Hittite Journal of Science and Engineering, 2017, 4 (1) 79-83
ISSN NUMBER: 2149-2123
DOI: 10.17350/HJSE19030000052

Optimization of the Schiff-Base Reaction of Acetylacetone with Biogenic Amines

Bediha Akmese[1]* and Adem Asan[2]
[1] Hitit University, Department of Chemistry, Corum, TURKEY
[2] Ondokuzmayis University, Department of Chemistry, Samsun, TURKEY

ABSTRACT

In this work, optimum conditions for the derivatization reaction of biogenic amines (histamine, tyramine, putrecine, tryptamine, phenylethylamine, cadaverine, spermidine and spermine) with acetylacetone have been determined. In this reaction, the amount of K2HPO4, reaction time, reagent amount, solvent choice and solvent amount were optimized. As a result of this study, optimum conditions were determined as amount of K2HPO4 2 g, reaction time 20 min, amount of acetylacetone 1 mL, solvent methanol and amount of solvent 10 mL.

Article History:
Received: 2017/02/29
Accepted: 2017/04/10
Online: 2017/06/30

***Correspondence to**: Faruk Maraşlıoğlu,*
Hitit University, Department of Chemistry,
Faculty of Arts and Science,
Corum, Turkey
Tel: +90 (364) 223-8000-3376
Fax: +90 (364) 223-8004
E-Mail: bedihaakmese@hitit.edu.tr

Keywords:
Biogenic amines; Acetylacetone; Derivatization; Schiff Base

INTRODUCTION

Amines are basic nitrogenous compounds formed by substituting alkyl or aryl groups of the one, two or three hydrogen atom in ammonia. Decarboxylation of aminoacids is the most common synthesis route of foods; aromatic amines may exhibit food toxicity [1]. These amines by decarboxylation of amino acids of living organisms (bacteria) when the operating results produced are called biogenic [2]. In the presence of bacterial biogenic amine decarboxylase and suitable environmental conditions, biogenic amine formation permits bacterial growth and production of decarboxylase enzymes [3]. Biogenic amines are produced as a result of various metabolic activities of plants, animals and microorganisms. Biogenic amines containing aliphatic (putrecine, cadaverine, spermine, spermidine), aromatic (tyramine, phenylethylamine) and especially heterocyclic (histamine, tryptamine) structures are described as small molecule toxic compounds which can also be present in foods.

Biogenic amines are formed in large quantities of protein rich foods and fermented foods [4-5]. The formation of biogenic amines;

- The presence of free amino acids,
- The presence of microorganisms showing decarboxylase high enzyme activity in the medium and their number,
- Development of microorganisms
- The formation of decarboxylases depends on the presence of suitable environmental conditions such as pH and temperature [1].

It is very important to analyze and identify biogenic amines present in various foods due to their potential toxicity. Absorption intensities of the biogenic amines in the UV-Vis region are very low or not at all. Therefore, their absorption strengths need to be increased. These substances must be derivatized using an organic chelator and thus increasing the absorption intensities in the UV-Vis spectrometer becomes possible by making the resultant indirect determinations. Various derivatization reagents have been used in literature for the determination of these substances [6-22]. In this study, the use of acetylacetone reagent, previously used by Nishikawa [23] in the derivatization of primary amines, was investigated for the derivatization of

Table 1. Classification of biogenic amines

According to chemical structures	*Aliphatic*	*Aromatic*	*Heterocyclic*
	Putrecine Cadaverine Spermine Spermidine	*Tyramine Phenylethylamine*	*Histamine Tryptamine*
According to number of nitrogen	*Monoamines*	*Diamines*	*Polyamines*
	Tyramine Phenylethylamine	*Histamine Putrecine Cadaverine*	*Spermine Spermidine*

