Palm Kernel Cake Amino Acid Composition Determination by High Performance Liquid Chromatography

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ABSTRACT

A reverse-phase High Performance Liquid Chromatography (HPLC) column (3.9 x 150.0 mm) was used to determine amino acid composition in palm kernel cake (PKC) in 40 min (based on Waters AccQ Tag method). The amino acid analysis was carried out in three steps i.e., *substrate hydrolysis, chromatographic separation and data analysis. 18 amino acids were separated after precolumn derivatisation. The amino acids separated were glutamic acid, aspartic acid, alanine, arginine, cystine, glycine, histidine, isoleucine, leucine, lysine, methionine. phenylalnine, proline, serine, threonine, tyrosine and valine. Glutamic acid and aspartic acid were the major non-essential amino acids in palm kernel cake accounting for 3.01% and 2.08% of the total recovered amino acids respectively. Trythopan, methionine and cystein which were destroyed during hydrolysis with Hydrochloric acid (HCl) required special method for the analysis.*

Keywords: high performance liquid chromatography, amino acid, palm kernel cake (PKC).

INTRODUCTION

Palm kernel cake (PKC) is the residue obtained after the oil has been extracted from oil palm fruits via mechanical pressing or solvent extraction. There is less residual oil in the meal obtained via solvent extraction than the expeller process. Malaysia produced about 4.89 million tonne of palm kernels in 2019 of which 2.59 million tonne of PKC produced as it's by-product (MPOB Statistic, 2020). Of this, 2.49 million tonne of PKC was exported and nearly most of them were sent to European countries, for use as ingredient in animal feed. PKC has in average 2.0%-9.0% oil and 14.0%-17.0% protein (*Table 1*).

TABLE 1. PROXIMATE ANALYSIS OF PALM KERNEL CAKE (PKC)

	Mechanical extraction	Solvent extraction
Protein (%)	14.8	17.0
Shell (%)	3.9	4.2
moisture (%)	7.0	9.4
Oil content (%)	8.9	2.0

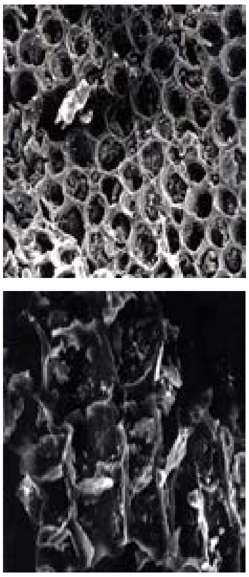
Source: Siew W L (1985).

Figure 1 are the images of PKC by scanning electron microscope (SEM). The image showed that the protein of PKC by mechanical extraction (*Figure 1a*) is fully protected by a tough cell wall, resulted less protein than by solvent extraction (*Figure 1b*).

Table 2 shows the amino acid profile of the PKC by mechanical press. Glutamic acid and arginine are the most abundant amino acid in PKC.

Composition of amino acid provides important quantitative parameter in protein characterisation. Two techniques available for amino acid analyse are ion-exchange separation coupled with post-column derivatisation and pre-column derivatisation with reversed-phase high performance liquid chromatography (HPLC). Three separate steps with highly interrelated function *i.e,* sample preparation and hydrolysis, separation and derivatisation and data analysis are needed before HPLC analysis.

Hydrolysis either chemical or enzymatic, liberates all the amino acids substrate which were then recovered in the hydrolysates. Chemical hydrolysis can be either acidic or basic but acidic hydrolysis seemed to more be popular (Bidlingmeyer *et al.,* 1984; Gehrke *et al.,* 1985; Zumwalt *et al.,* 1987).



- PKC by mechanical extraction
- Honeycomb like cell structure.
- Cell wall are tough and still intact.
- Cell content mainly oil and protein may still entrapped in the cell.

- PKC by solvent extraction.
- Preconditioning treatment weaken cell wall structure.
- Broken and fluffy cell wall.
- Cell content mainly oil and protein easily released.

