Amino Acid, Fatty Acid, and Carbohydrate Content of *Artocarpus altilis* (Breadfruit)

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Abstract

A study is conducted to determine the amino acid, fatty acid, and carbohydrate content of breadfruit using high-performance liquid chromatography (HPLC) and gas chromatography (GC). An HPLC method is used for the determination of amino acids and fatty acids in breadfruit. Representative amino acid samples are derivatized with phenylisothiocianate and the resulting phenylthiocarbamyl derivatives are separated on a reversed-phase column by gradient elution with a 0.05M ammonium acetate buffer and 0.01M ammonium acetate in acetonitrile–methanol–water (44:10:46, v/v). Representative fatty acid samples are derivatized with phenacyl bromide and the resulting fatty acid phenacyl esters are separated on a reversed-phase column by gradient elution with acetonitrile and water. Amino acid and fatty acid derivatives are detected by ultraviolet detection at 254 nm. The analysis of the carbohydrates in breadfruit employs a GC method. Carbohydrates are derivatized using trimethylchlorosilane and hexamethyldisilazane to form trimethylsilyl ethers. Compounds in the samples are separated by the temperature programming of a GC using nitrogen as the carrier gas. Percent recoveries of amino acids, fatty acids, and carbohydrates are 72.5%, 68.2%, and 81.4%, respectively. The starch content of the breadfruit is 15.52 g/100 g fresh weight.

Introduction

The biological value of food is determined by the nutrients it contains. The nutritive value of proteins (one of the most important components of the human diet) depends mainly on their amino acid composition, especially their content of essential amino acids. In addition to their dietary importance, proteins also influence food organoleptic properties. Proteins affect food texture, and small peptides and amino acids contribute to food flavor. Proteins, peptides, and amino acids undergo thermal and enzymic reactions during food preparation and storage that generate colored aromatic compounds (1).

Underivatized amino acids are not readily measured by high-performance liquid chromatography (HPLC) because of their high polarity and low response to ultraviolet or fluorescent detection. In order to effectively detect amino acids, it is first necessary to chemically modify them, which usually involves converting them into derivatives that absorb in the UV–vis wavelength range or compounds that fluoresce (2).

There are various methods that are described for the quantitation of amino acids in samples. These include precolumn and postcolumn derivatization techniques. Popular among the precolumn derivatization techniques are the dansyl chloride and *o*-phthaldialdehyde (OPA) derivatizing reagents.

Dansyl chloride forms fluorescent adducts with amino acids but lacks sensitivity (because it reacts with both -OH and $-NH_2$ groups) and requires long reaction times and high temperatures (3).

OPA is nonfluorescent itself and reacts rapidly with primary amino acids at room temperature to form highly fluorescent isoindoles. The disadvantage of this method is the lack of reactivity of OPA with secondary amino acids (4).

Phenylisothiocianate (PITC) reacts with both primary and secondary amino acids to yield phenylthiocarbamyl derivatives, which can be detected by their UV absorption at 254 nm. The disadvantage of this method is low sensitivity compared with methods based on fluorometric detection, which can be overcome by using a large amount of sample material (2). Fatty acids are very important compounds in biological systems. First, they are important components of structural molecules such as phospholipids and are an important source of energy. Separation and analysis of long-chain fatty acid mixtures have been applied extensively to obtain information on a number of biological systems. The inherent low absorptivity of fatty acids in the ultraviolet–violet range has led to the use of aromatic derivatives such as *p*-nitrobenzyl (5) and 2-napthacyl esters (6).

Gas–liquid chromatography (GLC) coupled with flame ionization detection is generally the method of choice for the routine quantitation of fatty acids usually after their conversion to methyl esters (7). However, more recently fatty acids or methyl ester mixtures have been resolved by reversed-phase HPLC using acetonitrile–water (8) or methanol–water (9).

Carbohydrates are among the most abundant compounds

found in nature, and the analysis of sugars and sugar mixtures is becoming a growing importance in the food industry (10). Human dietary intake of these carbohydrates is important because of their diverse biological roles, the most important of which is the provision of energy.

Underivatized carbohydrates are not suitable for analysis by GLC because of their low volatility and thermal instability. Consequently, they are converted into more volatile derivatives such as trimethylsilyl ethers, alditol acetates, and aldonitrile acetates. In contrast to trimethylsilyl derivatives, both the alditol acetate and aldonitrile acetate derivatives give single peaks for individual reducing sugars; however, they require long preparation times of 40–100 min (11).

In this study, the amino acid and fatty acid reducing and nonreducing sugar content of breadfruit is determined by precolumn derivatization techniques. Amino acids are derivatized using PITC, fatty acids are derivatized using phenacyl bromide, and carbohydrates by trimethylchlorosilane and hexamethyldisilazane.

Experimental

Breadfruit samples

Breadfruit (*Artocarpus altilis*, white heart cultivar) was obtained from a local market in Papine, St. Andrew, Jamaica. Samples were analyzed during three developmental stages of breadfruit: the mature green, ripe, and overripe stages.

Reagents

All chemicals used were of reagent grade unless otherwise noted.

Amino acid, fatty acid and carbohydrate standards, trimethylchlorosilane, hexamethyldisilazane, HPLC-grade acetonitrile, triethanolamine (TEA), PITC, and phenacyl bromide were obtained from Sigma (St. Louis, MO). Chloroform, pyridine, sodium hydroxide, acetone, oxalic acid, lead acetate, and ammonium acetate were obtained from BDH (Poole, U.K.). Isopropanol, methanol, hexane, acetic anhydride, and phosphoric acid were obtained from Fisher Chemicals (Fair Lawn, NJ). Hydroxylamine hydrochloride was obtained from Koch–Light Laboratories (Colnbrook, Bucks, U.K.).