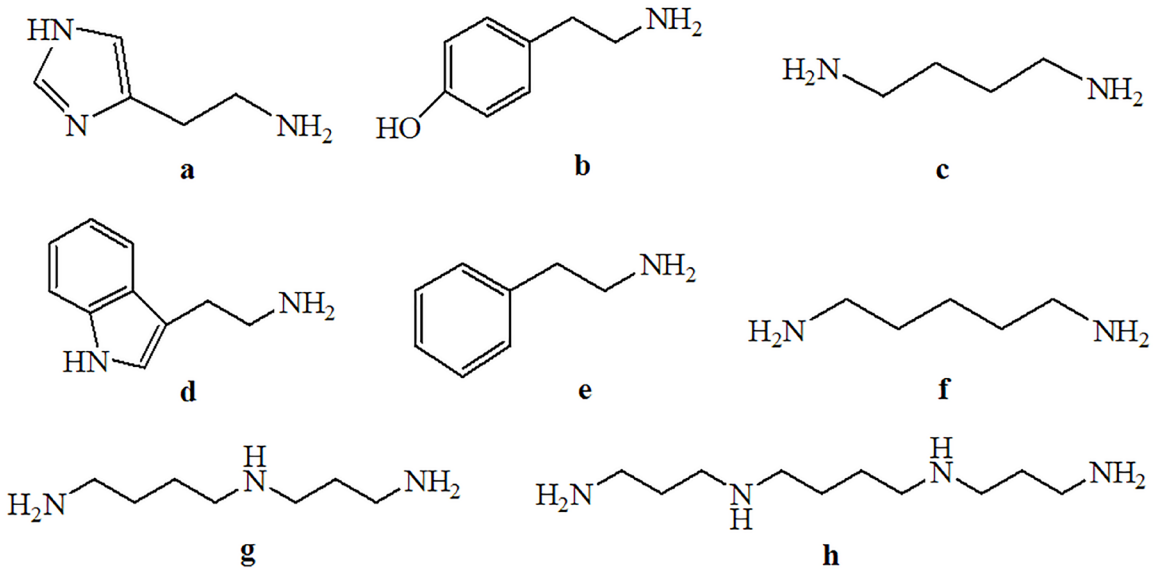

Figure 1. The chemical structure of biogenic amines (**a**: histamine, **b**: tyramine, **c**: putrecine, **d**: tryptamine, **e**: phenylethylamine, **f**: cadaverine, **g**: spermidine, **h**: spermine)

biogenic amines. The generic name for the derivatization reaction of biogenic amines with acetyl acetone is known as Schiff base formation reactions. In this study, optimum conditions for the derivatization reaction of the biogenic amines with acetylacetone have been determined.

The schiff bases can be represented by the general formula RCH=NR', which is obtained from the condensation of aldehydes or ketones with primary amines and also referred to as "imine" or "azomethine" compounds due to its C=N double bond as a characteristic feature in its structure [24]. R and R' is alkyl or aryl substituents. Schiff bases are also known as a good nitrogen donor ligand (>C=N-).

Figure 2. The formation reaction of Schiff base (R-R'-Alkyl or Aryl)

MATERIALS AND METHODS

Chemical and Reagents

Biogenic amine standards (histamine, tyramine, putrecine, tryptamine, phenylethylamine, cadaverine, spermidine, spermine), were obtained from Sigma–Aldrich. Acetylacetone was supplied by Merck. Dipotassium hydrogen phosphate (K_2HPO_4) was purchased from Merck. Acetone, acetonitrile, ethanol, methanol and tetrahydrofuran were obtained from Sigma-Aldrich (HPLC-grade).

Apparatus

The UV–Vis absorbance spectra were recorded at using a GENESYS™ 10S Thermo Scientific Perkin Elmer spectrophotometer, equipped with a 1 cm path length cell, controlled by a personal computer. This equipment has a degasser system (WiseClean). Mettler Toledo MA 235 pH/ion analyzer with Hanna HI 1332 Ag/AgCl combined glass electrode was used for pH measurements.

Preparation of standard solutions of biogenic amines

Stock solutions of histamine, tyramine, putrecine, tryptamine, phenylethylamine, cadaverine, spermidine, spermine were prepared by dissolving each biogenic amine in 10 % (v/v) methanol/water. The diluted solutions were taken from this stock solution and diluted to the desired concentration in the same mixture. Stock solutions were kept +4 ^{0}C and stored in the dark. Water-methanol mixture was used for the further dilutions of the solutions of biogenic amines.

Derivatization Process

The final concentration of each biogenic amine solution was taken from stock solution as 1x 10^{-4} M. To this, methanol, K_2HPO_4 and derivatization reagent acetylacetone were added in a certain amount and volume 100 mL completed. The mixture was allowed to stand in the dark and then their UV-Vis spectra were taken against the blank solution. Biogenic amines, the effect of all parameters of the reaction to optimize the derivatization reaction which is converted into schiff base using acetylacetone reagent were examined individually. For this purpose, parameters such as K_2HPO_4 amount, reaction time, acetylacetone amount, solvent effect and solvent amounts were investigated and optimum conditions were obtained for each.

