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HPLC determination of gamma amino butyric acid (GABA) and some biogenic amines (BAs) in controlled, germinated, and fermented brown rice by pre-column derivatization

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ABSTRACT

The gamma amino butyric acid (GABA) and biogenic amines (BAs) have great physiochemical importance due to their unique functions in human biological systems. The current study describes the investigation and separation of GABA and BAs including putrescine (PUT), cadaverine (CAD), dopamine (DOP), tyramine (TYRA), histamine (HIST), spermine (SPM) and spermidine (SPD) in some Pakistani brown rice. The GABA and BAs were analyzed in controlled, germinated and fermented conditions by using high performance liquid chromatographic (HPLC) technique after pre-column derivatization on a reversed phase C-8 column with diode-array detection at 230 nm. 2-Hydroxynaphthaldehyde was used as a pre-column derivatizing agent and the analytes were detected as Schiff base derivatives. A linear regression was found within 2.25–34.5 μg/mL for the standards GABA and DOP, 1.15–28.5 μg/mL for standards PUT, CAD, HIST, and 2.50–48.5 μg/mL for standards TYRA, SPD, SPM with the correlation coefficient (r^2) in the range of 0.997–0.998. GABA was detected in controlled, germinated and fermented samples, whereas analyzed biogenic amines were below the detection limit in controlled conditions and their concentration increased with the passage of germination time and also detected after the fermentation process. This method could be used for separation and quantification of GABA and other BAs simultaneously in food samples especially in cereal seeds during germination and fermentation to examine their levels.

1. Introduction

Gamma amino butyric acid (GABA) is a non-protein amino acid found in the human central nervous system (CNS). GABA and some biogenic amines (BAs) work in the central nervous system (CNS) as neurotransmitters and control a number of physiochemical functions in human body such as lowering the blood pressure, helping to promote relaxation, ease nervous tension and anti-stress effects in the human brain (Oh et al., 2003; Sakai et al., 2005; Zhang et al., 2006). Biogenic amines including putrescine, spermine and spermidine are also involved in a number of physiological processes in plants, such as cell division, flowering, fruit development, response to stress and senescence (Kaniou et al., 2001).

BAs are natural nitrogen containing low molecular weight compounds with heterocyclic, aliphatic and aromatic structures. These compounds have been reported in higher concentrations in fermented foods (Spano et al., 2010) and in wine samples from different origins (Anlıa et al., 2004; Yildirim et al., 2007; Anli and Bayram, 2009; Beneduce et al., 2010; Coton et al., 2010; Romano et al., 2012) including rice wines (Lu et al., 2007). In wine, BAs are formed by a variety of microorganisms associated with different stages of its production and storage (Smit et al., 2008). Histamine, putrescine and tyramine are the principal biogenic amines in wines (moDugo et al., 2006). BAs have also been reported in different fermented foods such as fish (Ruiz-Capillas and Moral, 2002), dairy products (Fernandez-Garcıa et al., 1999), sausages and meat (Maijala and Eerola, 1993) and sauerkraut (Kalac et al., 1999).

GABA and other BAs are most commonly synthesized from respected amino acids by decarboxylation (Shalaby, 1996). There

Abbreviations: GABA, gamma amino butyric acid; DOP, dopamine; HIST, histamine; TYRA, tyramine; PUT, putrescine; CAD, cadaverine; SPD, spermidine; SPM, spermine; BAs, biogenic amines.

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are several reviews published in the last two decades that explain the formation of BAs in different foods and their roles in nutrition. (Krizek and Pelikanova, 1998; Ayhan et al., 1999; Smela et al., 2003).

Some researchers suggest that the accumulation of GABA in food during germination or the fermentation process is beneficial for human health due to its function in the central nervous system as a neurotransmitter (Oh, 2003; Oh and Oh, 2003). On the other hand, some research papers suggest that the accumulation of BAs in foods may have detrimental effects on human health after their concentration has reached a certain level (Onal, 2007). The present study describes the development of a simple reverse phase HPLC method for simultaneous separation and quantification of GABA and BAs in brown rice samples. We have recently utilized 2-hydroxynaphthaldehyde (HN) as a pre-column derivatization reagent for the analyses of GABA in rice samples Hayat et al. (2014). The same derivatizing reagent was selected for this study due to its high sensitivity and response to minor GABA and biogenic amines for the formation of Schiff bases. This methodology was applied to rice samples in controlled, germinated and fermented conditions.

2. Experimental

2.1. Reagents

All chemicals used were of analytical grade and purchased from Merck (Darmstadt, Germany), Sigma Aldrich (Germany) and Dae-Jung (Korea). All standards including 4-aminobutyric acid (purity 99%), 1, 5-diamine pentane (purity 98%), 1, 4-diamine butane (purity 98%), histamine (purity 99%), tyramine (purity >97%), spermidine (purity 98%), spermine (purity 98%) and dopamine (purity 98%) were purchased as their hydrochloride salts. De-ionized water (Milli-Q) was used for solution preparation.

