Characterization of Jamaican Agro-Industrial Wastes. Part I: Characterization of Amino Acids Using HPLC: Pre-column Derivatization with Phenylisothiocyanate

Y.A. Bailey-Shaw[1,*], K.D. Golden[1], A.G.M. Pearson[1], and **R.B.R. Porter[2]**

[1]Department of Basic Medical Sciences (Biochemistry Section) and [2]Department of Chemistry, University of the West Indies, Mona, Kingston 7, Jamaica W.I.

Abstract

Jamaican agro-industries generate large quantities of wastes, which are either discarded or under-utilized. In order to evaluate the possible utilization of these wastes, it is necessary that the profiles of the major biochemical groups be developed. This paper describes the determination of the amino acid composition of coffee, citrus, and rum distillery wastes using a reversed-phase high-performance liquid chromatography method. Acid hydrolysates of the wastes are derivatized with phenylisothiocyanate. They are analyzed as their phenylthiocarbamyl derivatives and determined quantitatively using norleucine as the internal standard. The presence of all the 17 amino acids investigated, nine of which include those essential for animal nutrition, are observed in the samples investigated, suggesting a high quality of protein with implications in the formulation of animal feeds.

Introduction

Agro-industrial wastes contain carbohydrates, proteins, and lipids, among other biochemicals. However, these wastes are often discarded. A strategy of bioremediation is to utilize these materials as components of animal feeds. Approximately 1000 million tonnes of animal feed is produced globally on an annual basis, with feed for poultry produced in the highest tonnage, followed by pig and cattle feeds. Although feed production for aquaculture is currently relatively low (14 million tonnes), there has been an increasing demand for feed for farmed fish and crustaceans. Protein is the key building block for these feed formulation systems, with the three main sources being from oilmeals (316 million tonnes, soybean dominating as a protein source), animal by-products (10 million tonnes), and fishmeal (7 million tonnes) (1). However, due to increasing importation costs, world population growth, particularly in developing countries, and recent public concerns associated with genetically modified crops such as soybean and maize (corn), incidents of dioxin contamination of feeds and bovine spongiform encephalopathy (BSE) or mad cow disease, which is said to be linked to contaminated meat and bone meal fed to animals, the utilization of cheaper, indigenous feed materials from alternative sources is now becoming highly desirable (2).

Jamaica, being a developing country, is no exception to these adverse effects. Currently, Jamaica has three animal feed mills [Newport Mills, Masterblend, and Jamaica Livestock Association (JLA) Feed Mills], all utilizing corn and soybean in their manufacturing processes. However in recent times, the sustained high importation prices for corn and soybeans, for which there is no domestic production, has resulted in a 12% increase in domestic feed prices, which helped to curb the 11.3 and 6.5% expansion in the poultry industry during 2000 and 2001, respectively, to a mere 1.1% during 2002. Due to this escalation in feed prices, a significant proportion of smaller independent poultry farmers, who represent ~ 30% of the poultry industry, shifted production to a seasonal basis. In addition, there was restricted growth in mixed feed production, resulting in an expansion in feed production of less than 1% in 2003 (351,000 MT) and a similar percentage in 2004 (356,000 MT) (3). On this basis, the search for alternative sources of protein for animal feed is therefore timely.

The quality of the protein in terms of animal feed is determined by the amino acid composition and their availability to the animals. These are usually supplied in the form of protein or crystalline amino acids in the feed (4). However, the requirements will vary, depending on the species and the age of the animals. The objective of this study was, therefore, to characterize the amino acids in coffee, citrus, and rum distillery wastes in terms of their composition using high-performance liquid chromatography (HPLC).

Determination of amino acid composition has been achieved by the use of several techniques. These include gas chromatography (5–7), capillary electrophoresis (8), and HPLC (9–30). In recent times, most amino acid analyses have been conducted using

* Author to whom correspondence should be addressed: yvonneb@src-jamaica.org

HPLC, in which a universal detection method such as refractive index, light scattering, or very low UV wavelengths are used, or derivatization with substances that absorb in the visible/UV wavelengths or fluoresce. Derivatization is done after elution from an HPLC column (post-column derivatization) or prior to HPLC separation (pre-column derivatization). Post-column derivatization methodologies involving the use of reagents such as ninhydrin (9) and *o*-phthaladehyde (OPA) (10,11) have been published. The relative instability of the ninhydrin reagent has however been cited as a major disadvantage in using this method (12). The remaining techniques which involve the use of pre-column derivatization with phenylisothiocyanate (PITC) (13–16), 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) (17,18), OPA (19–22), dimethylaminoazobenzenesulfonyl chloride (DABSYL Chloride) (23,24), dimethylaminonaphthalenesulfonyl chloride (DANSYL Chloride) (25, 26), 9-fluorenylmethyl chloroformate (FMOC-Cl) (27, 28) and 4-fluoro-7-nitrobenzo-2-oxa-1,3-diazole or NBD-F (29, 30) have also been published.

Pre-column derivatization procedures are generally faster and more sensitive than post-column derivatization (31). Nonetheless, these applications have been limited by several factors. These include: the lack of reactivity with secondary amines such as proline, instability of the fluorescent products, and difficulties in quantitation due to their sensitivity to quenchers in the case of OPA (13,14). The lack of selectivity (reacts with both hydroxyl and amine groups), long reaction times, and high reaction temperatures required by dansyl chloride and the need to use excess FMOC-Cl reagent that must be extracted prior to chromatography (often results in hydrolysis and loss of FMOC–amino acid adducts) (13), are but a few of the limitations of pre-column derivatization. However, the choice of any one technique is ultimately determined by the sensitivity required from the assay. PITC was on this basis the reagent of choice for use in this study because it reacts with both primary and secondary amino acids to yield stable phenylthiocarbamyl (PTC) derivatives that can be detected by UV absorption at 254 nm. In addition, the analysis is rapid, sensitive, and simple to quantify, overcoming the disadvantages of detection using dansyl chloride, OPA, among others.

