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Identification and quantification of amino acids in coconut water using high performance thin layer chromatographic method

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ABSTRACT

Amino acids are minor compounds present in coconut water but they have pronounced effects on protein quality of foods. Accurate standardized methods for measuring amino acid levels are required to assess the nutritional safety and compositional adequacy of sole source foods and nutritionals. Standard conditions have been optimized based on simulation in R_f values under experimental conditions of nature of mobile phase and saturation time of solvent chamber. The coconut water contains 0.07, 0.03 and 0.05% of aspartic acid, lysine and leucine respectively.

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INTRODUCTION

Chemical analysis of agricultural and food products is becoming of great importance due to achieving the adequate quality of agricultural and food products. Proteins are the indispensable agents of biological function and amino acids are the building blocks of proteins[1]. Amino acids are important for nutrition as vitamins and minerals. They are critical to life and have many functions in metabolism. Amino acids are used by the human body to make tissues, enzymes, hormones and other vital body substances[2]. They furnish the material from which proteins are synthesized. The ultimate value of a food protein lies in its amino acid composition[3].

The purification and analysis of individual amino acids from complex mixtures was once a very difficult process. Today, however, the biochemists have a wide variety of methods available for the separation and analysis of amino acids in food and herbal products. The most popular and widely used methods are gel electrophoresis, amino acid analyzer, capillary electrophoresis and HPLC[4,5]. All these methods require preliminary treatment of the test material to hydrolyze the proteins to the free amino acids which are time consuming process[6]. High Performance Thin Layer Chromatography (HPTLC) is recently introduced technique which include separations based on partition properties (the tendency to associate with one solvent or phase over another). This technique has the advantages of simplicity, no pre chemical treatment of the sample, speed, reproducibility and cost effectiveness[7,8]. It is an offline technique: the subsequent steps are relatively independent, allowing parallel treatment of multiple samples during chromatography, derivatization and detection. Unlike other methods, HPTLC produces visible chromatograms in which the complex information about the entire sample is available at a glance. HPTLC based separations are fast, simple and require very less amount of analyte (μl).

The present work reports standardization of HPTLC method for the separation and estimation of amino acids present in coconut water. Simulation in R_f values as a function of nature of mobile phase and time of satura-

tion of solvent chamber has been carried out. The proposed HPTLC method has been validated based on selectivity, linearity, limit of detection and quantification, accuracy in terms of recovery % and precision.

EXPERIMENTAL

Chemicals and reagents

Standard of amino acids were obtained from E. Merck (Darmstadt, Germany). Coconut water available in the market was considered for the study. HPTLC plates (cellulose, 20×10 cm) purchased from E. Merck (Darmstadt, Germany) were used for analysis. Plates were developed in a chromatographic chamber using optimized solvent system comprising of n-butanol: acetic acid: water. The solvent was allowed to migrate up to a height of 80 mm from the lower edge of the plate and then dried it.

Sample preparation and analysis

Standard solutions of amino acids (aspartic acid, lysine and leucine) were freshly prepared by dissolving individual amino acids (5mg) in water (1ml) and sonicated for 10 minutes over an ultrasonic bath and then makeup with ethanol (5ml). Sample (coconut water) was mixed with the aqueous alcohol (100mg/ml) and sonicated for 10 minutes over an ultrasonic bath for proper mixing. Standard and sample were loaded on the HPTLC plates for the analysis. HPTLC system (Camag, Muttanz, Switzerland) consisted of a TLC scanner which is connected to a PC running WinCATS; an auto sampler Linomat V using 100µL and 500µL syringes, connected to a nitrogen cylinder; a UV scanner. Each HPTLC plate contains different tracks of samples and standards. Amino acids are not UV active, so to visualize the bands plates were derivatized using ninhydrin reagent and then the plates were placed in Camag, TLC Scanner 3 for quantification.

RESULTS AND DISCUSSION

Preliminary tests on silica gel, alumina and cellulose coated HPTLC plates indicated that cellulose layer gave the best resolution of the amino acids. Therefore, all subsequent analyses were done on cellulose layers. Optimization of solvent system has been achieved based on simulation in R_f values obtained in differently designed solvent system as a function of polarity (TABLE 1a) and saturation time (TABLE 1b).