Figure 1. Scanning Electron Microscope (SEM) of palm kernel cake (PKC) by solvent extraction (1a) and PKC by expeller extraction (1b).
Source: Osman A (2002).

TABLE 2. AMINO ACID CONTENTS OF PKC (%) (g/16 g N)

Alanine	3.83
Arginine	11.56
Aspartic acid	3.63
Cystine	1.13
Glycine	4.17
Glutamic acid	16.80
Histidine	1.91
Isoleucine	3.22
Leucine	6.07
Lysine	2.68
Methionine	1.75
Phenyalanine	3.96
Proline	3.31
Serine	4.11
Threonine	2.75
Tryptophan	1.06
Tyrosine	2.60
Valine	5.05

Several amino acids undergo modifications during hydrolysis with 6 M HCl for 24 hr at 110°C. Aspargine and glutamine hydrolysed quantitively to aspartic acid and glutamic acid while trythophan is largely destroyed particularly in the presence of carbohydrate. Protein hydrolyzed with methanesulfonic acid probably gives the best recovery for trythopan (Hugli *et al.,* 1972).

Hydrolysate containing amino acids needs to be derived prior to analysis. Post-column technique involves separation of free amino acids by ion exchange chromatography and subsequent visualisation by reaction with a chromaphore. Pre-column system, derives the amino acids in the hydrolysate and then separates them on a reverse-phase HPLC column (Husek, 1975). Reversed-phase HPLC coupled with fluorescent detection is suitable for analysing samples containing less than 20 amino acids.

This article reports on the amino acid composition in PKC as determined by reversed phase HPLC based on Waters AccQ tag method. The technique involved precolumn amino acids derivatisation using 6-aminoquinolyl-n-hydroxysuccinimidyl carbamate (AQC), which gave them a fluorescene tag (Cohen and Michaud, 1993). Amino acids derivatives were then separated by reversed-phase chromatography and detected by fluorescence detector at 395 nm wavelength.

MATERIALS AND METHODS

Materials

PKC by mechanical press obtained were provided by a mill in Klang, Selangor.

Chemicals and Reagents

AccQTag Chemistry packages for amino acid analysis consists of 6-aminoquinolyl-N-hydroxysuccinimidyyl carbamate (AccQ) Fluor reagent, AccQTag eluent and amino acid standard were obtained from Waters, USA. The internal standard, α amino acid butyric (AABA) were from Sigma-Aldrich, USA. Hydrochloric acid and acetonitrile of HPLC were purchased from Merck, Germany. High purity water was supplied by a Milli-Q purification system.

Proximate Analysis

Ash, moisture, shell and oil contents were determined according to Association of Official Analytical Chemists (AOAC) method (1990).

Total Protein Content

Crude protein in PKC was determined by conventional acid hydrolysis and Kjeldahl digestion using selenium catalyst according to method describde in AOAC, 1995. Ammonia was distilled and collected in a boric acid solution, which was then titrated using 0.1 N HCl. Digestion and distillation were carried out using Kjeltec apparatus (Model 2200 Auto distillation, Foss Tecator, Denmark). Protein was calculated as N x 6.25 and reported as percent protein.

Instrument

Waters Breeze HPLC System (USA) consisting of a Waters Breeze Gradient System, Waters 1525 Binary HPLC pump, Waters 7725 injector and AccQTag reversed–phase column (3.9 x 150 mm) was used. The column was maintained at 36°C by Waters 1500 column heater. Detection was carried out by Waters 2475 multi-Fluorescent detector with excitation at wavelength 250 nm and emission at 395 nm.

Acid Hydrolysis

PKC samples were dissolved in 6 M hydrochloric acid. The solutions were gassed with nitrogen and sealed in tubes before incubated in an oven at 110°C for 24 hr. The samples were then allowed to cool upon hydrolysis completion of hydrolysis.