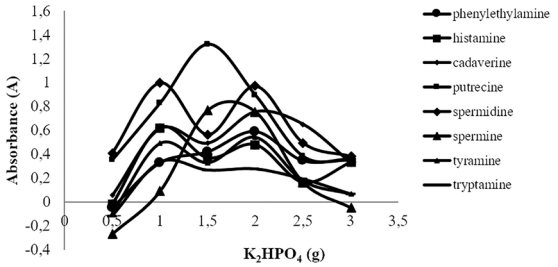

Figure 3. The effect of the amount of K_2HPO_4 on the derivatization reaction of biogenic amines with acetylacetone

RESULTS AND DISCUSSION

Effect of the Amount K_2HPO_4

The reaction of biogenic amines with acetylacetone takes place in mildly basic medium. For this purpose, K_2HPO_4 was added in different amounts and the changes in absorption were evaluated according to spectrophotometric measurement results. For this, an appropriate amount of each biogenic amine was added to a solution containing 10 mL methanol, different amounts of K_2HPO_4 (0.5; 1.0; 1.5; 2.0; 2.5 and 3.0 g) and 1 mL of acetylacetone. The mixture was then diluted to 100 mL with ultrapure water. The mixture was kept in dark for 40 min to complete the reaction. The absorption changes of different amounts of K_2HPO_4 obtained on the spectra are as shown in Figure3.

The amount of K_2HPO_4 from which the highest absorptions were obtained was determined to be 1 g and 1 g K_2HPO_4 was used for all derivatization reaction with acetylacetone of biogenic amines throughout the study.

Effect of Time

To determine the extent to which the reaction of the derivatives of the biogenic amines with acetylacetone reactives was carried out, the solutions in which the derivatives were obtained at certain time intervals were left in the dark and the UV-Vis spectra were taken. Reaction time was optimized taking into account the absorption intensities in the spectra. An appropriate amount of each biogenic amine was added to a solution containing 10 mL methanol, 1 g K_2HPO_4 and 1 mL of acetylacetone. The mixture was then diluted to 100 mL with ultrapure water. Each solution was kept in the dark at 10, 20, 30, 40, 50 and 60 min intervals and UV-Vis spectrum were taken. The absorption changes obtained at different times in the spectrum are the same as in Figure 4.

The reaction time at which the highest absorptions were obtained was determined as 20 min. During the study, the solutions for the derivatization reaction of biogenic amines with acetylacetone were left in the dark for 20 min.

Effect of the Amount Acetylacetone

To optimize the amount of acetylacetonate used as the reagent for the derivatization of biogenic amines, the optimum amount was determined by adding acetylacetone medium at various ratios. An appropriate amount of each biogenic amine was added to a solution containing 10 mL methanol, 1 g K_2HPO_4 and different amounts of acetylacetone (0.6; 0.8; 1.0; 1.2 and 1.4 mL). The mixture was then diluted to 100 mL with ultrapure water. The mixture was kept in dark for 20 min to complete the reaction. The absorption changes of different amounts of acetylacetone obtained on the spectra are as shown in Figure 5.

The amount of acetylacetone in which the highest absorption intensity was obtained was determined to be 1 mL and this value was used for the derivatization reaction throughout the study.

Effect of Different Solvents

The optimal solvent was determined using various solvents to reveal the solvent to be used in the derivatization reaction of the biogenic amines and the effect on the reaction. For this purpose, different solvent containing derivatization reactions were performed for each compound. An appropriate amount of each biogenic amine was added to a solution containing 10 mL solvent (methanol, ethanol, acetone, acetonitrile and tetrahydrofuran) 1 g K_2HPO_4 and 1 mL of acetylacetone. The mixture was then diluted to 100 mL with ultrapure water. The mixture was kept in dark for 20 min to complete the reaction. The absorption changes of

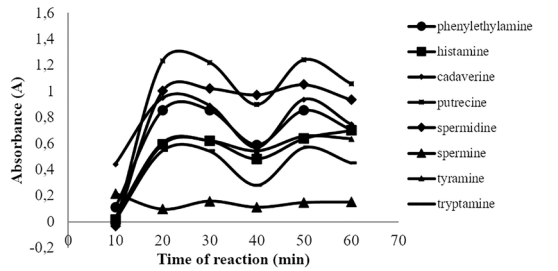

Figure 4. The effect of time on the derivatization reaction of biogenic amines with acetylacetone

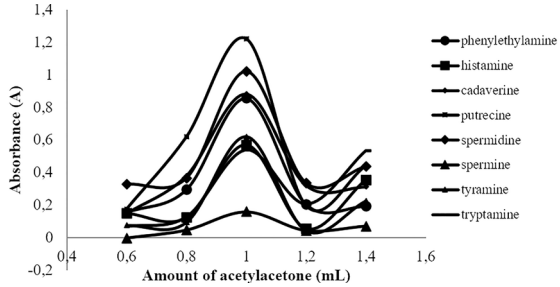

Figure 5. The effect of the amount of acetylacetone on the derivatization reaction of biogenic amines with acetylacetone