Experimental

Samples

Coffee processing wastes [coffee pulp, coffee pulping water (water that takes the pulp from the pulper) and wash water containing mucilage (water that takes the mucilage from the aquapulper)] were obtained from a coffee factory (traditional aquapulper, wet processing) in St. Andrew, Jamaica.

Citrus wastes [juice extractor residues-orange and grapefruit pulp, wash water (water used to wash fruits and floors) and press liquor (waste from pressing of juice extractor residues during production of citrus meal)] were obtained from a commercial citrus processing plant in St. Catherine, Jamaica.

Liquid distillery wastes [pot stills and fermentor bottoms (light and heavy) and continuous still effluent] were obtained from a sugar factory/rum distillery in Trelawny, Jamaica.

Reagents

All reagents used, unless otherwise stated, were analytical grade. A kit of individual amino acid standards, HPLC-grade methanol and triethylamine (TEA) were obtained from Sigma Chemical (St. Louis, MO). HPLC-grade acetonitrile was obtained from Acros (New Jersey, NJ). PITC and ammonium acetate were obtained from Aldrich Chemical (Milwaukee, WI). Water used was deionized then purified with a Milli-Q ultrapure water system (Millipore, Bedford, MA).

HPLC Apparatus

HPLC analyses were performed using a Beckman System Gold-Nouveau HPLC Unit (Beckman Instruments, Fullerton, CA) equipped with a 126 Programmable Solvent Module with a binary pump, a 168 Photodiode Array Detector, a 508 Autosampler fitted with a 20 µL loop and utilizing Gold Nouveau Software. Chromatographic separation was performed on an analytical reverse phased column (Waters Spherisorb ODS2, 5 µm, 250 × 4.6 mm) at room temperature (26°C).

Sample Preparation

Prior to extraction, solid samples (coffee pulp and citrus juice extractor residues) were freeze dried and stored in sealed plastic bags in a dessicator at 0–4°C.

Citrus press liquor, citrus wash water, coffee pulping water, and wash water containing mucilage were stored at –10°C without prior preparation. The distillery wastes, on the other hand, were separated into fractions of dissolved (supernatant) and suspended solids (pellets) by centrifugation procedures prior to analysis. Both fractions were stored at 4°C.

Reference to "solid wastes" from here on will relate to coffee pulp, citrus juice extractor residues, and suspended solids of distillery wastes, whereas "liquid wastes" will be used to refer to press liquor, washing water (coffee and citrus), and dissolved solids of distillery wastes.

Protein extraction from solids

Total soluble protein was extracted from samples of solids using 20 mM sodium phosphate buffer (pH 7) containing 0.1% Triton-X-100 (TX-100). Approximately 0.5 g of freeze dried sample was homogenized for 30 s at high speed with 15 mL of chilled extractant in a Waring laboratory blender pre-chilled to ~ 4°C. The homogenate was then sonicated (Heat Systems Ultrasonics Inc. Sonicator/Cell Disruptor, Model W 220F fitted with a microtip) for 10 min at 60 W using pulses of 45 s while keeping the tube immersed in an ice bath. The sonicated sample was then centrifuged at 27,200 × g, for 30 min at 4°C (Beckman J2-21 Centrifuge, JA – 20 rotor). The supernatant was decanted and the homogenization, sonication, and centrifugation procedures repeated using the pellet. The pellet was washed twice only where possible. The supernatants obtained were pooled, and the combined solution used as the soluble protein extract.

Protein extraction from liquids

Total soluble proteins were extracted from liquid wastes using 0.15 M sodium chloride. Approximately 5 mL of liquid sample was diluted with 5 mL of 0.15 M solution of sodium chloride. The resulting mixture was then sonicated for 1 min at 60 W using

pulses of 45 s. The sonicated sample was then incubated with 1 mL of 0.1% TX-100 at room temperature (26°C) for 30 min. This final solution was used as the soluble protein extract.

Hydrolysis and derivatization of amino acids

Soluble protein extracts were hydrolyzed according to the method of González-Castro et al. (13) with minor modifications. Approximately 6 mL of 12 N HCl was added to 6 mL of sample extract in a 16 × 125 mm, teflon-lined screw cap Pyrex tube, to produce a solution with a final concentration of 6 N. The tube was thoroughly flushed with nitrogen (N_2), quickly capped, and placed in an oven at 110°C for 24 h. The contents of the tube were vacuum filtered, (Whatman No. 1 filter paper, Whatman International Ltd., Kent, U.K.) to remove solids after hydrolysis. Aliquots of the solution were further filtered through 0.45-µm pore-size membrane (Millipore Corp., Bedford, MA).

Derivatization was carried out according to the method of González-Castro et al. (13) to which minor modifications were made. A standard mixture containing 2.5 µmol/mL of each amino acid in 0.10 N HCl was prepared. Twenty microliters (20 µL) of this standard or 100 µL of hydrolyzed sample extract were then mixed with 10 µL of norleucine (10 µmol/mL) in an Eppendorf tube. The norleucine was used as an internal standard. The mixture was then dried in vacuo using a Savant Speedvac. Sixty microliters (60 µL) of methanol–water–triethylamine (TEA) (2:2:1, v/v) was added to the residue, the contents of the tube were then mixed and dried in vacuo. Sixty microliters (60 µL) of the derivatizing reagent, methanol–water–TEA–PITC (7:1:1:1, v/v) was added, the tube was agitated and left to stand at room temperature for 20 min. A stream of N_2 was used to remove the solvents and the tube was closed and stored at 4°C prior to analysis. Prior to injection, 300 µL of 0.05 M ammonium acetate was added to each tube.

