Quantitative method for assessing biogenic amines in fermented food products by colorimetry

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Abstract

In this study, a method of enzyme coupling assay using a colorimetric method the biogenic amines contents of fermented food products was investigated. The reaction solution composed of diamine oxidase, bromoperoxidase, potassium bromide and phenol red in the buffer pH 7.0. The method was used for determination of aqueous biogenic amine concentrations in the range of 0-8 mg/ml of putrescine, cadaverine and histamine, 0-14 mg/ml of tryptamine, 0-30 mg/ml of tryramine and 0-2 mg/ml of β-phenylethylamine. In this study, 3 M perchloric acid was used as extraction medium for nham and fermented pork sausage. Perchloric acid at the concentration of 1.5 M was used as extraction medium for fermented fish and 4 M hydrochloric acid was used as extraction medium for fermented snail. The ratio of sample and extraction medium was 1: 2 (w/v). Re-extraction for 3 times resulted in the highest recovery of 74.80, 64.65 and 50.60% for putrescine, cadaverine and histamine, respectively. The fermented fish contained the highest biogenic amine concentations of 432.06 mg/kg whereas its content of 381.06 mg/kg, 340.70 mg/kg, 267.21 mg/kg and 183.33 mg/kg were found in nham and fermented snail, nham, fermented pork sausage and fermented rice, respectively. The stability of the reaction solution containing glycerine and the reaction solution in the lyophilized form could prolong the reaction of the enzymes at least 24 and 16 week, respectively, at 4°C. The procedure can be used as a rapid screening for determination of the biogenic amine contents in fermented food products.

Keywords: Biogenic amines, fermented foods, determination

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Introduction

Biogenic amines (BAs) are the basic nitrogenous compounds with aliphatic, aromatic or heterocyclic structure. BAs can also be found in a variety of foods, beverages and fermented foods especially in protein-rich foods that are formed by microbial decarboxylation of the corresponding amino acids. Consumption of foods containing high amounts of BAs may cause problems such as headaches, nausea, hypotension, hypertension, cardiac palpitation, etc (Shalaby, 1996). Several analytical methods for the determination of BAs in foods such as thin layer chromatography, biosensors, capillary electrophoresis and reversed phase high performance liquid chromatography (HPLC) or gas chromatographic separation have been reported. Of these HPLC is the most popular and frequently reported for the separation and quantification of BAs. Existing tests for BAs can take several hours, are cumbersome and require large, expensive instruments found only in laboratories. The aim of this study was to develop the reagent kit for determining the biogenic amine contents in fermented foods by using enzyme coupling assay between diamine oxidase and bromoperoxidase.

Materials and Methods

1. Extraction of fermented food samples

The fermented food sample (20 g) was homogenized and mixed with 15 mL of extraction medium; 4 M hydrochloric acid and 0.6, 1.5, 3 M perchloric acid. After filtration through Whatman No. 1 filter paper (Whatman Internation Ltd., Maidstone, England), the filtrate was recovered. The precipitate was again treated with 15 mL of extraction medium as described above. The recovered filtrates were combined and neutralized to pH 7.0 by sodium hydroxide.

2. Determination of BA contents

BA contents of the samples were determined by reaction of bromoperoxidase and diamine oxidase. Reaction solutions (200 μl) contained diamine oxidase (0.2 U), bromoperoxidase (18.36 mU), 20 mM KBr and 0.2 mM color reagent in 0.1 M Tris-HCl buffer (pH 7.0). The reaction was initiated by the addition of 300 μl sample extract. The absorbance of the solution was measured against a blank at 590 nm using a spectrophotometer. A calibration curve was constructed using 0-30 mg/ml of putrescine, cadaverine, histamine, tryptamine, tyramine and α - phenylethylamine (Sigma) as standards. The total BA contents in sample is expressed as milligrams per kilogram of sample food product. All measurements in this study were made in triplicate and the data are reported as the mean value ± one standard deviation.

3. Effect of additive on reaction solution stability

Aqueous solutions with glycerol (2%) was added to reaction solution which contained diamine oxidase (0.2 U), bromoperoxidase (18.36 mU), 20 mM KBr and 0.2 mM color reagent in 0.1 M Tris-HCl buffer (pH 7.0). For storage stability, the reaction solution with and without additive in liquid form and the reaction solution in the lyophilized form stored at 4°C were assayed periodically for 16 weeks.