Table I. Content of Selected Amino Acids in Unhydrolyzed Breadfruit Samples*

Amino acid	RRT[†] (standards)	RRT (sample)	Mature green (g/100 g FW)	Ripe (g/100 g FW)	Over-ripe (g/100 g FW)
Asparagine	0.19 ± 0.02	0.18 ± 0.00	0.01 ± 0.00	2.13 ± 0.23	1.19 ± 0.01
Glutamine	0.24 ± 0.01	0.21 ± 0.01	0.35 ± 0.02	0.15 ± 0.03	0.19 ± 0.41
Tryptophan	0.01 ± 0.00	0.01 ± 0.00	0.39 ± 0.10	0.47 ± 0.09	1.21 ± 0.45
Cysteine	1.21 ± 0.01	1.19 ± 0.00	0.03 ± 0.00	0.31 ± 0.01	0.63 ± 0.03

* Values represent an estimate in mean ± standard deviation of the content of those amino acids that are destroyed by acid hydrolysis.
[†] RRT, relative retention time.

Table II. Amino Acid Content of Hydrolyzed Breadfruit Samples*

Amino acid	RRT[†] (standards)	RRT (sample)	Mature green (g/100 g FW)	Ripe (g/100 g FW)	Over-ripe (g/100 g FW)
Aspartic acid	0.14 ± 0.04	0.16 ± 0.00	1.55 ± 0.06	2.57 ± 0.52	2.71 ± 0.13
Glutamic acid	0.19 ± 0.05	0.21 ± 0.00	0.52 ± 0.06	0.82 ± 0.11	4.22 ± 0.15
Hydroxyproline	0.28 ± 0.01	0.29 ± 0.01	0.01 ± 0.00	0.18 ± 0.01	0.15 ± 0.01
Serine	0.33 ± 0.01	0.35 ± 0.01	0.14 ± 0.00	0.24 ± 0.09	0.33 ± 0.01
Glycine	0.36 ± 0.00	0.38 ± 0.01	0.40 ± 0.07	0.66 ± 0.06	0.80 ± 0.05
Threonine	0.46 ± 0.00	0.46 ± 0.00	0.24 ± 0.01	0.24 ± 0.09	0.33 ± 0.01
Alanine	0.48 ± 0.00	0.48 ± 0.00	0.33 ± 0.06	1.59 ± 0.47	2.92 ± 0.09
Histidine	0.51 ± 0.00	0.51 ± 0.00	0.18 ± 0.08	0.44 ± 0.08	0.65 ± 0.17
Proline	0.54 ± 0.00	0.56 ± 0.00	0.09 ± 0.00	0.53 ± 0.09	0.26 ± 0.01
Arginine	0.59 ± 0.02	0.61 ± 0.05	0.10 ± 0.03	0.33 ± 0.07	0.41 ± 0.02
Tyrosine	0.76 ± 0.00	0.71 ± 0.00	0.06 ± 0.01	0.61 ± 0.03	0.70 ± 0.11
Valine	0.77 ± 0.00	0.77 ± 0.00	0.19 ± 0.05	0.03 ± 0.01	0.16 ± 0.03
Methionine	0.82 ± 0.00	0.82 ± 0.00	0.21 ± 0.05	0.02 ± 0.01	0.13 ± 0.02
Isoleucine	0.95 ± 0.00	0.95 ± 0.00	0.10 ± 0.01	0.23 ± 0.03	0.25 ± 0.04
Leucine	0.96 ± 0.00	0.96 ± 0.00	0.22 ± 0.03	0.61 ± 0.01	0.71 ± 0.16
Phenylalanine	1.08 ± 0.00	1.09 ± 0.01	0.15 ± 0.03	0.32 ± 0.01	0.72 ± 0.11
Lysine	1.15 ± 0.00	1.14 ± 0.00	0.03 ± 0.02	0.80 ± 0.22	1.53 ± 0.00

* Presented in mean ± standard deviation.
[†] RRT, relative retention time.

Preparation of amino acid standards and samples

   Prior to derivatization, breadfruit proteins were extracted by homogenizing 50 g of breadfruit tissue in water (150 mL) and passing the extract through two layers of cheesecloth. Each sample (6 mL) was pipetted into a screw cap Pyrex vial (16 × 125 mm) and 6 mL of 12N HCl added. The tube was flushed with nitrogen and then quickly capped and placed in an oven at 140°C for 32 h. After hydrolysis, the contents of the tube were filtered (Whatman No. 1, Whatman International Ltd., Kent, U.K.) to remove solids and stored at 4°C (2). A standard solution containing 2.5 µmol/mL of each amino acid was prepared in 0.1N HCl. Norleucine (10 µmol/mL) was used as the internal standard (IS). Unhydrolyzed samples (6 mL each) were treated with dilute acid (6 mL of 0.1N HCl) and stored at 4°C.

Preparation of fatty acid standards and samples

   Free fatty acids from breadfruit tissue (40 g) were extracted using 40 mL of a chloroform–isopropanol mixture (7:11, v/v). Extracts were centrifuged at 2000 × g for 5 min before use for derivatization. The extract (10 mL) was reduced to dryness under a stream of nitrogen and treated with 0.5N NaOH in methanol–water (90:10, v/v). Saponification was carried out overnight (7).