2.2. Instrumentation

A double beam Lamda-135 Spectrophotometer (Perkin Elmer Ltd. United Kingdom) with dual 1 cm quartz cuvettes was used for the spectrophotometric study. A pH meter Orion (420 A, Orion (Pvt) Ltd., Boston, USA) with internal reference electrode and combined glass electrode was used for pH measurements. Sample extraction was done by using a Vortex mixer and an Eppendorf Centrifuge-5804 R (Eppendorf, USA) was used for the centrifugation. A Rotavapor with heating bath and vacuum pump R-210 (Buchi, Switzerland) was used to evaporate the ethanol from the rice extract. An Agilent 1200 HPLC system (Agilent Technology Inc. Palo Alto, CA, and USA) with an online degasser, an auto sampler, a binary pump and photo diode array detection (DAD) system and SB-C-8 (2.1 × 50 mm id, Agilent Technology Inc., USA) column was used for HPLC analysis which was connected to a computer and controlled by a Chemstation software system.

2.3. Preparation of standard solution and derivatization process

All Stock standard solutions (0.5 mg/mL) were prepared by dissolving standards (GABA, DOP, TYRA, PUT, CAD, HIST, SPD and SPM) in separate 50 mL volumetric flasks and kept in the dark at 4 °C. Six working solutions of 1 μg/mL, 2.5 μg/mL, 5 μg/mL, 10 μg/mL, 20 μg/mL and 40 μg/mL for each standard were prepared through dilution with water.

Each working solution (1 mL) was treated with 1 mL of 2-hydroxynaphthaldehyde (2.5% *w/v*) in methanol followed by addition of 0.5 mL boric acid-NaOH buffer (pH 8.5) in 5 mL volumetric flask. The resultant mixture was heated at 85 °C for 15 min in a water bath, and the solution was allowed to cool at room temperature. The volumes were adjusted to 5 mL with methanol and were kept at 4 °C till analysis. The detailed derivatization procedure has been already published Hayat et al. (2014). The absorption spectra were recorded using a spectrophotometer against a reagent blank which was prepared with the same procedure without standards.

2.4. Germination and fermentation of rice samples

Five Pakistani brown rice samples including basmati super, basmati-385, basmati-2000, Irri-6, and Irri-9 rice were provided by National Agriculture Research Center (NARC) Islamabad, Pakistan and Rice Research Institute Dokri, Sindh, Pakistan. The germination of rice samples were carried out by the method used by Jannoey et al. (2010) with minor modifications. Rice seeds (100 g) were soaked in 500 mL water at 30 °C for 96 h and water was changed after every 24 h. Samples (5 g) for the determination of GABA and BAs level were drawn after 12, 24, 48, 72 and 96 h for the analyses to be carried out in triplicate for each sample.

The fermentation process was carried out according to the method described by Qureshi and Anwar (2011) with minor modification. Rice seeds (250 g) were soaked in water for 30 min and boiled in distilled water for 10 min. The boiled rice was inoculated with a 2.5 mL suspension of *Rhizopus oligosporus* (M−90) and packed in a sterilized, closed glass jar for incubation at 35 °C under aerobic conditions for 96 h. GABA and BAs were extracted followed by analyses by the methods as described below.

2.5. Extraction and derivatization of rice samples

The sample extraction procedure was carried out with slight modification of the reported method of Komatsuzaki et al. (2007). The powdered rice (1 g) was placed in a falcon tube with 5 mL of 80% *(v/v)* ethanol and shaken on a vortex mixer for 5 min. The sample was centrifuged (5000 rpm) for 10 min at 4 °C and the supernatant was filtered with Millipore filter paper (pore size 0.45 μm, diam. 47 mm). The extraction step was repeated twice. The resultant extracts were dried on a Rotary Evaporator till the complete evaporation of ethanol from extract and the dried residue was dissolved in 1 mL water for derivatization followed by HPLC analysis.

2.6. HPLC analysis of GABA and BAs and method validation

All brown rice samples including controlled, germinated and fermented forms were subjected to the extraction processes and were analyzed in triplicate. Resulting data was recorded as the mean of three analyses of each sample with standard deviation. 1 mL aliquots of rice extract were treated with the derivatization reagents as described in the above section. The standards and samples were subjected to HPLC analyses (Agilent 1200 series) with a linear gradient system of solvent A (water) and solvent B (methanol). The gradient elution procedure was 60% A + 40% B at 0 min, 50% A + 50% B at 2 min, 40%A + 60% B at 5 min 30% A + 70% B at 10 min, 25% A + 75% B at 12 min and 20% A + 80% B at 15 min. This system allowed the separation in 15 min using a C-8 reversed phase column and peak detection at 230 nm.

The HPLC method was validated with respect to linearity, accuracy, precision, and selectivity. Linearity of the method was examined for GABA and BAs standards (with five replicates) of concentration ranging 0.5 μg/mL−8.0 μg/mL. Method precision was examined by the analysis of samples in triplicate and expressed as RSD%. Recovery analysis was performed by spiking a known amount of standard to the rice samples and then the recoveries of GABA and BAs were determined on HPLC with triplicates and the mean was calculated on Microsoft Excel. Selectivity of the method