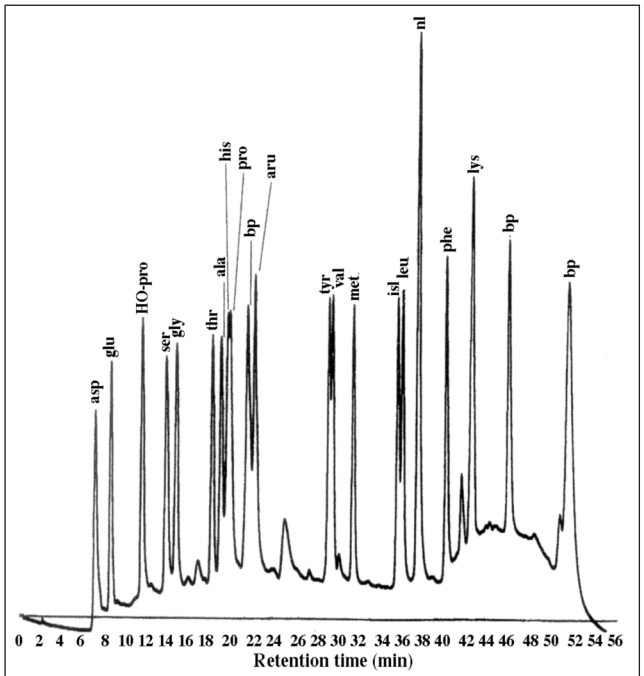

Figure 1. Chromatogram of PTC standard amino acids. Peak labels are as follows: asp, aspartic acid; glu, glutamic acid; oh-pro, hydroxy-proline; ser, serine; gly, glycine; thr, threonine; ala, alanine; his, histidine; pro, proline; arg, arginine; tyr, tyrosine; val, valine; met, methionine; isl, isoleucine; leu, leucine; nl, norleucine; phe, phenylalanine; lys, lysine; bp, by-products (PITC-TEA derivatives).

Recovery of amino acids

The efficiencies of the extraction, hydrolysis, and derivatization procedures were investigated by replacing 1 mL of the extractant with 1 mL of 5.88 µmol/mL standard amino acid mixture (spiking), extracting total soluble proteins, and then subjecting the extracts to hydrolysis and derivatization as described earlier.

Recoveries were calculated based on the difference between the total amount determined in the spiked and that in the samples extracted without the addition of amino acids (non-spiked).

HPLC analysis

A step-wise gradient elution program developed in our laboratory was used. Solvent A consisted of an aqueous solution of 0.05 M ammonium acetate and Solvent B was 0.1 M ammonium acetate in acetonitrile–methanol–water (44:10:46), both adjusted to pH 6.8 with phosphoric acid (10). The profile was as follows: 0–0.2 min, 0% B; 0.2–35 min, 50% B; 35–40 min, 75% B; 40–45 min, 75% B; 45–55 min, 0% B, followed by the next injection. The flow rate was 1 mL/min, and the detector was set at a wavelength of 254 nm. Twenty microliters (20 µL) of each sample was injected for analysis. All HPLC determinations were conducted in duplicate.

Results and Discussion

The elution profile of the PTC standard amino acid mixture showed a good separation of each component (Figure 1). PITC–TEA-derived components were detected in both standard and samples; however, these components did not cause any

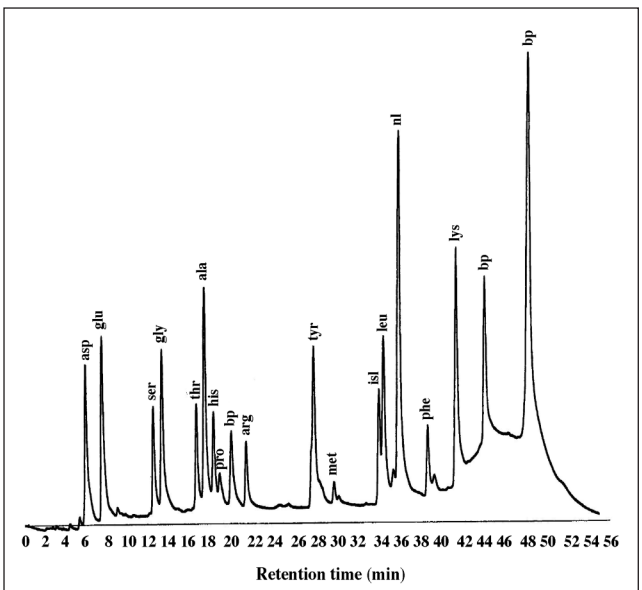

Figure 2. Chromatogram of PTC amino acids in continuous still effluent suspended solid (pellet). Peak labels are the same as Figure 1.

interference in the detection of the PTC amino acids. These peaks were observed to elute near arginine and after all the other PTC-amino acids had eluted. González-Castro et al. (13) and Heinrikson and Meredith (14) made similar observations. However, the latter authors suggested that distillation of the pyridine and triethylamine would reduce the number and extent of these peaks. Similar profiles were obtained for sample extracts.

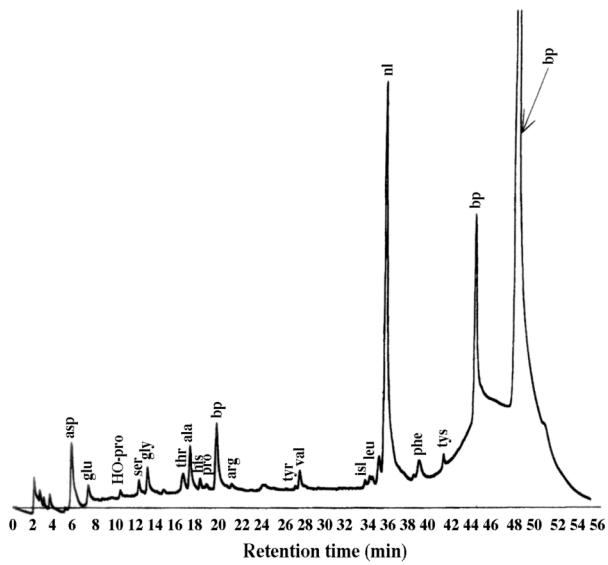

Figure 3. Chromatogram of PTC amino acids in continuous still effluent dissolved solids (supernatant). Peak Labes are the same as Figure 1.

Representative chromatograms of some liquid and solid waste samples are illustrated in Figures 2 and 3.

The content of each amino acid was calculated using the norleucine internal standard addition method, and the percentage recoveries calculated by spiking duplicate samples with a standard mixture of amino acids and then subjecting them to extraction, hydrolysis, and derivatization procedures.