   Samples were then filtered (Whatman No. 1), extracted with 3 × 2 mL hexane, and reduced to dryness. The dried residue was used during derivatization. A mixture of fatty acids was prepared containing 5 mg/mL of each fatty acid in acetone. Petroselinic acid (5 mg/mL) was used as the IS.

Preparation of carbohydrate standards and samples

   Breadfruit tissue (5 g) was extracted twice with 30 mL of 80% ethanol and finally with 30 mL of pure distilled water (12). The extracts were pooled and heated at 90°C for 10 min. The extract was then treated with 10% lead acetate in order to remove precipitated proteins, and 10% oxalic acid was added to remove unreacted lead acetate (13). A standard solution of monosaccharides, disaccharides, and the trisaccharide raffinose was prepared containing 5 mg/mL of

Table III. Fatty Acid Content of Breadfruit Samples*

Fatty acid	RRT[†] (standards)	RRT (sample)	Mature green (mg/100 g FW)	Ripe (mg/100 g FW)	Over-ripe (mg/100 g FW)
Caproic	0.22 ± 0.00	0.26 ± 0.01	0.09 ± 0.02	0.24 ± 0.00	0.44 ± 0.08
Capric	0.31 ± 0.00	0.35 ± 0.01	n.d.[‡]	6.67 ± 0.23	7.06 ± 0.03
Lauric	0.44 ± 0.00	0.48 ± 0.02	1.36 ± 0.04	6.94 ± 0.35	16.57 ± 0.41
Myristic	0.64 ± 0.01	0.66 ± 0.03	5.44 ± 0.04	11.18 ± 0.21	31.95 ± 0.14
Palmitic	0.92 ± 0.01	0.89 ± 0.05	1.47 ± 0.33	4.91 ± 0.20	7.23 ± 0.22
Linolenic	1.09 ± 0.01	1.10 ± 0.04	0.01 ± 0.00	2.13 ± 0.23	1.19 ± 0.01
Linoleic	1.30 ± 0.00	1.32 ± 0.01	0.35 ± 0.02	0.15 ± 0.03	0.19 ± 0.41
Oleic	1.37 ± 0.02	1.39 ± 0.00	3.92 ± 0.27	10.34 ± 0.15	34.61 ± 0.07
Stearic	1.71 ± 0.03	1.74 ± 0.02	0.22 ± 0.06	2.24 ± 0.10	4.75 ± 0.41
Arachidic	2.31 ± 0.00	2.36 ± 0.00	0.39 ± 0.10	0.47 ± 0.09	1.21 ± 0.45
Behenic	2.84 ± 0.00	2.86 ± 0.03	0.03 ± 0.00	0.31 ± 0.01	0.63 ± 0.03

* Presented in mean ± standard deviation.
[†] RRT, relative retention time.
[‡] n.d., not detected.

Table IV. Carbohydrate Content of Breadfruit Samples*

Carbohydrate	RRT[†] (standards)	RRT (sample)	Mature green (mg/100 g FW)	Ripe (mg/100 g FW)	Over-ripe (mg/100 g FW)
Arabinose	0.45 ± 0.00	0.44 ± 0.00	0.22 ± 0.04	0.31 ± 0.02	0.47 ± 0.01
Xylose	0.58 ± 0.00	0.53 ± 0.00	0.19 ± 0.01	0.20 ± 0.01	0.32 ± 0.01
Fructose	0.70 ± 0.02	0.70 ± 0.01	n.d.[‡]	0.07 ± 0.03	0.16 ± 0.03
Galactose	0.83 ± 0.01	0.84 ± 0.00	0.18 ± 0.02	0.36 ± 0.00	0.34 ± 0.03
Glucose	0.90 ± 0.00	0.94 ± 0.02	0.22 ± 0.04	0.44 ± 0.03	0.37 ± 0.01
Sucrose	1.63 ± 0.00	1.66 ± 0.01	0.25 ± 0.04	0.62 ± 0.11	0.42 ± 0.08
Maltose	1.69 ± 0.01	1.70 ± 0.00	n.d.	0.01 ± 0.00	0.01 ± 0.00
Cellobiose	1.73 ± 0.00	1.73 ± 0.00	n.d.	0.01 ± 0.00	0.01 ± 0.00
Trehalose	1.77 ± 0.01	1.77 ± 0.02	n.d.	0.01 ± 0.00	0.01 ± 0.00
Gentiobiose	1.89 ± 0.02	1.85 ± 0.04	0.02 ± 0.00	0.03 ± 0.00	0.01 ± 0.00
Raffinose	2.28 ± 0.02	2.29 ± 0.03	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00

* Presented in mean ± standard deviation.
[†] RRT, relative retention time.
[‡] n.d., not detected.

each sugar in pyridine. Myo–inositol (10 mg/mL) was used as the IS.

HPLC apparatus

The HPLC equipment consisted of a chromatographic system (Beckman System Gold Nouveau, Beckman Coulter, Fullerton, CA) comprised of dual pumps, a solvent module (Model 126), a 20-µL Rheodyne (Rohnert Park, CA) injection loop, a UV–vis detector (Model 168), and an autosampler (Model 508) all linked to a computer running Gold Nouveau software. Amino acids were separated using a 5-µm Spherisorb (Supelco, Bellefonte, PA) ODS2 column (4.6 × 250 mm), and

Table V. Percent Recovery of Amino Acids, Fatty Acids, and Carbohydrates from Breadfruit Samples*

Sample	Expected value (mg)	Observed value (mg)[†]	%Recovery
Amino acid	10.0	7.25 ± 0.14	72.5
Fatty acid	10.0	6.82 ± 0.05	68.2
Carbohydrate	5.0	4.07 ± 0.33	81.4

* All values represent the average of triplicate analysis.
[†] Presented in mean ± standard deviation.

fatty acids were separated using a 5-µm Spherisorb S5 C_8 column (4.6 × 250 mm).