Results and Discussion

The capacity of the enzyme coupling assay between diamine oxidase and bromoperoxidase to determine the concentration of putrescine, cadavarine and histamine were in the range of 0-8 mg/ml while the concentration of tryptamine, tyramine and α-phenylethylamine were in the range of 0-14, 0-30 and 0-2 mg/ml, respectively (Figure 1).

1. Extraction yield and BA content

Extraction yields of different magnitude were obtained after the extraction of fermented foods with different acids (Figure 2). The higher extraction yields were from 3 M perchloric acid for nham and fermented pork sausage. The fermented fish extracted by 1.5 M perchloric acid resulted in higher yield of biogenic amines concentration as compared to the samples extracted in all the extraction medium while the fermented snail treated in 4 M hydrochloric acid for extraction, maximum yield of biogenic amines was obtained, suggesting that most of the biogenic amines present in the fermented snail are hydrochloric acid soluble. The biogenic amine contents obtained in the fermented food was quite high in the acid fraction with repeated three times. Since most of the biogenic amines present in the fermented foods are in bound form, the initial biogenic amines content was low in the acid (data not shown). The suitable acid for extraction of BAs from fermented meat and Thai fermented sausage was 3 M PCA and fermented rice was 1.5 M PCA, while 4 N HCl was suitable acid for extraction BAs from fermented fish and fermented shell due to each kind of acid has different efficiency to extract the biogenic amines from fermented food products (Figure 2). Shalaby (1996) referred to a variety of substances for extraction BAs such as perchloric acid, trichloroacetic acid, methanesulfonic acid, hydrochloric acid, petroleum ether and other solvents depended on efficiency of extraction on each analysed method. Limsuwan et al. (2007) found out that Nham was extracted with PCA with higher % recovery value of tryptamine, phenylethylamine, putrescine, cadaverine and tyramine than that of extraction with HCl and TCA.

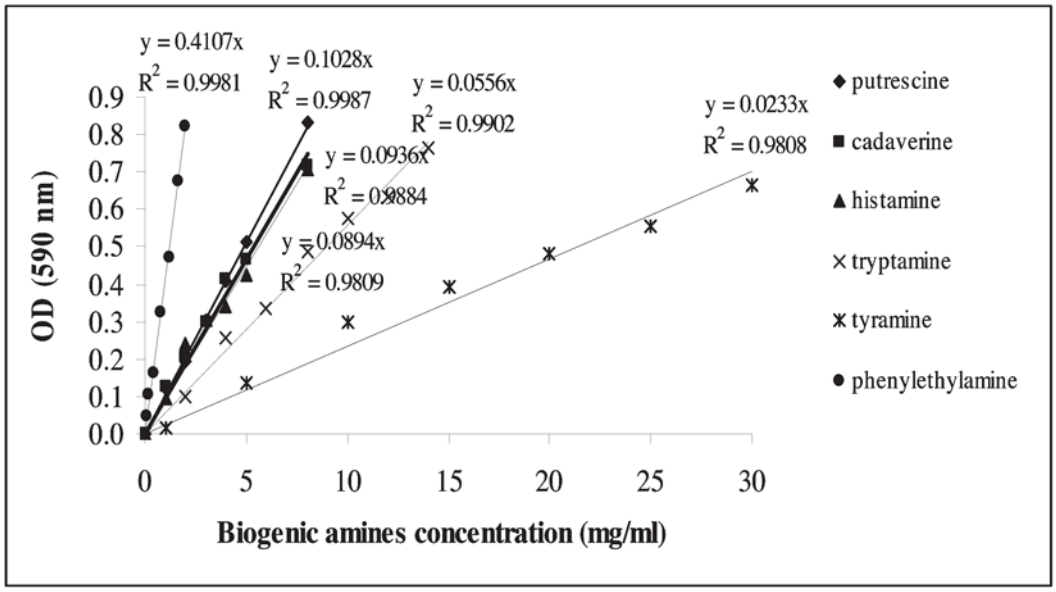

Figure 1. Standard curves of putrescine, cadavarine, histamine, tryptamine, tyramine and α-phenylethylamine for enzyme coupling assay between diamine oxidase and bromoperoxidase.