Tables I–IV list the amino acid contents and percentage recoveries for all wastes examined. Of importance is the fact that all amino acids essential for animal nutrition: phenylalanine, histidine, arginine, leucine, threonine, lysine, methionine, isoleucine, and valine (32), with the exception of tryptophan, which was completely destroyed during acid hydrolysis (33), were detected in the samples analyzed, suggesting a high quality of protein. However, it should be noted that due to variations in digestion and absorption, the ration which has the highest protein quality is usually the one that supplies all the essential amino acids needed, in proportions similar to those in which they exist in the protein to be formed (34).

Continuous still suspended solids effluent had the highest total amino acid content of all the solid wastes (119.8 mg/100 g), while heavy fermentor bottom suspended solids effluent was found to have the lowest content of total amino acid (16.4 mg/100 g). With the exception of coffee pulp (43.8% of the total amino acid detected were essential amino acids), more than 50% of total amino acids detected in the solid waste samples were amino acids essential to animal nutrition. Orange pulp, which had a total amino acid content of 83.7 mg/100 g, contained the highest percentage (71.3%) of essential amino acids.

On the other hand, analysis of data with respect to liquid wastes revealed that heavy pot still effluent (dissolved solids) contained the highest total amino acid content (49.7 mg/L). Citrus press liquor was found to contain the lowest content of total amino acid (3.20 mg/L). All liquid wastes, with the exception of pot stills: heavy and light, and heavy fermentor bottom effluents (39.7%, 45.3%, and 45.0% essential amino acids, respectively), were found to contain greater than 50% of their total amino acids essential to animal nutrition.

Profiles of the amino acid compositions obtained for coffee pulp, citrus pulp, and distillery wastes were similar to those reported in the literature (35,36). Other comparisons were not possible due to the unavailability of published data. All 17 amino acids investigated were detected in the samples. However, no comparisons of the quantitative distribution of amino acids in the experimental samples to those reported in literature were done because reported values were expressed on a constant nitrogen basis, that is, g/16 g N or as % crude protein, assuming that all proteins contain exactly 16% nitrogen.

In comparing the quantitative distribution of amino acids among the solid waste samples, it was

Table I. Amino Acid Content and Recoveries of Coffee Processing Wastes as Determined by RP- HPLC of PTC-Amino Acids*

Amino Acid	Coffee Pulp mg/100 g Dry Weight	Recovery (%)	Pulping Water (mg/L)	Recovery (%)	Wash Water with Mucilage (mg/L)	Recovery (%)
Asp	5.1 ± 0.2	97.0	0.81 ± 0.06	99.1	1.45 ± 0.02	97.9
Glu	3.9 ± 0.2	96.6	2.0 ± 0.1	99.1	3.20 ± 0.02	97.5
Phe†	1.5 ± 0.4	96.8	0.55 ± 0.02	99.1	0.180 ± 0.001	97.2
Gly	0.99 ± 0.05	97.0	1.06 ± 0.09	99.3	1.16 ± 0.08	97.7
His†	5.9 ± 0.1	94.4	4.1 ± 0.2	98.6	7.11 ± 0.04	96.3
Arg†	0.66 ± 0.09	98.0	1.89 ± 0.09	99.5	2.1 ± 0.2	98.4
Pro	1.13 ± 0.07	96.9	1.8 ± 0.1	99.3	2.4 ± 0.5	97.8
Leu†	1.0700 ± 0.0004	95.1	0.88± 0.05	98.9	1.11 ± 0.03	96.7
Thr†	1.60 ± 0 .02	97.8	1.42 ± 0.04	99.4	1.5 ± 0.5	98.0
Ala	1.77 ± 0.05	96.1	1.71 ± 0.05	99.2	2.1 ± 0.4	97.2
Lys†	0.18 ± 0.01	98.3	1.07 ± 0.09	99.1	1.00 ± 0.04	96.7
Met†	0.1000 ± 0.0005	98.6	1.14 00 ± 0.0003	99.1	0.8840 ± 0.0002	97.1
Isl†	0.34 ± 0.04	96.9	0.31 ± 0.01	99.3	0.43 ± 0.03	97.9
Ser	1.83 ± 0.02	98.4	1.7 ± 0.1	99.5	0.85 ± 0.03	98.1
Val†	0.56 ± 0.02	96.3	0.82 ± 0.03	99.0	0.79 ± 0.04	97.1
HO-Pro	0.221± 0.008	97.4	0.0700 ± 0.0008	99.3	0.1700 ± 0.0002	98.1
Tyr	0.36 ± 0.06	96.3	0.460 ± 0.003	99.3	2.2 ± 0.2	98.2
Total	**27.2**		**21.8**		**28.6**	
EAA %	**43.8**		**55.9**		**52.8**	

* Values in table represent the mean of 2 determinations ± standard error of the mean. Abbreviations are as follows: aspartic acid (Asp); glutamic acid (Glu); phenylalanine (Phe); glycine (Gly); histidine (His); arginine (Arg); proline (Pro); leucine (Leu); threonine (Thr); alanine (Ala); lysine (Lys); methionine (Met); isoleucine (Isl); serine (Ser); valine (Val); hydroxyl-proline (HO-Pro); and tyrosine (Tyr).

† EAA = Essential Amino Acids.

observed that histidine was the major amino acid in all samples, while methionine and hydroxyl proline were present in the lowest concentrations. The content of the remaining amino acids were variable. In observations made with respect to the quantitative distribution of amino acids in liquid wastes, it is important to note that, while histidine was not the major amino acid in all liquid wastes, it was present in a relatively high concentration in the majority of samples. Methionine and hydroxyl proline were also found to be present in very low concentrations in several liquid waste samples.

Methionine has been recognized as one of three limiting amino acids being most likely to be deficient in the rations of swine and poultry; the others being lysine and tryptophan (36). Lysine, methionine, and histidine have also been identified as limited in cattle diets (37,38). The low concentrations of methionine in these samples should not, however, be taken as a negative indicator because the use of these materials will ultimately be decided by their digestibility and how well the amino acids supplied match the nutrient requirements of the animals.