GLC apparatus

The GLC equipment consisted of a Pye Unicam Series 204 gas chromatograph (GC) (Cambridge, U.K.) fitted with a flame ionization detector. Monosaccharides, disaccharides, and trisaccharides were separated using 3% OV 17 (80–100) mesh packed into a glass column (6-mm o.d., 1.5-m × 4-mm i.d.). The GLC was interfaced with an MK II digital integrator coupled with an LKB 2210 recorder.
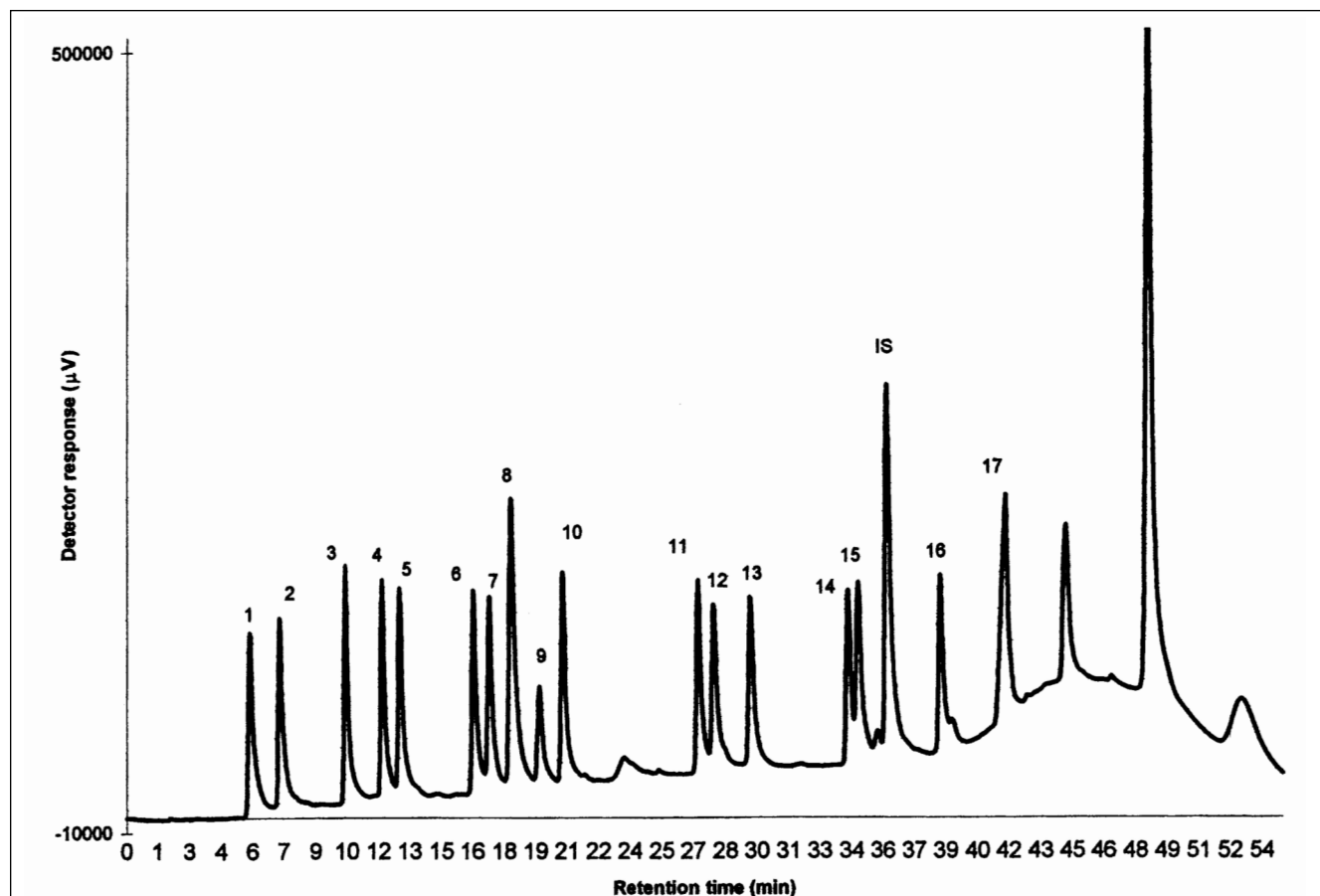
Derivatization procedures

Derivatization of amino acids

The method used for the derivatization of amino acids was a modification of a method used by González–Castro et al. (2). A standard amino acid solution (20 µL) containing 2.5 µmol/mL of each amino acid in 0.10N HCl was pipetted into a screw cap vial, and 10 µL of norleucine (10 µmol/mL) was then added. The latter was used as an IS. The solvent was driven off under a stream of nitrogen in a heating block at 70°C. To the residue, 60 µL of methanol–water–TEA (2:2:1, v/v) was added and the solvent removed under nitrogen. Then, 60 µL of the derivatizing reagent consisting of methanol–water–TEA–PITC (7:1:1:1, v/v) was added. The tubes were agitated and left to

