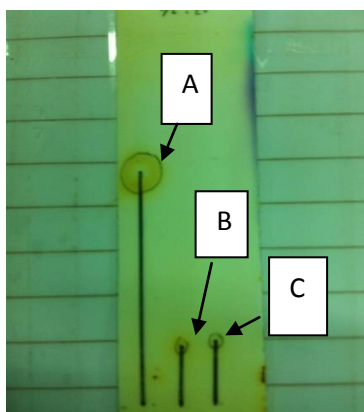


Figure 4. Chromatogram of standard biogenic amines

Note: a represent histamine standard spot ; b represent putrescein standard spot and c represent cadaverine standard spot

The spot on TLC chromatogram could be detected by 2 methods as iodine vapor and 1.5% ninhydrin in ethanol solution. However, the iodine vapor could easily prepared and lower cost than ninhydrin solution, so this work chose the iodine vapor for spot detection. The optimization method was applied to analyse the three biogenic amines in all those sauces as showed the chromatogram from some sauce sample as in Fig. 5.

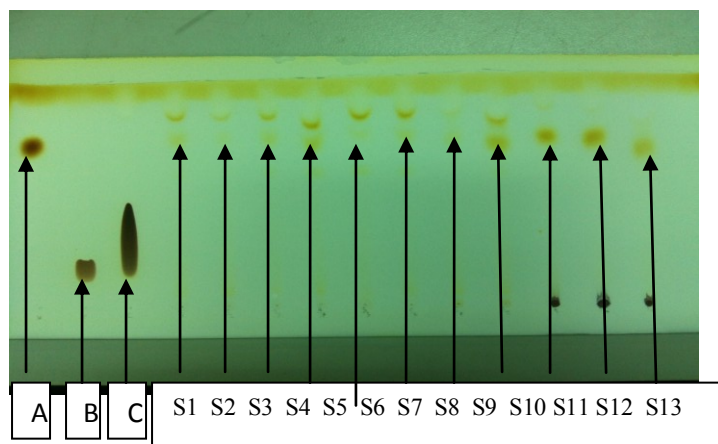


Figure 5 Example Chromatogram of sample compare with standard biogenic amines

Note: a represent histamine standard spot ; b represent putrescein standard spot and c represent cadaverine standard spot, lane S1-13 represent sample spot.

From the qualitative TLC chromatogram showed that all sample sauces contain only histamine as a predominant biogenic amine since the Rf value of sample nearest 0.7 which relate to Rf value of histamine standard. This chromatogram could not detected the other 2 biogenic amines of putrescine and cadaverine, since both amine may be detected in storage product as referred by Beneduce et al[27].Bover-Cid et al. [28,] The sample sauce in this experiment was freshly prepared from the factories as the manufacturing date.

After the qualitative screening TLC test on sauce samples, those samples were analysed only histamine content by compare with the standard histamine calibration curve.The result from scanning the histamine standard with Spectrofluorometer showed in Fig.6.

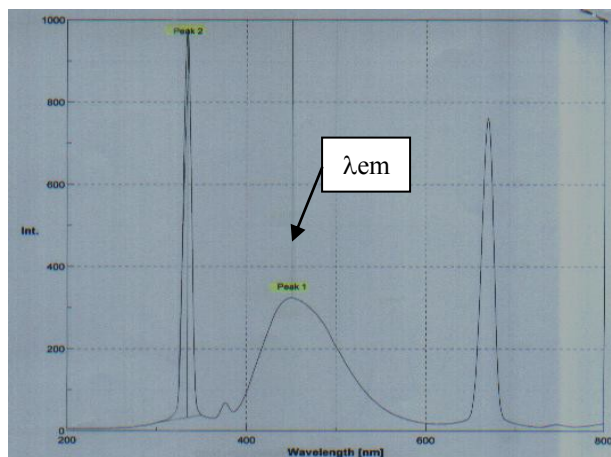


Figure 6. Spectrum of histamine standard solution with emission spectrum at 452 nm

The fluorescence intensities of the histamine standard solutions were recorded and created a standard calibration curve that plotted between the fluorescence intensity (I_f) and the concentration of histamine standard. The standard calibration curve showed the linearity range between 1.6×10^{-5} - 12.8×10^{-5} M with correlation coefficient of ± 0.993 . The limit of determination (LOD) and limit of quantitation (LOQ) were 1.30×10^{-5} and 2.52×10^{-5} M, respectively. The relative standard deviation (RSD) was less than 10.0%. So this method showed good results in terms of linearity, accuracy, precision, limits of detection, and limit of quantitation.