High levels of histidine were observed in the samples. However, these results were not in keeping with published data on the amino acid profiles of coffee pulp (35), citrus pulp (36), and distillery wastes (36), which indicated that although histidine is present, it is not a major constituent of these materials. The significance of the findings of high levels of histidine in a great majority of the samples, should however not be overlooked, since it has been recognized that supplying histidine to diets deficient in metabolizable histidine can improve protein deposition by growing cattle. A determination of the nutritional factors that influence the efficiency with which histidine is utilized in cattle could, therefore, improve on current methods of balancing diets to meet protein requirements (37). Wastes from these three agro-industries could therefore positively impact on Jamaica's dairy industry.

Recoveries of amino acid standards in solid wastes were generally good with the exception of orange waste, which had the lowest overall percentage recovery, ranging from 61.5% (proline)–94.0% (methionine). In the case of liquid wastes, recoveries of amino acids were also good with most being in the high nineties; continuous still effluent (suspended solids), however, had recoveries of amino acids that were mostly in the eighties. The extraction, hydrolysis, and derivatization procedures can therefore be described as good, and the results can be used with confidence. This is highly significant because no published information was found, indicating the use of these methods on materials of this nature.

No comparisons of the quantitative distribution of amino acids in the experimental samples were made with the amino acid profiles of two most common animal feeds, corn and soybean, since literature values were calculated on a per gram of nitrogen basis, which would have included all non-protein nitrogen, thus resulting in values higher than those in the samples investigated. It is important to note, however, that investigations by Elías (35) revealed that coffee pulp protein contained similar or higher levels of amino acids than soybean flour and corn. Coffee pulp was also found to be deficient in sulfur-containing amino acids, but had relatively high levels of lysine, as high as those found in soybean meal on a per gram of nitrogen basis.

Distillery wastes contain dead yeast cells; hence, observations with respect to the high levels of total amino acids in some distillery samples were not surprising. Yeast protein has been described as excellent for a vegetable protein and is about equivalent in quality to soybean protein. Both are rich in lysine and deficient in sulphur-containing amino acids. In addition,

Table II. Amino Acid Content and Recoveries of Citrus Processing Wastes as Determined by RP-HPLC of PTC-Amino Acids*

Amino Acid	Grapefruit Pulp mg/100 g	Rec. (%)	Orange Pulp (mg/100 g)	Rec. (%)	Press Liquor (mg/L)	Rec. (%)	Wash Water (mg/L)	Rec. (%)
Asp	10.7 ± 1.0	99.6	3.4 ± 0.5	77.5	0.120 ± 0.007	94.1	0.2650 ± 0.0003	99.2
Glu	6.9 ± 0.6	97.6	3.0 ± 0.7	68.2	0.36 ± 0.04	93.4	0.7020 ± 0.0003	99.1
Phe†	3.3 ± 0.1	98.3	0.92 ± 0.08	68.1	0.89 ± 0.02	94.9	0.23 ± 0.02	99.1
Gly	1.5 ± 0.3	98.4	0.99 ± 0.01	76.0	0.120 ± 0.008	94.2	0.25 ± 0.01	99.2
His†	25.2 ± 4.6	NR	44.4 ± 1.6	NR	0.24 ± 0.02	82.7	4.0. ± 0.5	98.6
Arg†	2.1 ± 0.4	97.9	2.9 ± 0.1	90.7	0.120 ± 0.009	96.1	0.41 ± 0.03	99.5
Pro	6.4 ± 1.2	NR	11..3 ± 0.4	61.5	0.080 ± 0.002	91.8	1.0 ± 0.1	99.2
Leu†	3.4 ± 0.6	97.4	1.9 ± 0.2	77.0	0.076 ± 0.003	93.3	0.34 ± 0.04	99.0
Thr†	4.6 ± 0.1	95.1	4.3 ± 0.2	80.1	0.17 ± 0.02	94.7	0.580 ± 0.001	99.4
Ala	4.2 ± 0.8	95.2	3.1 ± 0.1	74.5	0.22 ± 0.01	93.1	0.38 ± 0.01	99.0
Lys†	4.9 ± 0.3	97.9	2.2 ± 0.5	85.2	0.181 ± 0.009	93.6	0.31 ± 0.02	99.1
Met†	0.389 ± 0.002	99.0	0.68 ± 0.04	94.0	0.040 ± 0.002	95.1	0.28 ± 0.07	99.3
Isl†	0.87 ± 0.02	97.0	0.8 ± 0.2	84.2	0.0600 ± 0.0009	94.4	0.060 ±0.003	99.3
Ser	2.1 ± 0.2	98.2	1.3 ± 0.5	80.5	0.115 ± 0.007	95.4	0.235 ± 0.003	99.4
Val†	1.3 ± 0.4	95.7	1.6 ± 0.9	76.6	0.13 ± 0.02	94.3	0.32 ± 0.01	99.1
HO-Pro	0.234 ± 0.003	98.0	0.29 ± 0.08	87.2	0.142 ± 0.004	95.5	0.2650 ± 0.0003	99.4
Tyr	0.6 ± 0.1	96.7	0.6 ± 0.1	69.1	0.17 ± 0.03	94.7	0.360 ± 0.001	99.3
Total	**78.7**		**83.7**		**3.20**		**10.0**	
EAA%	**58.5**		**71.3**		**59.6**		**65.3**	

* Values in table represent the mean of 2 determinations ± standard error of the mean. Abbreviations are the same as in Table I.
† EAA = Essential Amino Acids

Table III. Amino Acid Content and Recoveries of Distillery Wastes (Dissolved Solids) as Determined by RP-HPLC of PTC-Amino Acids*