Figure 1. Chromatogram of a PITC amino acid standard: aspartic acid, 1; glutamic acid, 2; hydroxyproline, 3; serine, 4; glycine, 5; histidine, 6; arginine, 7; threonine, 8; alanine, 9; proline, 10; tyrosine, 11; valine, 12; methionine, 13; isoleucine, 14; leucine, 15; phenylalanine, 16; lysine, 17; norleucine, IS; and the unlabelled peaks were reaction byproducts.

stand at room temperature for 20 min. The solvents were removed under a stream of nitrogen, and the tubes were sealed and stored at 4°C pending analysis. Prior to injection, 150 µL of 0.05M ammonium acetate was added to each tube. The non-hydrolyzed and hydrolyzed breadfruit samples (200 µL each) containing 10 µL of a stock solution of norleucine (10 µmol/mL) were derivatized in the same way as was the standard amino acids.

Derivatization of fatty acids

The method employed for the derivatization of the fatty acids was a modification of a method used by F. Borsch (14). A standard solution (1 mL) containing 5 mg/mL of each fatty acid was pipetted into a screw cap vial. Five microliters of a stock solution of petroselinic acid (5 mg/mL) was added as the IS. To this solution, 0.5 mL of phenacyl bromide in acetone (12 mg/mL) and 0.5 mL of TEA in acetone (10 mg/mL) were added. Vials were allowed to incubate overnight (12 h) at room temperature. Samples were then ready for HPLC analysis. For the breadfruit samples, 5 mL of the extract was spiked with 5 µL of a stock solution of petroselinic acid (5 mg/mL). Derivatization was the same as for the standard fatty acids.

Derivatization of carbohydrates

The method used for the derivatization of the carbohydrates was a modification of methods used by P.M. Holligan (15) and Chen and McGinnis (11). A solution was prepared containing the monosaccharides, disaccharides, trisaccharide (raffinose), and the IS (myo-inositol) all at a concentration of 1 mg/mL in pyridine. One milliliter of the solution was placed in a reaction vial and treated with 100 µL of hexamethyldisilazane and 100 µL of trimethylchlorosilane. The vial was sealed and heated at 60°C for 30 min to allow for the formation of trimethylsilylethers. The vial was allowed to cool, and the solvent evaporated under a stream of nitrogen. The residue was redissolved in 100 µL of hexane. For the breadfruit samples, 5 mL of the extract was placed in a reaction vial and reduced to dryness. The residue was redissolved with 1 mL of pyridine, which contained myo-inositol at a concentration of 1 mg/mL. Derivatization was carried out as mentioned previously.

Determination of starch content

The method used for the determination of the starch content of breadfruit was a modification of a method used by J.B. Harbourne (16). Breadfruit tissue (200 g) was homogenized with 1% NaCl (800 mL). The homogenate was filtered through four layers of muslin. The residue was re-extracted twice with 150 mL of 1% NaCl and the extracts pooled. The mixture was allowed to stand for 1 h to facilitate the settling of the starch granules. The supernatant was discarded and the wet starch washed thrice with 150 mL of 1% NaCl, once with 150 mL of 0.01M NaOH, and once with 150 mL of water. Finally, the starch was drained and dried at 50°C until a constant weight was obtained.
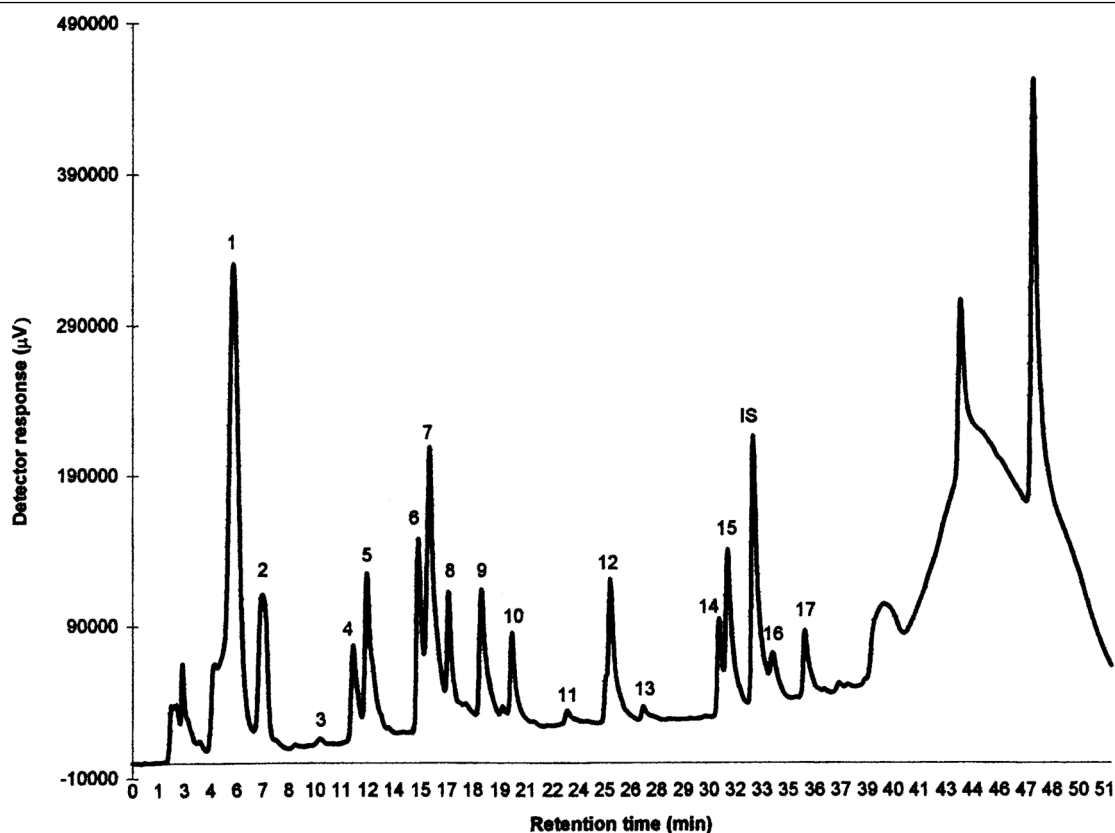
Figure 2. A typical chromatogram showing the amino acids detected in hydrolyzed breadfruit samples: aspartic acid, 1; glutamic acid, 2; hydroxyproline, 3; serine, 4; glycine, 5; histidine, 6; arginine, 7; threonine, 8; alanine, 9; proline, 10; tyrosine, 11; valine, 12; methionine, 13; isoleucine, 14; leucine, 15; phenylalanine, 16; lysine, 17; norleucine, IS; and the unlabelled peaks were reaction byproducts.