TABLE 1a : Effect of nature of solvent system on the R_f value of amino acids using cellulose as stationary phase

Solvent system (v/v/v)	Saturation	R_f		
		Aspartic acid	Lysine	Leucine
Acidic				
n-butanol: water: acetic acid (3:1:1)	No	0.41	0.10	0.73
Basic				
EtOH: water: ammonia (10:2.25:1.25)	No	0.25	0.16	0.74

TABLE 1b : Effect of saturation time on the R_f value of amino acids using cellulose as stationary phase

Solvent system (v/v/v)	Saturation	R_f		
		Aspartic acid	Lysine	Leucine
n-butanol: water: acetic acid (3:1:1)	No	0.41	0.10	0.73
n-butanol: water: acetic acid (3:1:1)	15 minutes	0.25	0.08	0.72
n-butanol: water: acetic acid (3:1:1)	30 minutes	0.21	0.07	0.70

Amino acids exhibit different R_f values depending upon the pH of the solvent system. However, the separation of the mixture of three amino acids has not been found good in basic solvent system. Therefore, acidic solvent was considered to observe the saturation effect on R_f value. R_f value of amino acids decreases with the increase in saturation time. Presence of amino acids (aspartic acid, lysine and leucine) in coconut water was confirmed by recording their chromatogram after the derivatization with ninhydrin reagent (Figure 1).

Based on our observation the optimum solvent system was found to be n-butanol- acetic acid- water (3:1:1, v/v) with the R_f value of 0.41, 0.10 and 0.73 respectively for aspartic acid, lysine and leucine. There was no overlap with any other analyte of the sample at λ max (ε): 520nm (Figure 2).

The proposed chromatographic method was validated. Validation parameters include selectivity, linearity, limit of detection and quantification, accuracy in terms of recovery % and precision. Selection of wavelength (520 nm) is specific for the detection of amino acids. The linearity of the proposed method was confirmed in the range (83-996 ng) for aspartic acid and leucine,

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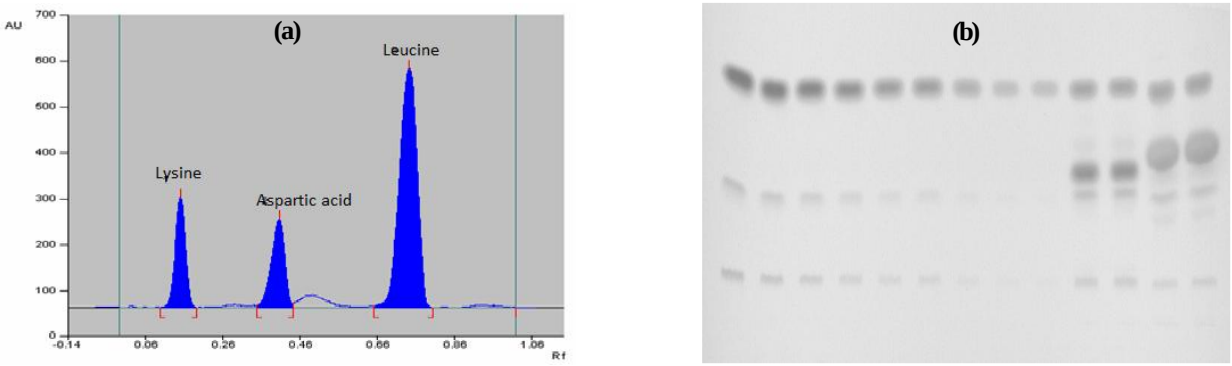

Figure 1 : (a) Chromatogram of amino acids; (b) appearance of band after derivatization