Amino Acid	CS (mg/L)	Rec. (%)	LPS (mg/L)	Rec. (%)	HPS (mg/L)	Rec. (%)	LFB (mg/L)	Rec. (%)	HFB (mg/L)	Rec. (%)
Asp	3.4 ± 0.8	98.9	6.4 ± 0.4	99.7	10.80 ± 0.04	99.1	3.0 ± 0.2	99.4	6.8 ± 1.6	99.3
Glu	1.42 ± 0.08	98.7	2.0 ± 0.3	99.4	6.13 ± 0.09	99.4	4.5 ± 0.1	99.3	7.69 ± 0.06	99.3
Phe†	0.33 ± 0.01	98.4	1.01 ± 0.05	98.9	2.93 ± 0.02	99.3	2.16 ± 0.08	99.2	3.3 ± 0.1	98.9
Gly	0.59 ± 0.03	98.5	0.65 ± 0.03	99.4	2.03 ± 0.49	99.2	0.94 ± 0.02	99.3	2.3 ± 0.9	99.4
His†	2.6 ± 0.2	96.5	2.5 ± 0.7	99.0	2.700 ± 0.003	97.5	3.7000 ± 0.0005	99.2	2.1 ± 0.2	96.6
Arg†	3.5 ± 0.3	99.4	1.8 ± 0.1	99.7	6.500 ± 0.004	100.0	4.1 ± 0.4	99.8	6.5 ± 0.5	100.0
Pro	0.65 ± 0.04	98.3	0.57 ± 0.02	99.4	3.16 ± 0.02	99.5	2.9 ± 0.3	99.6	1.5 ± 0.4	99.4
Leu†	1.1 ± 0.3	98.5	1.00 ± 0.04	99.0	2.3 ± 0.1	99.1	1.360 ± 0.006	99.0	2.6 ± 0.4	99.0
Thr†	0.83 ± 0.07	98.7	1.6 ± 0.3	99.5	1.6 ± 0.1	99.2	1.330 ± 0.008	99.4	1.7 ± 0.2	99.1
Ala	1.7 ± 0.1	98.2	2.5 ± 0.2	99.4	3.0 ± 0.1	98.6	4.3 ± 0.8	99.2	4.4 ± 0.3	98.3
Lys†	0.45 ± 0.02	99.3	1.38 ± 0.07	98.9	0.9 ± 0.1	99.0	2.5 ± 0.4	99.2	2.1 ± 0.1	99.2
Met†	0.3340 ± 0.0002	98.7	0.392 ± 0.005	99.9	ND‡	ND‡	0.680 ± 0.005	100.0	ND‡	ND‡
Isl†	0.42 ± 0.02	98.5	0.63 ± 0.01	99.3	1.2 ± 0.5	99.2	0.61 ± 0.01	99.3	1.34 ± 0.04	99.2
Ser	1.07 ± 0.07	98.8	0.82 ± 0.02	99.5	3.4 ± 0.2	99.6	0.57 ± 0.05	99.4	1.8 ± 0.5	99.7
Val†	0.83 ± 0.01	98.3	1.1 ± 0.2	99.0	1.6 ± 0.1	99.0	1.0 ± 0.3	99.0	1.88 ± 0.08	98.9
HO-Pro	0.27 ± 0.02	98.8	0.39 ± 0.03	99.4	1.0 ± 0.2	99.3	0.280 ± 0.002	99.3	0.65 ± 0.02	99.2
Tyr	0.23 ± 0.03	98.9	0.40 ± 0.01	99.4	0.462 ± 0.008	99.3	0.3 ± 0.0	99.3	1.1 ± 0.1	99.2
Total	**19.7**		**25.1**		**49.7**		**34.2**		**47.8**	
EAA %	**52.6**		**45.3**		**39.7**		**51.0**		**45.0**	

* Values in table represent the mean of 2 determinations ± standard error of the mean. Abbreviations are the same as in Table I. additional abbreviations: continuous still effluent (CS); light pot still (LPS); heavy pot still (HPS); light fermentor bottom (LFB); heavy fermentor bottom (HFB)
† EAA = Essential Amino Acids
‡ ND = Not Detected

Table IV. Amino Acid Content and Recoveries of Distillery Wastes (Suspended Solids) as Determined by RP-HPLC of PTC-Amino Acids*

Amino Acid	CS (mg/100 g)	Rec. (%)	LPS (mg/100 g)	Rec. (%)	HPS (mg/100 g)	Rec. (%)	LFB (mg/100 g)	Rec. (%)	HFB (mg/100 g)	Rec. %
Asp	10.4 ± 0.5	87.0	8.6 ± 1.3	95.1	5.1 ± 0.5	94.0	1.1 ± 0.1	99.0	1.4 ± 0.4	97.8
Glu	16.3 ± 4.4	87.5	15.4 ± 2.1	95.9	10.8 ± 0.5	95.2	2.2 ± 0.4	98.4	2.0 ± 0.2	96.5
Phe†	5.0 ± 0.9	81.8	4.52 ± 0.07	91.1	3.9 ± 0.8	93.5	0.6 ± 0.1	98.0	0.7 ± 0.1	98.5
Gly	5.3 ± 0.1	88.8	3.9 ± 0.3	92.6	3.1 ± 0.2	92.0	0.78 ± 0.07	98.3	0.74 ± 0.08	97.1
His†	15.60 ± 0.05	80.3	19.2 ± 1.8	94.6	11.5 ± 3.9	88.4	2.6 ± 0.4	99.2	2.2 ± 0.4	93.4
Arg†	3.8 ± 0.8	93.8	3.56 ± 0.09	96.5	1.9 ± 0.3	96.2	0.58 ± 0.09	98.9	0.542 ± 0.004	98.2
Pro	2.8 ± 0.1	88.9	4.9 ± 0.5	97.8	2.4 ± 0.1	94.1	0.66 ± 0.01	98.6	0.5 ± 0.2	96.9
Leu†	11.5 ± 1.9	89.0	11.2 ± 0.5	95.9	6.7 ± 0.4	92.4	1.7 ± 0.1	97.4	1.4 ± 0.2	95.2
Thr†	5.8 ± 0.9	91.7	4.85 ± 0.07	96.1	3.5 ± 0.1	95.1	0.74 ± 0.08	98.4	0.82 ± 0.01	98.1
Ala	9.7 ± 0.2	88.7	8.8 ± 0.9	95.0	5.81 ± 0.04	91.9	1.33 ± 0.03	97.4	1.2 ± 0.2	95.8
Lys†	14.4 ± 2.1	84.7	8.0 ± 0.3	90.2	5.5 ± 0.2	96.3	1.2 ± 0.1	97.9	1.6 ± 0.2	97.2
Met†	1.0400 ± 0.0005	84.8	1.01 ± 0.05	93.3	0.65 ± 0.05	95.3	0.16 ± 0.06	97.8	0.18 ± 0.01	98.6
Isl†	5.8 ± 0.2	89.8	6.8 ± 0.2	97.4	4.09 ± 0.01	95.1	0.87 ± 0.01	98.8	0.78 ± 0.02	98.6
Ser	4.5 ± 0.9	91.4	4.1 ± 0.1	96.3	2.4 ± 0.2	95.1	0.62 ± 0.06	98.7	0.61 ± 0.05	97.7
Val†	4.3 ± 0.3	81.6	8.1 ± 0.1	94.8	5.4 ± 0.3	92.7	1.23 ± 0.08	97.2	1.1 ± 0.1	96.1
HO-Pro	ND‡	ND‡	ND‡	ND	0.4890 ± 0.0002	95.9	0.0300 ± 0.0007	98.7	ND	ND
Tyr	3.6 ± 0.1	90.0	3.6 ± 0.5	96.0	3.3 ± 0.7	95.9	0.61 ± 0.03	98.8	0.61 ± 0.07	98.5
Total	**119.8**		**116.5**		**76.5**		**17.0**		**16.4**	
EAA%	**56.1**		**57.7**		**56.3**		**57.1**		**56.8**	