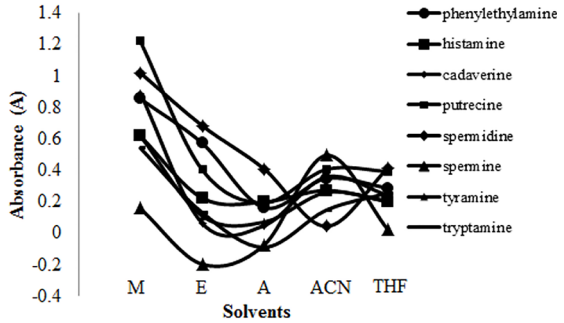

Figure 6. The effect of solvent on the derivatization reaction of biogenic amines with acetylacetone (M: methanol, E: ethanol, A: acetone, ACN: acetonitrile, THF: tetrahydrofuran)

different solvents obtained on the spectra are as shown in Figure 6.

It is seen that the solvent in which the highest absorptions are obtained is methanol. Therefore, the methanol-water mixture was used as this solvent during the derivatization of the biogenic amines with acetylacetone.

Effect of Solvent Volume

After determining that the most suitable solvent for the derivatization reaction of the biogenic amines with acetylacetone was methanol, the effect of this solvent volume on the absorption intensity was investigated. An appropriate amount of each biogenic amine was added to a solution containing different volumes of methanol (6, 8, 10, 12 and 14 mL), 1 g K_2HPO_4 and 1 mL of acetylacetone. The mixture was then diluted to 100 mL with ultrapure water. The mixture was kept in dark for 20 min to complete the reaction. The absorption changes of different volumes of methanol obtained on the spectra are as shown in Figure 7.

It is seen that the solvent in which the highest absorptions are obtained is 10 mL of methanol. For this reason, 10 mL of methanol was used during the derivatization of biogenic amines.

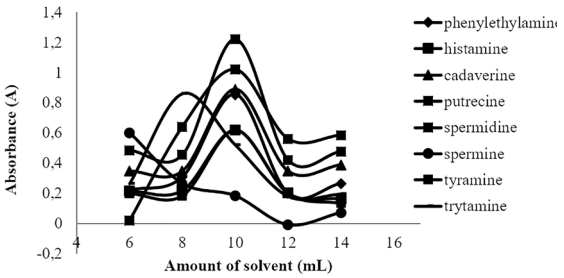

Figure 7. The effect of the volume of methanol on the derivatization reaction of biogenic amines with acetylacetone

CONCLUSION

In this study, the derivatization reaction of biogenic amines (phenylethylamine, histamine, cadaverine, putrecin, spermidine, spermine, tyramine and tryptamine) with acetylacetone reagent was optimized. The amount of K_2HPO_4, the reaction time, the amount of acetylacetone, the solvent and the solvent amount were determined in the optimization studies. This reaction was carried out by adding 1 g of K_2HPO_4, 10 mL of methanol, 1 mL of acetylacetone on the biogenic amine solution taken at a given concentration and diluting to 100 mL with a pure and standing for 20 minutes in the dark to complete the reaction. The end result is that the biogenic amines are converted to schiff bases.

ACKNOWLEDGEMENTS

The authors are grateful for the financial support of the scientific Research projects of Ondokuz Mayis University (project no. PYO.FEN.1904.12.009).

REFERENCES

1. Shalaby AR. Significance of biogenic amines to food safety and human health. Food Research International 29 (1996) 675–690.
2. Preti R, Antonelli ML, Bernacchia R, Vinci G. Fast determination of biogenic amines in beverages by a core-Shell particle column. Food Chemistry 187 (2015) 555-562.
3. ten Brink B, Damink C, Joosten HM, Huis in't Veld JH. Occurrence and formation of biologically active amines infFoods. International Journal of Food Microbiology 11 (1990) 73-84.
4. Hernandez–Borges J, D'orazio G, Aturki Z, Fanali S. Nano–liquid chromatography analysis of dansylated biogenic amines in wines. Journal of Chromatography A 1147 (2007) 192-199.
5. Saaid M, Saad B, Hashim NH, Ali ASM, Saleh MI. Determination of biogenic amines in selected Malaysian food. Food Chemistry 113 (2009) 1356-1362.
6. Anlı RE, Vural N, Yılmaz S, Vural YH. The determination of biogenic amines in Turkish red wines. Journal of Food Composition and Analysis 17 (2004) 53-62.
7. Bauza T, Blaise A, Daumas F, Cabanis JC. Determination of biogenic amines and their precursor amino acids in wines of the Vallke du RhSne by high–performance liquid chromatography with precolumn derivatization and fluorimetric detection. Journal of Chromatography A 707 (1995) 373–379.
8. Busto O, Miracle M, Guasch J, Borrull F. Determination of biogenic amines in wines by high–performance liquid chromatography with on–column fluorescence derivatization. Journal of Chromatography A 757 (1997) 311-318.