Spiked samples for percent recovery

Three separate vials were prepared: a known amount of alanine (10 mg) and norleucine (0.66 mg) were added to vial 1, palmitic acid (10 mg) and petroselinic acid (5 mg) were added to vial 2, and gentibiose (5 mg) and myoinositol (5 mg) were added to vial 3. Five milliliters of the breadfruit extract was added to each vial, and the extraction and derivatization processes were carried out on each as per usual. For vials 1, 2, and 3, percent recovery was obtained by comparing the amounts that were found with the amounts that were added (all based on the IS method).

Identification and quantitation

The amount of each component in the breadfruit sample was quantitated using the following formula (17):

amount of component = RF × area of component ×
(area of IS in calibration / area of IS in analysis)   Eq. 1

The response factor (RF) was calculated during the calibration of each component. The ratio of the area of the IS peak (in the calibration) to that of the sample was used to adjust for any changes in the size of the IS peak.

The relative retention time (RRT), which is the ratio of the retention time of the standard compounds to that of the IS, was used to identify the amino acids, fatty acids, and carbohydrates present in the samples. Although there may have been a significant variation in the retention times of the components in the samples, the RRT of these components remained relatively constant when compared with the RRT of the standards. This is illustrated in Tables I, II, III, and IV.

Analysis of samples
Analysis of amino acids

Chromatography was carried out at ambient temperature using a gradient elution as follows: eluent A was an aqueous buffer consisting of 0.05M ammonium acetate, eluent B consisted of 0.01M ammonium acetate in acetonitrile–methanol–water (44:10:46, v/v), and both eluents were adjusted to pH 6.8 with phosphoric acid.

In the gradient program employed, the pumps were operated with 0% of B initially for 2 min at a rate of 1 mL/min. This was increased to 50% of B in a period of 30 min, after which there was a further increase from 50% B to 75% in 5 min. Finally, 75% B was maintained for 5 min and reduced to 0% in 10 min.

Analysis of fatty acids

Fatty acids were separated by HPLC at ambient temperature using a gradient elution. Eluent A was acetonitrile and eluant B was water. The pumps were operated initially with 30% B at a rate of 2 mL/min. Then, 30% B was reduced to 0% over 50 min.

Analysis of carbohydrates

The trimethylsilyl ethers formed were separated by the temperature programming of the GC. For the analysis of monosaccharides, disaccharides, and trisaccharides, the column temperature was operated at 150°C for 4 min initially, programmed at a rate of 8°C/min to 290°C, and maintained at this
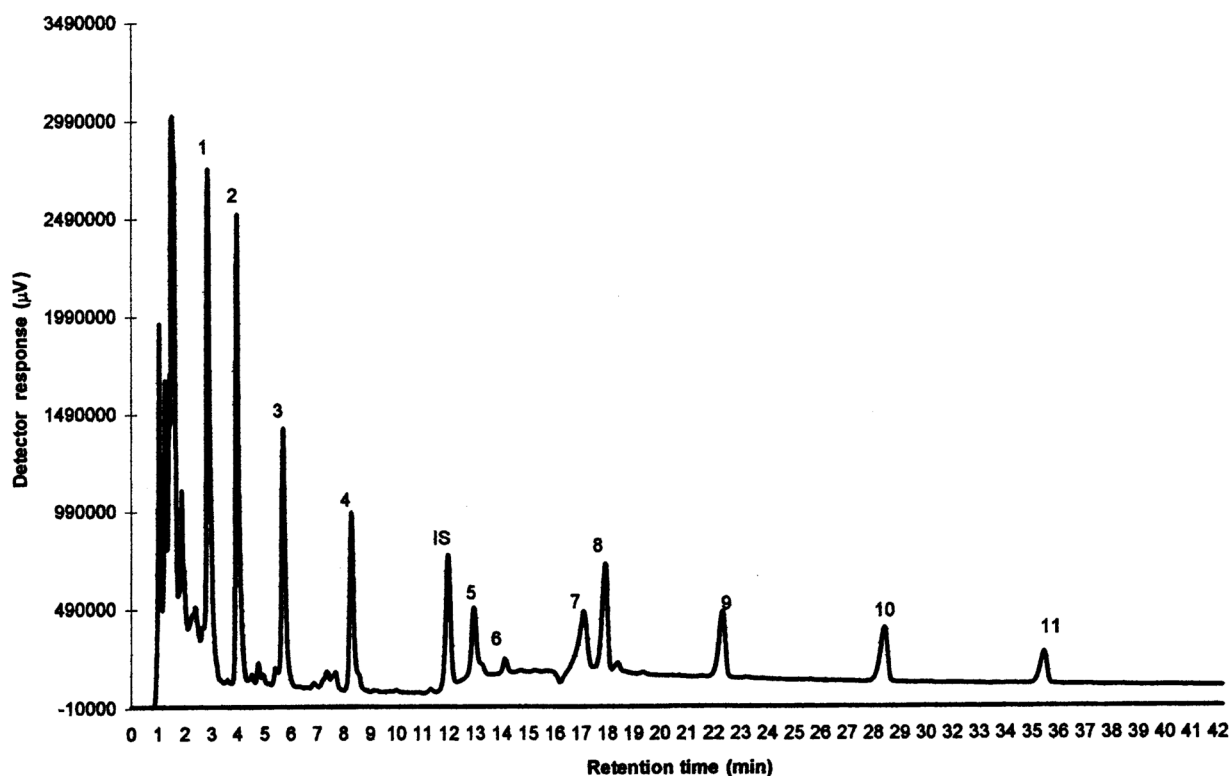