* Values in Table represent the mean of 2 determinations ± standard error of the mean. Abbreviations are the same as in Table I and III.
† EAA = Essential Amino Acids
‡ ND = Not Detected

because 20% of the crude protein nitrogen in yeast is in the form of nucleic acids, there is a limit as to how much can be fed because excessive nucleic acid intake can result in elevated uric acid levels in the blood (39).

Conclusion

The supply of protein and amino acids in animal diets represents a significant cost of production. Supplementation of animal feeds with citrus, coffee, and rum distilleries wastes, which contain valuable amino acids can no doubt considerably reduce the cost of imported feeds, especially from the standpoint of Jamaica. The total amino acid profile is a good indicator of the potential nutritive value of these agro-industrial wastes. The results of this study suggest that the HPLC determination and quantitation of the phenylthiocarbamyl derivatives of amino acids can undoubtedly be used in the development of the total amino acid profiles of agro-industrial wastes.

Acknowledgement

This study was financed by grants provided by the Environmental Foundation of Jamaica (EFJ) and the University of the West Indies, Mona, Jamaica. The authors would also like to thank, Dr. Lawrence Willliams, who assisted with editing the manuscript and Mr. Timon Waugh, Dr. Percival Miller and Ms. Paulette Tavares who facilitated sample collection and provided technical information relevant to the coffee and citrus industries respectively.