9. Casal S, Oliveira MBPP, Fereira MA. Determination of biogenic amines in coffee by an optimized liquid chromatographic method. Journal of Liquid Chromatography & Related Technologies 25 (2002) 2535-2549.
10. Henriquez-Aedo K, Vega KM, Prieto-Rodriguez S, Aranda M. Evaluation of biogenic amines content in chilean reserve varietal wines. Food and Chemical Toxicology 50 (2012) 2742-2750.
11. Loukou Z, Zotou A. Determination of biogenic amines as dansyl derivatives in alcoholic beverages by high-performance liquid chromatography with fluorimetric detection and characterization of the dansylated amines by liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. Journal of Chromatography A 996 (2003) 103-113.
12. Marcobal A, Polo MC, Marti'n-A'Ivarez PJ, Moreno-Arribas MV. Biogenic amine content of red Spanish wines: comparison of a direct ELISA and an HPLC method for the determination of histamine in wines. Food Research International 38 (2005) 387-394.
13. Mo Dugo G, Vilasi F, La Torre GL, Pellicano TM. Reverse phase HPLC/DAD determination of biogenic amines as dansyl derivatives in experimental red wines. Food Chemistry 95 (2006) 672-676.
14. Papavergou EJ. Biogenic amine levels in dry fermented sausages produced and sold in Greece. Procedia Food Science 1(2011) 1126-1131.
15. Romero R, Sanchez-Vinas M, Gazquez D, Bagur MG. Characterization of selected Spanish Table Wine Samples according to their biogenic amine content from liquid chromatographic determination. Journal of Agricultural and Food Chemistry 50 (2002) 4713+4717.
16. Simat V, Dalgaard P. Use of small diameter column particles to enhance HPLC determination of histamine and other biogenic amines in seafood. LWT - Food Science and Technology 44 (2011) 399-406.
17. Smela D, Pechova P, Komprda T, Klejdus B, Kuban V. Liquid chromatographic determination of biogenic amines in a meat product during fermentation and long-term storage. Czech Journal of Food Science 21 (2003) 167-175.
18. Tameem AA, Saad B, Makahleh A, Salhin A, Saleh MI. A 4-hydroxy-N_-[(E)-(2-hydroxyphenyl)methylidene] benzohydrazide-based sorbent material for the extraction HPLC determination of biogenic amines in food samples. Talanta 82 (2010) 1385-1391.
19. Tang T, Qian K, Shi T, Wang F, Li J, Cao Y, Hu Q. Monitoring the contents of biogenic amines in sufu by HPLC with SPE and pre-column derivatization. Food Control 22 (2011) 1203-1208.
20. Vidal-Carou MC, Lahoz-Portole's F, Bover-Cid S, Marine-Font A. Ion-pair high-performance liquid chromatographic determination of biogenic amines and polyamines in wine and other alcoholic beverages. Journal of Chromatography A 998 (2003) 235-241.
21. Yassoralipour A, Bakar J, Rahman RA, Bakar FA. Biogenic amines formation in barramundi (Lates calcarifer) fillets at 8 ^{0}C kept in modified atmosphere packaging with varied CO_2 concentration. LWT - Food Science and Technology 48 (2012) 142-146.
22. Yiğit M, Ersoy L. Determination of tyramine in cheese by LC-/UV. Journal of Pharmaceutical and Biomedical Analysis 31 (2003) 1223-1228.
23. Nishikawa Y. Liquid chromatographic determination of aliphatic diamines in water via derivatization with acetylacetone. Journal of Chromatography A 392 (1987) 349-353.
24. Sedighipoor M, Kianfar AH, Mahmood WAK, Azarian MH. Epoxidation of alkenes by an oxidovanadium(IV) tetradentate Schiff base complex as an efficient catalyst with tert-butyl hydroperoxide. Inorganica Chimica Acta 457 (2017) 116-121.