Figure 3. Chromatogram of standard fatty acid phenacyl esters: caproic acid, 1; capric acid, 2; lauric acid, 3; myristic acid, 4; linolenic acid, 5; palmitic acid, 6; oleic acid, 7; linoleic acid, 8; stearic acid, 9; arachidic acid, 10; behenic acid, 11; and petroselinic acid, IS.

temperature for 30 min. The nitrogen carrier gas flow rate was 45 mL/min. The injector port and detector temperatures were 220°C and 300°C, respectively.

Results and Discussion

In this study, chromatographic methods were employed for the separation and identification of the amino acids, fatty acids, and carbohydrates in breadfruit. Fatty acids and amino acids were analyzed by HPLC, and monosaccharides, disaccharides, and trisaccharides were analyzed by GC. In analyzing the amino acid, fatty acid, and carbohydrate content of the breadfruit sample, the ripe developmental stage was selected for closer scrutiny because breadfruit is generally eaten most often during this developmental stage.

Figures 1 and 2 show the chromatograms of the PITC–amino acid standard mixture and a typical breadfruit sample, respectively. The specific amounts of amino acids detected in the samples are listed in Tables I and II. Table I lists the mean ± standard deviation in grams per 100 g fresh weight (FW) for asparagine, glutamine, tryptophan, and cysteine. These values reflect the typical values of these amino acids, which are sensitive to the acid hydrolysis treatment employed in the quantitation of the other amino acids. Upon hydrolysis, asparagine and glutamine are converted to aspartic acid and glutamic acid, respectively, and tryptophan and cysteine are destroyed (1).

Table II lists the mean ± standard deviation (grams per 100 g FW) for the amino acids in hydrolyzed breadfruit samples. The amino acids with the highest concentrations were aspartic acid (2.57 ± 0.52 g/100 g FW, ripe stage) and alanine (1.59 ± 0.47 g/100 g FW, ripe stage).

All of the essential amino acids were detected in the breadfruit sample in varying amounts. The essential amino acids that were found in the greatest amounts were leucine (0.61 ± 0.01 g/100 g FW) and lysine (0.80 ± 0.22 g/100 g FW) during the ripe developmental stage. These essential amino acids accounted for approximately 30% of the total amino acid content of the breadfruit sample, rendering it a relatively good source of essential amino acids in a diet.