Sample Preparation and Addition of Internal Standard for Amino Acid Composition

Hydrolysate samples were filtered using Whatman filter No.1 into 100 ml volumetric flask (*Figure 2*). 400 µl AABA was pipeted into the flask and pure water was used to add up to the mark. 10 µl samples was added with 70 µl AccQFluor buffer and vortexed for 1 min for derivatisation. The mixture was then mixed thoroughly with 20 µl AccQFluor reagent. 10µl of the derivative sample was then injected into a AccQTag column (150 x 3.9 mm) at 36°C with detection at 395 nm. The AccQ Tag eluent diluted in 10% MilliQ water and used as eluent A, and 60% acetonitrile as eluent B in separation gradient with flow rate of 1.0 ml min^{-1}. The separation gradient used was 0-0.5 min (100% A), 2.0 min (98% A),15.0 min (90% A), 19.0 min (87% A), 32.0-33.0 min (65% A), 34.0-37.0 min (0% A), and 38.0%-50.0% min (100% A)

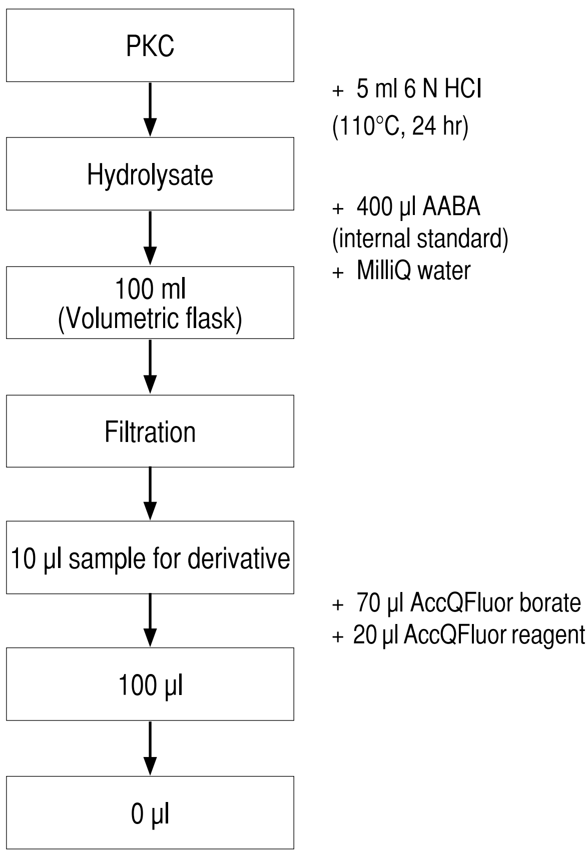


Figure 2. Process flow of sample preparation and addition of internal standard for amino acid composition determination.

RESULTS AND DISCUSSION

Results showed that PKC has high oil content averaging 8%-11% and protein content in the range of 12%-16% (*Table 3*). Besides protein and oil, PKC also has high shell which is only suitable for ruminant feed.

TABLE 3. PROXIMATE ANALYSIS OF PALM KERNEL CAKE (PKC) BY MECHANICAL PRESS

	(%)
Protein	12-16
Oil content	8-11
Moisture	7-8
Shell	11-13
Ash	4-5

Eighteen amino acids in PKC were separated and identified as glutamic acid, aspartic acid, alanine, arginine, cystine, glycine, histidine, isoleucine, leucine, lysine, methionine. phenylalnine, proline, serine, threonine, tyrosine and valine. Trythophane was not shown in the chromatogram as it has been destroyed by acid hydrolysis. Cystein and methionine could not be detected as they were below the detection limit. The HPLC chromatogram of amino acid of PKC and its internal standard were presented in, *Figures 3* and *4.*

The amino acid composition in PKC is depicted in *Table 4*. Glutamic acid is the most abundant amino acid in PKC while lysine is the least amino acid present. Glutamic acid and arginine were the major non-essential amino acids in PKC accounting for 3.01% and 2.08% of the total recovered amino acids respectively, while essential amino acids such as leucine, lysine and phenylalanine accounted for 1.11%, 0.59% and 0.73% of the total recovered amino acids in PKC respectively.