The content of BA in fermented meat, Thai fermented sausage, fermented rice, fermented fish and fermented shell are shown in Figure 3. In fermented fish, BA was the most abundant BA content (432.06 mg/kg), followed by fermented shell (381.06 mg/ mg/kg), fermented meat (340.7mg/kg), Thai fermented sausage (267.21 mg/kg) and fermented rice (183.33 mg/kg). As seen from the figure, there are significant differences in the contents of BA in the samples maybe because of the influent factors such as materials, pH, salt concentration, storage temperature, production process, acid addition (ex. citric acid, malic acid or succinic acid (Kang and Park, 1984) and preservative addition (ex. potassium sorbate or sodiumhexametaphosphate (Taylor and Speckhard, 1984). Spanjer and Van Rood (1991) suggested that for regulation purposes, in fish and fish products, the sum of histamine, putrescine and cadaverine should be limited to 300 mg/kg. As for BA contents of fermented meat, Thai fermented sausage, fermented rice and fermented shell was 340.70, 267.21, 183.33 and 381.06 mg/kg, respectively. Similar results were also determined by HPLC. BAs contents (the sum of putrescine, cadaverine and tyramine) in fermented meat ("Nham") were 383.89 mg/kg (Limsuwan et al., 2007). However, this study must be detected BA contents in sample fermented foods that had the same factors such as sources, analysis time, materials and production process by HPLC in order to confirm their results.

2. Storage stability of reaction solution

To evaluate the stability of BAs test kits, the stored BAs test kit for 0-16 weeks at 4°C in both liquid form added with 2% glycerol as stabilizer and lyophilized forms were evaluated by comparing with test kit in liquid form no adding with glycerol (control sample). The data as shown in Figure 4 was found that the test kit in liquid form (control sample) remained less stable, with significant loss of activity of enzymes when storage for 16 weeks. While, test kits in both liquid form (added with 2% glycerol) and lyophilized forms remained extremely stable, with no significant loss of activity of enzymes at least 16 weeks.

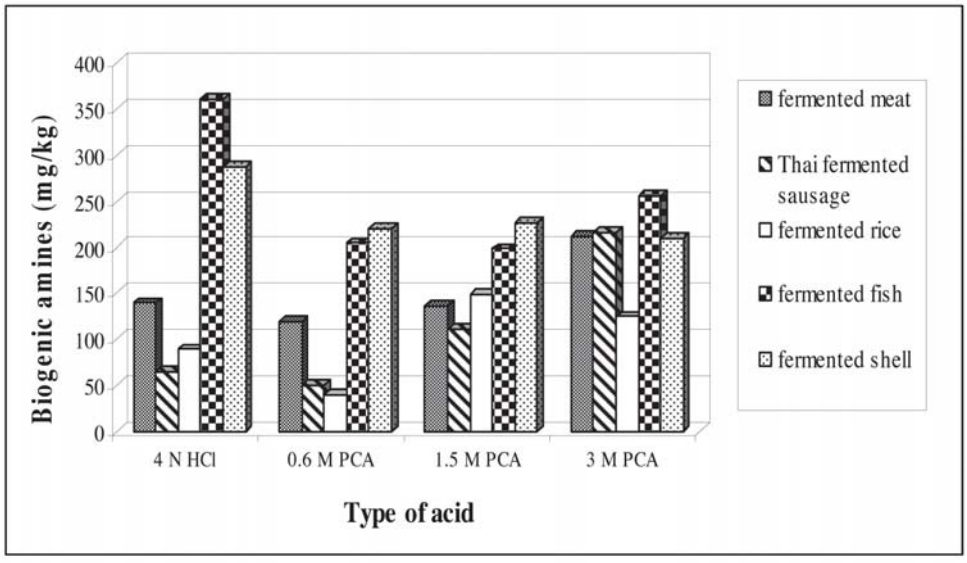

Figure 2. Effect of acid on biogenic amines extraction from fermented foods.