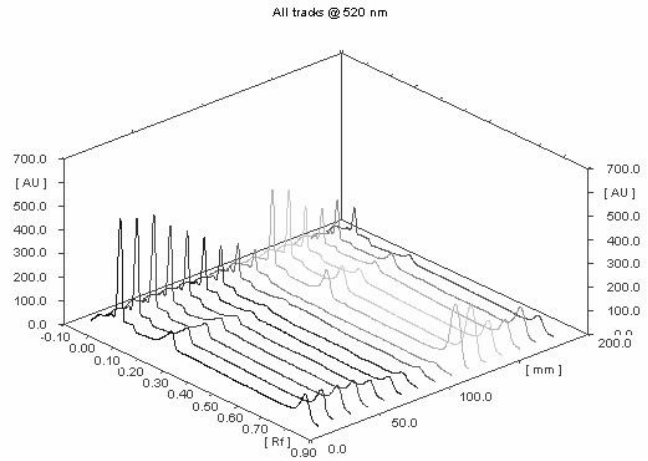

Figure 2 : 3D display of amino acids peaks

while (66-792ng) for Lysine. This range was suitable for the determination of amino acid contents in coconut water. Regression analysis of the calibration data for amino acids showed that the dependent variable (peak area) and the independent variable (concentration) were represented by the equations Y = 4.713 +14.158x, Y = 1430.156 + 13.59x and Y = 9115.893 + 28.082x for aspartic acid, lysine and leucine respectively. The correlation of coefficient (r^2) obtained was 0.999, 0.998 and 0.997 respectively for aspartic acid, lysine and leucine, shows a good linear relationship (Figure 3). The correlation coefficient was found to be greater than 0.997 which manifests a linear relationship between concentration and the peak area.

The concentration of aspartic acid, lysine and leucine in the coconut water was found to 0.07, 0.03 and 0.05% respectively with standard deviation of ± 2.78 (TABLE 2).

The calibration curve was accurate within the specified concentration range with a mean recovery[9] of 90.32, 92.75 and 91.60 % respectively for aspartic

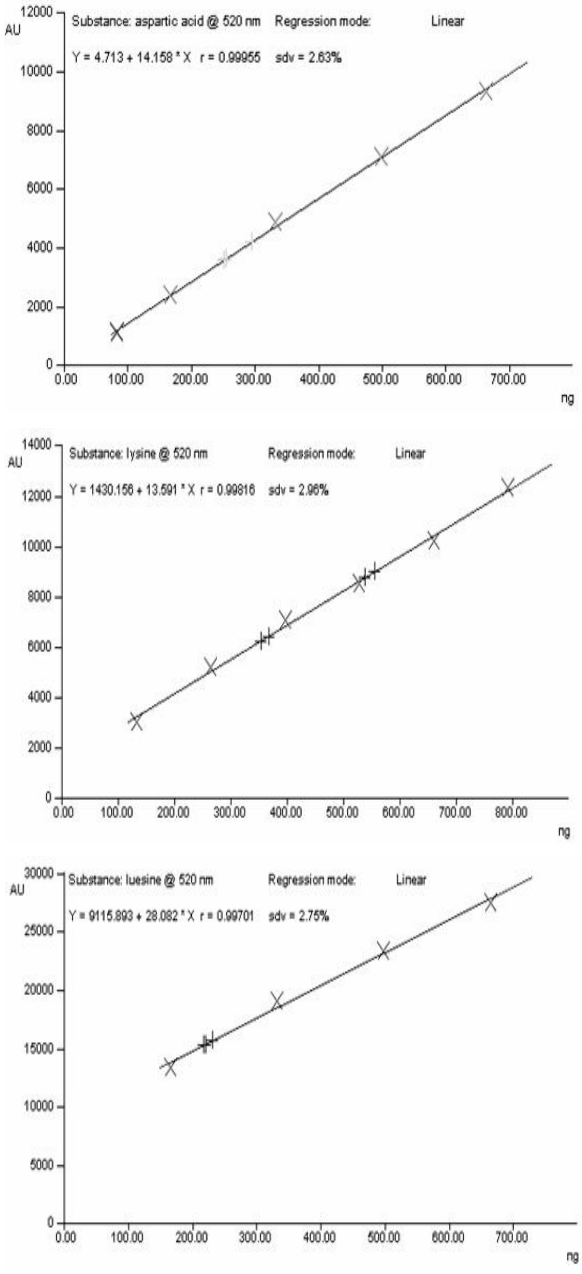

Figure 3 : Calibration curve of amino acids (aspartic acid, lysine and leucine)

TABLE 2 : Amino acid levels in coconut water

Amino acids	Cholesterol content [%]
Aspartic acid	0.07
Lysine	0.03
Leucine	0.05

acid, lysine and leucine. The limit of detection (LOD) and quantification (LOQ) was found to be 8 and 80 ng for aspartic acid and leucine, while for lysine 6 and 60 ng respectively. Precision (repeatability) was determined by running a minimum of four analyses and the coefficient of variability was found to be 0.05-0.1 % for all the amino acids selected for the study.

CONCLUSION

The proposed HPTLC method is simple, sensitive, rapid and effective for identification and quantification of amino acid contents in coconut water.

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