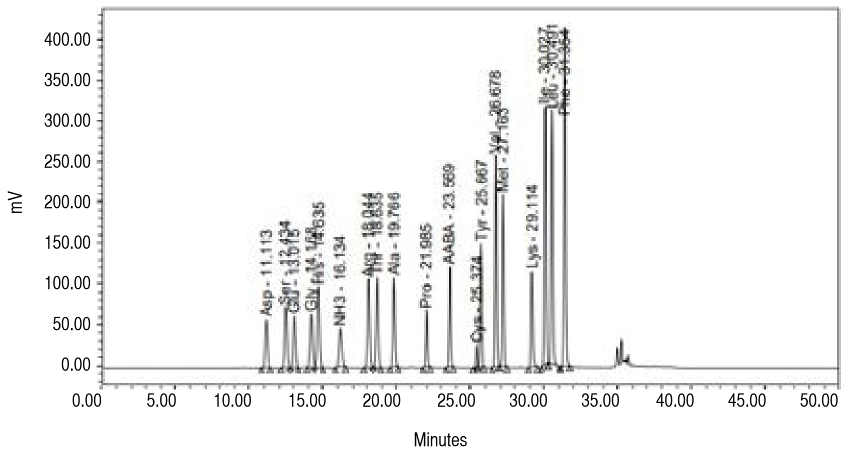


Figure 3. Chromatogram of amino acid internal standard.

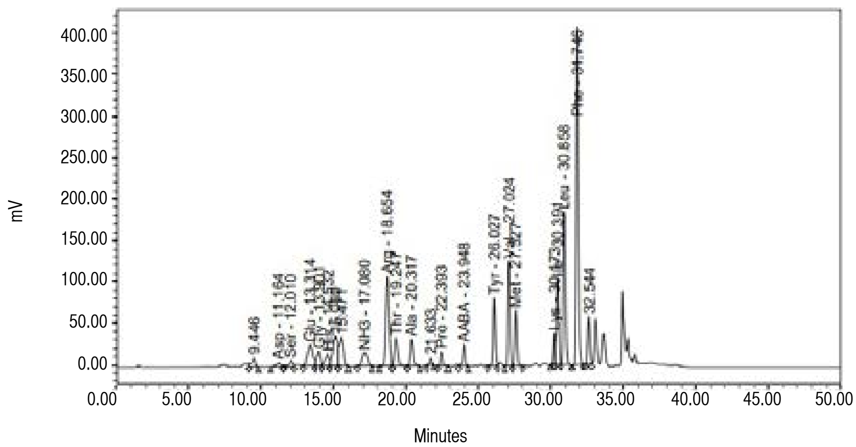


Figure 4. Amino acid chromatogram of palm kernel cake (PKC).

TABLE 4. AMINO ACID COMPOSITION OF PROTEIN IN PALM KERNEL CAKE (PKC)

Amino acid	PKC (%)
Protein (N x 6.25)	14.06
Alanine	0.72
Arginine	2.08
Aspartic acid	0.55
Cystine	0.20
Glutamic acid	3.01
Glycine	0.63
Histidine	0.29
Isoleucine	0.62
Leucine	1.11
Lysine	0.53
Methionine	0.20
Phenylalanine	0.73
Proline	0.63
Serine	0.69
Theorinne	0.55
Tyrosine	0.38
Valine	0.73

CONCLUSION

The PKC amino acid profile was almost identical with published data. Not all amino acids were recovered quantitatively under standard hydrolysis conditions. Cystein, methionine and trythophane were totally destroyed upon hydrolysis but still can be quantified by different hydrolysis procedure.

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