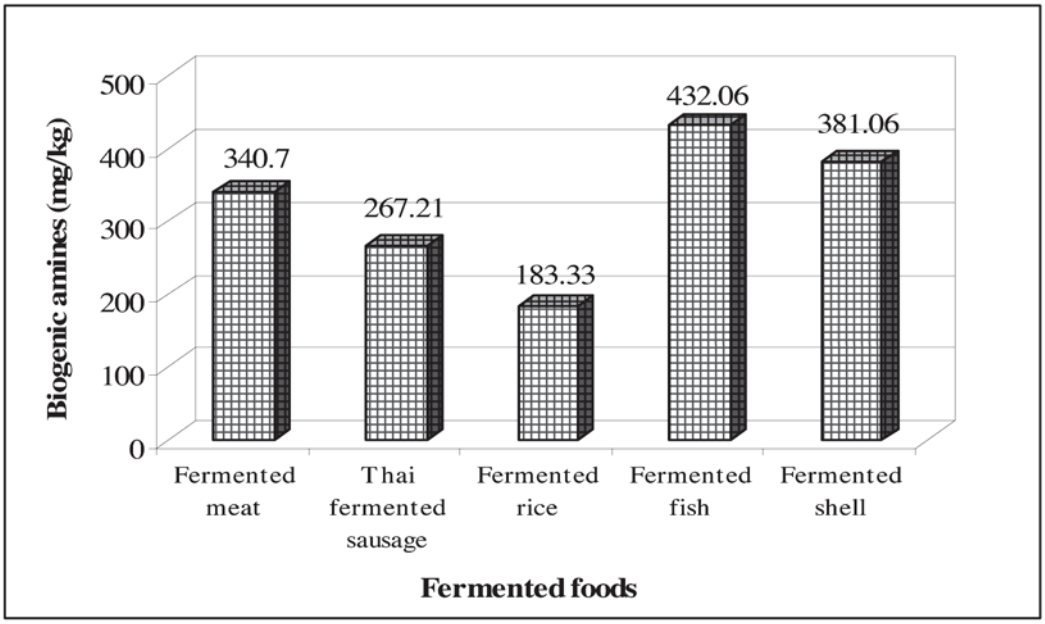

Figure 3. Contents of Biogenic amines in the fermented foods.

2% Glycerol was studied in order to evaluate their effect on the reaction solution storage stability at pH 7.0 and 4°C in liquid form and lyophilized form (Figure 4). 2% Glycerol did not affect significantly the reaction solution activity at the above conditions. Our experimental results showed that glycerol was the appropriate additives for reaction solution stabilization against storage conditions. The glycerol treated enzyme retained 100% activity compared to the non stored native reaction solution. When glycerol was not added in the reaction solution, the enzyme activity decreased. Possibly the reaction of the glycerol with the enzymes, promoted preferential hydration, domain interface changes and reduction of backbone mobility of the enzyme chains, and there from resulting in enzyme activities during the stabilization process. The storage stability of native, additives treated enzymes and lyophilized form were studied at 4°C (Figure 4). At 4°C, the highest stabilities were obtained in the presence of glycerol (2% v/v), glycerol induced the high stabilization effect. The denaturation of the enzymes in aqueous solution proceeds through hydratation of the protein. The role of glycerol in enzyme stabilization is as a water-structure maker, which depresses the hydratation of the protein. The glycerol molecules are preferentially excluded from the surface layer of the protein molecule and the water shell around the protein molecule is preserved, so that the conformation of the protein becomes more rigid (Michiaki et al., 1997; Longo and Combes, 1999). Unfolding of the protein structure takes place under thermal stress, due to disruption of the hydrogen bonds and the other interactions, responsible for the maintenance of the tertiary enzyme structure. The precise orientation of the residual groups in the protein backbone is lost, and interaction of the water molecules with the newly exposed regions occurred, resulting in protein denaturation. The high capability of glycerol to form hydrogen bonds should play the most important role in enzyme stabilization, increasing the degree of organization of water molecules (Noriko

et al., 1999; Yuji et al., 1984). Glycerol, was added in reaction solution, can be stabilized enzymes in reaction solution, especially at suboptimal temperatures because glycerol can be depressed the freezing point, altered the conformational and also changed in association constants of protein. Moreover, glycerol can be inhibited bacterial growth, precipitate formation and may be inhibited protease.