Figures 3 and 4 show the chromatograms for the fatty acid phenacyl esters of the fatty acid standards and the breadfruit sample, respectively. The amounts of each fatty acid detected are listed in Table III as the mean ± standard deviation in milligrams per 100 g FW. The fatty acids detected with the highest concentrations at the ripe stage were myristic acid (11.18 ± 0.21 mg/100 g FW) and oleic acid (10.34 ± 0.15 mg/100 g FW). The essential fatty acids, linoleic acid (0.15 ± 0.03 mg/100 g FW) and linolenic acid (2.13 ± 0.23 mg/100 g FW), were also
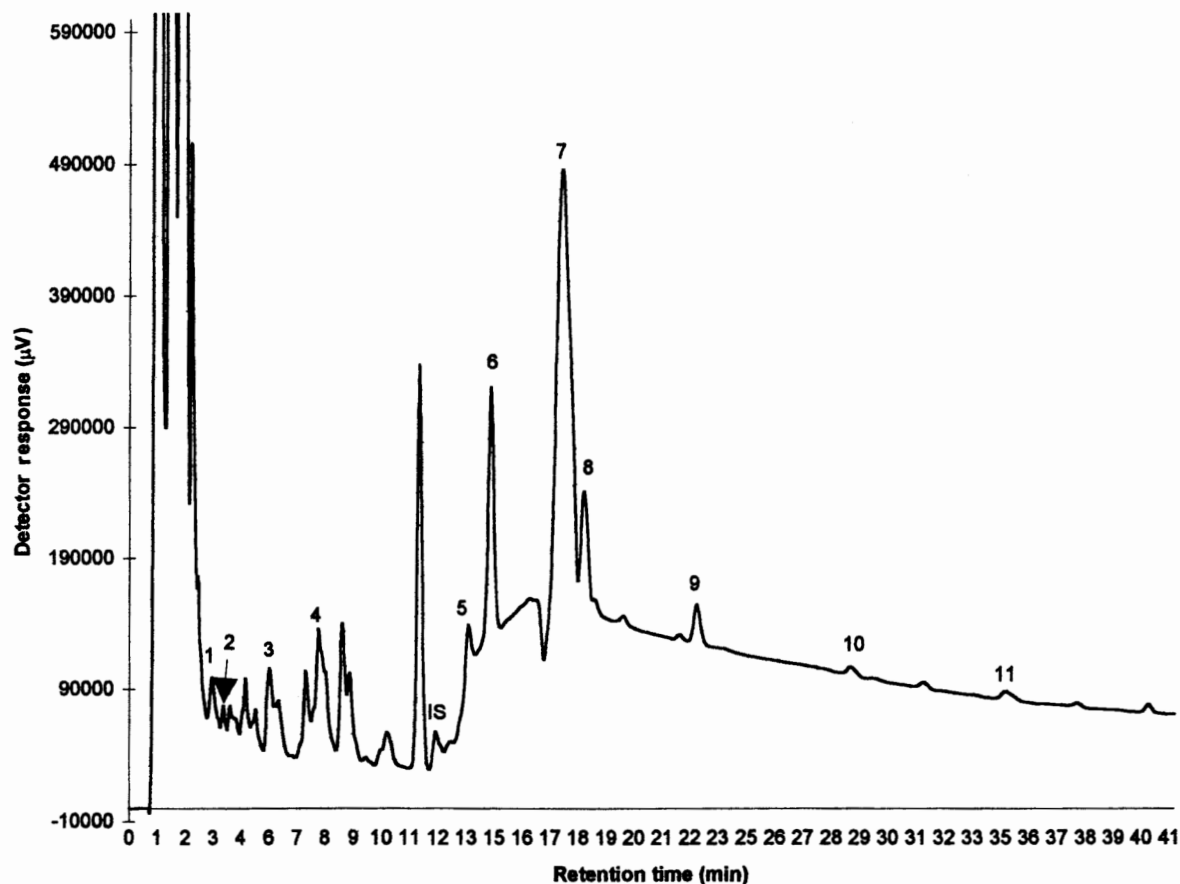
Figure 4. A typical chromatogram showing the fatty acids detected in breadfruit samples: caproic acid, 1; capric acid, 2; lauric acid, 3; myristic acid, 4; linolenic acid, 5; palmitic acid, 6; oleic acid, 7; linoleic acid, 8; stearic acid, 9; arachidic acid, 10; behenic acid, 11; and petroselinic acid, IS.

detected at the ripe stage. These essential fatty acids accounted for approximately 5% of the total fatty acid content of the breadfruit sample (at the ripe stage), which was not surprising because the best sources for essential fatty acids and fatty acids in general are oils (18). The total saturated fatty acids at the ripe stage was 72.3% and the total unsaturated fatty acids was 27.7%. It is rather interesting that this fruit contains less unsaturated fatty acids than saturated fatty acids, because the opposite is usually the case with respect to plants (18).

Table IV lists the mean ± standard deviation for the carbohydrates in milligrams per 100 g of FW in the breadfruit sample. The monosaccharides with the highest concentration at the ripe stage were glucose (0.44 ± 0.03 g/100 g FW) and galactose (0.36 ± 0.00 g/100 g FW). The disaccharides with the highest concentration at the ripe stage were sucrose (0.62 ± 0.11 g/100 g FW) and gentiobiose (0.03 ± 0.007 g /100 g FW). The starch content of the breadfruit sample was determined to be 15.52 g/100g FW.

The percent recoveries for the amino acids, fatty acids, and carbohydrates from the breadfruit sample (Table V) were evaluated by spiking triplicate samples of breadfruit extracts with known standard(s) and subjecting them to the usual extraction and derivatization procedure. The carbohydrates gave the highest recovery (81.4%) followed by the amino acids (72.5%) and the fatty acids (68.2%).

There was a general increase in the amino acid, fatty acid, and carbohydrate content from the green to the overripe developmental stages. This apparent increase in the case of the amino acids can be attributed to the increase in enzyme synthesis and activity, which is known to be associated with the ripening process. In the case of the monosaccharides, the increasing reduction of the sugar content in the ripe and overripe developmental stages can be attributed to the degradative processes associated with ripening. Another consideration is the action of hydrolytic enzymes such as invertase that converts sucrose into glucose and fructose (19). This in turn results in a decrease in the content of some polysaccharides and oligosaccharides.

With respect to the fatty acids, the softening of the plant matrix during ripening allows the free fatty acids to be extracted with greater ease from the ripe and overripe stages than from the green developmental stage.

Conclusion

This paper reports on the nutritive value of the breadfruit sample as a staple based on the amino acids, fatty acids, and monosaccharides it contains. This is of extreme importance because breadfruit is a major staple of the Jamaican people and is of growing importance to the food industry. The results reported in this study indicate that breadfruit is a relatively good source for essential amino acids and fatty acids and is also a good source of energy with the monosaccharides, disaccharides, and trisaccharide it contains.

The relatively low percentage recoveries for the fatty acids (68.2%) and to a lesser extent the amino acids (72.5%) seems to imply that with improved extraction procedures, a more accurate estimate of the fatty acid and amino acid content of breadfruit can be achieved.

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