The extracted sample from all sauce samples were measured and recorded the fluorescence intensity of emission peak at emission wavelength of 452 nm and calculated the histamine content in those sauces as in Fig. 7.

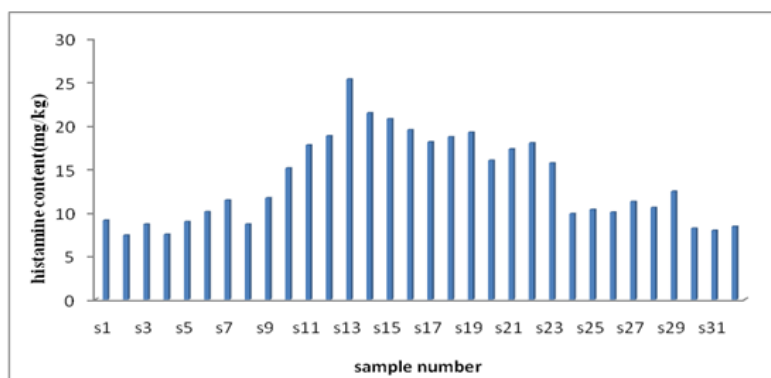


Figure 7. Histamine content (mg/kg) in Thai sauces samples

- Note:
- s1-s5 represent as mixed fish sauces
 - s6-s10 represent as pure fish sauces
 - s11-s15 represent as soy sauces
 - s16-s19 represent as sweet soy sauces
 - s20-s24 represent as black soy sauces
 - s25-s29 represent as oyster sauces
 - s30-s32 represent as mushroom vegetarian sauces

From those results presented that soy sauces and sweet soy sauces contain highest histamine contents approximately 17.76-25.33 mg/kg, but fish sauce samples group contained histamine approximately 7.50 -15.11 mg/kg. This may depend on the difference fermentation process and type of microorganisms that effected the histamine content as discussed by Stratton et al.[29-31]. This work supported the report from [25], that refer the histamine content in Japanese soy sauce in trace amount level. The oyster sauces showed histamine content in the same level as in fish sauces samples group. Oyster is also sea food which was referred to contain biogenic amine too [32]. The mushroom vegetarian sauce contained the lowest histamine content approximately 8.02 - 8.11 mg/kg. It is very interesting that this sauces which was produced from shitake mushroom contained low histamine content. However, there also had the report about the histamine in mushroom [5]. Then histamine was also be detected in low level in mushroom sauces too.

IV. Conclusion

This work showed that after the qualitative screening TLC test in this work was the rapid screening method to rapid detect the toxic three types of biogenic amine in Thai sauce products by using the mobile phase system as methanol : ammonia at the ratio 75 :25 and using iodine vapour for detection spot on chromatogram. The screening time for TLC test not exceed than 10 minute. The result from the screening TLC in all sauces showed that the histamine was the one biogenic amine that be detected in this experiment. After the optimization method of analysis histamine by spectrofluorometry technique, it revealed that Thai sauce products showed the histamine content in the range of 8.02-25.33 mg/kg. This is very low content. The allowed content of histamine in general fish sauce not exceed than 400 mg/kg[23]. This work also proved that Thai sauces that sold in Thailand contained the low level of biogenic amines as histamine. The consumer that use freshly Thai sauce which sold in Thailand will be safe. However, if that sauces were stored for a long time the content of histamine may increase as referred by M.E.Arena ,et al.[16].

Acknowledgments

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