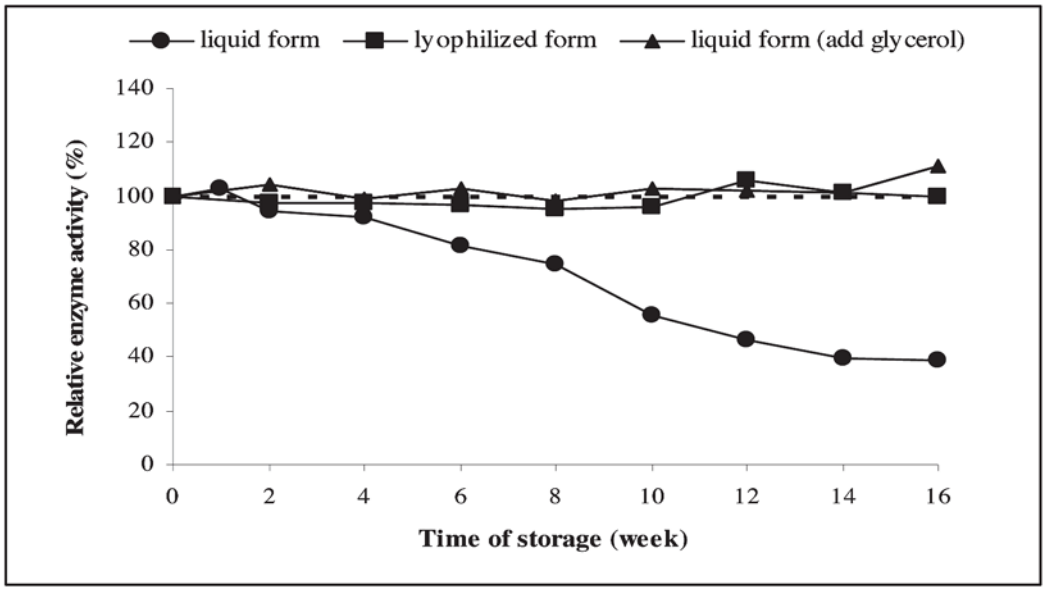

Figure 4. Effect of storage time on stability of biogenic amines test kits.

The activity of the lyophilized form of reaction solution retained 100% activity, in comparison to the non stored native reaction solution. The stabilizing effect of the lyophilized form against storage denaturation of the enzyme might be explained with the conformation of the protein becomes more rigid so it causes the preservation of the conformation of the protein molecule. The lyophilized form also causes a number of conformations of the chains, and probably the accumulation of all the displacements of the molecules impeded the accessibility of the active center of the enzyme. In other words inactivation, without denaturation of the protein occurred. Another explanation could be that the lyophilized form limits the movement of the molecules and greater stress, which could not be uniformly distributed along the protein chains, is accumulated in the polypeptide structure, leading to its disruption. In our experiment the enzyme was denaturated, which fact was confirmed by the irreversible loss of activity, and therefore the second mechanism was more reliable.

Conclusions

In conclusion, the suitable medium for extraction of BAs from fermented meat and Thai fermented sausage was 3 M PCA and 1.5 M PCA was for fermented rice while 4 N HCl was suitable medium for extraction of BAs from fermented fish and fermented shell. BAs content of fermented fish was higher than the other fermented foods in this study. This study evaluates the stabilization efficiency of additives on the biocatalyst activity of native

enzyme. The experiments revealed that the additives, even in low concentration (2% v/v) provided better long term storage stability at neutral pH and 4°C, without significant loss in enzyme activity. The test kits in the lyophilized form or in the liquid form added with glycerol, could prolong the shelf life at least 16 weeks at 4°C. The glycerol appeared to be appropriate for long-time storage stabilization of the enzyme during storage. From this study, the good additive stabilizing performance was induced by the glycerol. From the above considerations it could be suggested that the stabilized additive could be potentially applied for enzyme commercial kit reagent, thereby providing high valued product for determination of biogenic amine in fermented food products.

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