References

1. R. Gilbert. The world feed industry. In: FAO Animal Production and Health: Protein sources for the animal industry: Expert Consultation and Workshop, Bangkok, 29 April – 3 March 2002. Food and Agriculture Organization of the United Nations, Ed. Rome pp. 1-8 (2004)
2. Food and Agriculture Organization (FAO). Executive Summary. In: FAO Animal Production and Health: Protein sources for the animal industry: Expert Consultation and Workshop, Bangkok, 29 April – 3 March 2002. Food and Agriculture Organization of the United Nations, Ed. Rome pp. ix-xxv (2004)
3. S. Thomas. Jamaica and Dep: Oilseeds and Products Annual 2004. USDA Foreign Agriculture Service Global Agriculture Information Network Report. pp. 1–9 (2004).
4. Y. Toride. Lysine and other amino acids for feed: production and contribution to protein utilization in animal feeding. In: FAO Animal Production and Health: Protein sources for the animal industry: Expert Consultation and Workshop, Bangkok, 29 April – 3 March 2002. Food and Agriculture Organization of the United Nations, Ed. Rome pp. 161–184 (2004).
5. P. Hušek. Rapid derivatization and gas chromatographic determination of amino acids. *J. Chromatogr.* **552:** 289–299 (1991).
6. K.L. Woo and D.K. Chang. Determination of 22 protein amino acids as n (o)-tert.-butyldimethylsilyl derivatives by gas chromatography. *J. Chromatogr.* **638:** 97–107 (1993).
7. J. Wang, Z.H. Huang, D.A. Gage, and J.T. Watson. Analysis of amino acids by gas chromatography–flame ionization detection and gas chromatography–mass spectrometry: simultaneous derivatization of functional groups by an aqueous-phase chloroformate-mediated reaction. *J. Chromatogr. A* **663:** 71–78 (1994).
8. J. Smith. Developments in amino acid analysis using capillary electrophoresis. *Electrophoresis* **18:** 2377–2392 (1997).
9. D.H. Spackman, W.H. Stein and S. Moore. Automatic reading apparatus for use in the chromatography of amino acids. *Anal. Chem.* **30(1):** 190–1205 (1958).
10. S.E. Moller. Quantification of physiological amino acids by gradient ion-exchange high-performance liquid chromatography. *J. Chromatogr.* **613:** 223–230 (1993)
11. M.W. Dong and J.R. Grant. High-speed liquid chromatographic analysis of amino acids by post-column sodium hypochlorite, o-phthaldehyde reaction. *J. Chromatogr.* **327:** 17–25 (1985).
12. J. Le Boucher, C. Charret, C. Coudray-Lucas, J. Giboudeau, and L. Cynober. Amino acid determination in biological fluids by automated ion-exchange chromatography: performance of Hitachi L-8500A. *Clin. Chem.* **43(8):** 1421–1428 (1997).
13. M.J. González-Castro, J. López-Hernandez, J. Simal-Lozano, and M.J. Oruña-Concha. Determination of amino acids in green beans by derivatization with phenylisothiocyanate and high-performance liquid chromatography with ultraviolet detection. *J. Chromatogr. Sci.* **35:** 181–185 (1997).
14. R.L. Heinrikson and S.C. Meredith. Amino acid analysis by reverse-phase high-performance liquid chromatography: precolumn derivatization with phenylisothiocyanate. *Anal. Biochem.* **136:** 65–74 (1984).
15. C.-Y. Yang and F.I. Sepulveda. Separation of phenylthiocarbomyl amino acids by high-performance liquid chromatography on spherisorb octadecylsilane columns. *J. Chromatogr.* **346:** 413–416 (1985).
16. V. Fierabracci, P. Masiello, M. Novelli, and E. Bergamini. Application of amino acid analysis by high-performance liquid chromatography with phenylisothiocyanate derivatization to the rapid determination of free amino acids in biological samples. *J. Chromatogr.* **570:** 285–291 (1991).
17. S.A. Cohen and D.P. Michaud. Synthesis of a fluorescent derivatizing reagent, 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate, and its application for the analysis of hydrolysate amino acids via high-performance liquid chromatography. *Anal. Biochem.* **211(2):** 279–287 (1993).
18. M. Reverter, T. Lundh, and J.E. Lindberg. Determination of free amino acids in pig plasma by precolumn derivatization with 6–aminoqinolyl-*N*-hydroxysuccinimidyl carbamate and high-performance liquid chromatography. *J. Chromatogr. B: Biomed. Sci. Appl.* **696(1):** 1–8 (1997).
19. P. Lindroth and K. Mopper. High performance liquid chromatographic determination of subpicomole amounts of amino acids by precolumn fluorescent determination with o-phthaldialdehyde. *Anal. Chem.* **51:** 1667–1674 (1979).
20. B.N. Jones and J.P. Gilligan. O-phthaldialdehyde precolumn derivatization and reversed-phase high performance liquid chromatography of polypeptide hydrolysates and physiological fluids. *J. Chromatogr.* **266:** 471–482 (1983).
21. G. Ali Qureshi, L. Fohlin, and J. Bergström. Application of high-performance liquid chromatography to the determination of free amino acids in physiological fluids. *J. Chromatogr.* **297:** 91–100 (1984).
22. D. Fekkes, A. van Dalen, M. Edelman, and A. Voskuilen. Validation of the determination of amino acids in plasma by high – performance liquid chromatography using automated pre-column derivatization with o-phthaldialdehyde. *J. Chromatogr. B: Biomed. Sci. Appl.* **669(2):** 177–186 (1995).
23. J.Y. Chang, R. Knecht, and D.G. Braun. Amino acid analysis at the picomole level. *Biochem. J.* **199:** 547–555 (1981).
24. J.Y. Chang, R. Knecht, and D.G. Braun. Methods in Enzymology 91. C.H.W. Hirs, and S. N. Timasheff, Eds. Academic Press, New York, NY, pp. 41–48 (1983).
25. Y. Tapuchi, D.E. Schmidt, W. Lindner, and B. L. Karger. Dansylation of amino acids for high-performance liquid chromatography analysis. *Anal. Biochem.* **115:** 123–129 (1981)
26. N. Kaneda, M. Sato, and K. Yagi. Analysis of dansyl amino acids by reverse phase high-performance liquid chromatography. *Anal. Biochem.* **127(1):** 49–54 (1982).
27. S. Einarsson, B. Josefsson, and S. Lagerkvist. Determination of amino acids with 9-fluorenylmethyl chloroformate and reversed-phase high performance liquid chromatography. *J. Chromatogr.* **282:** 609–618 (1983).
28. S. Einarsson, S. Folestad, B. Josefsson, and S. Lagerkvist. High-resolution reversed phase liquid chromatography system for the analysis of complex solutions of primary and secondary amino acids. *Anal. Chem.* **58:** 1638–1643 (1986).
29. H. Kotaniguchi and M. Kawakatsu. Automatic amino acid analysis utilizing 4-fluoro-7-nitrobenzo-2-oxa-1,3-diazole. *J. Chromatogr.* **420:** 141–145 (1987).
30. C. Aoyama, T. Santa, M. Tsunoda, T. Fukushima, C. Kitada, and K. Imai. A fully automated amino acid analyzer using NBD-F as a fluorescent derivatization reagent. *Biomed. Chromatogr.* **18(9):** 630–636 (2004).
31. S.A. Cohen. Amino acid analysis using pre-column derivatization with 6-aminoquinolyl-N-hydroxylsuccinimidyl carbamate: Analysis of hydrolyzed proteins and electroblotted samples. In: Methods in Molecular Biology, 211: Protein Sequencing Protocols, 2nd ed. B.J. Smith, Ed. Humana Press Inc., Totowa, NJ. pp. 143–154 (2003).
32. S.J. Watson. The constituents of animal products. In: *Feeding of Livestock*. Thomas Nelson and Sons Ltd., New York, pp.16-31 (1949).
33. P. Hušek and K. Macek. Gas chromatography of amino acids. *J. Chromatogr.* **113(2):** 139–230 (1975).
34. L.A Maynard and J.K. Loosli. The proteins and their metabolism. In: Animal Nutrition. McGraw-Hill Book Company, New York, pp. 115–153 (1969).
35. L.G. Elías. Chemical composition of coffee berry by-products. In: Coffee Pulp-Composition, Technology and Utilization. R. Bressani & J.E. Braham (Eds.). Ottawa, Ont. IDRC-108e, pp. 11–16 (1979).
36. C.M. Lyman, K.A. Kuiken, and F. Hale. Amino acids in feedstuffs: Essential amino acid content of farm feeds. *J. Agric. Food Chem.* **4(12):** 1008–1013 (1956).
37. K.C. McCuistion, E.C. Titgemeyer, M.S. Awawdeh, and D.P. Gnad. Histidine utilization by growing steers is not negatively affected by increased supply of either ammonia or amino acids. *J. Anim. Sci.* **82:** 759–769 (2004).
38. R.H. Greenwood and E.C. Titgemeyer. Limiting amino acids for growing Holstein steers limit-fed soybean hull-based diets. *J. Anim. Sci.* **78:** 1997–2004 (2000).
39. C.W. Stone. Yeast products in the feed industry: A practical guide for feed professionals. Diamond V. Mills, Cedar Rapids, Iowa, pp. 1–16 (1998).

Manuscript received June 23, 2008;
revision received January 19, 2009.