

OPTIMISATION OF ALKALI EXTRACTION OF PALM KERNEL CAKE PROTEIN

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ABSTRACT

Palm kernel cake (PKC) is commonly used in animal feed, particularly as ruminant feed to supply protein and energy. There is little information on the properties of protein concentrate produced from the PKC which constitute 14%-17% of the meal. Protein concentrates can be produced from PKC using alkali extraction, where PKC is extracted with an alkali solution and followed by precipitation at the isoelectric point. Thus, this study examined the effects of extraction using several extractants at different conditions; meal ratio of 0.5:50-3.0:50 g ml⁻¹, concentration of 0.1-1.0 M, pH 1-12, temperature of 30°C-80°C and time duration of 30-180 min. Sodium hydroxide (NaOH) was found to be the most suitable alkali for protein extraction. Optimum conditions for protein extraction were obtained at 1.0 M NaOH concentration, 50°C temperature, meal to solvent ratio of 2:50 (g ml⁻¹), pH 12 and 120 min. The extracted protein was isolated by isoelectric precipitation at pH 3.5 using 1.0 M hydrochloric acid (HCl). The percentage of protein recovery was 80%-86%. Protein content in the recovery ranged from 45%-50%. Analysis by high performance liquid chromatography (HPLC) showed that arginine, glutamic acid, phenylalanine and leucine were the most abundant amino acids in the concentrates.

Keywords: alkali extraction, isoelectric precipitation, palm kernel cake, protein concentrate.

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INTRODUCTION

Malaysia produced about 4.86 million tonnes of palm kernels in 2018 of which 2.59 million tonnes of meal, also known as palm kernel cake (PKC) was produced as a by-product (Kushairi *et al.*, 2019). Of this, 2.29 million tonnes of PKC were exported and nearly most of them were sent to European countries mostly The Netherland and Germany for use as ingredient in animal feed. Thus, PKC is one of the most important by-products in the palm oil industry that generates substantial export earnings for Malaysia at approximately RM1016 million.

PKC is produced from kernel by extracting the oil through screw press or by solvent extraction. Extraction with solvent leaves lesser residual oil in the meal than that of the expeller extraction. PKC

is light to dark brown colour, where the expeller-extracted PKC is darker than those extracted by solvent. Most of the PKC are from mechanical extraction using screw press because solvent extraction is expensive. PKC has high oil content, 2%-9% on average and protein content in the range of 14%-17% (Table 1). Besides oil and protein, PKC also has high fibre content (Alimon, 2004) and high phosphorous to calcium ratio (Tang, 2001). It is suitable for use in animal feed, particularly as ruminant feed to supply protein and energy. Table 2 shows the amino acid profile of PKC. Glutamic acid and arginine are the most abundant amino acids in PKC.

The extraction of protein from oilseeds such as soybean, canola and peanut has been the subject of research over the last five decades. Soya protein, in particular, has gained many applications in food, fibre and paper products industries. The suitability of PKC protein for both food and non-food applications is not yet known. A large number of researchers are concerned with the use of meal

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TABLE 1. PROXIMATE ANALYSIS OF PALM KERNEL CAKE

Extractant (%)	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 (1989).

as animal feed sold at a low price to Europe. Over 2 million tonnes of PKC are produced annually in Malaysia. Assuming that the PKC has protein content of 17% and 55%-60% of these proteins can be extracted, this will ultimately produce almost 200 000 t of protein from the PKC and generate more value-added palm oil products.

There are several methods that can be used to extract protein, such as isoelectric precipitation, ultrafiltration and membrane technology. Ultrafiltration can be used to recover low molecular weight protein. The ultrafiltration and membrane technology have been successfully used to produce protein isolates and concentrates from jojoba meal (Abbott *et al.*, 1991; Nabetani *et al.*, 1995), *Crambe abyssinica* (Massoura *et al.*, 1998), *Rosa rubiginosa* seeds (Moure *et al.*, 2001), canola (Ghodsvali *et al.*, 2005), oilseed flour (Lawhon *et al.*, 2006) and soybean (De Moura *et al.*, 2010).

Furthermore, separation of protein from alkali solution by isoelectric precipitation is very common. To date, this method has been successfully applied to flaxseed, soybean, canola, *Lupinus campestris*, peanuts, sunflowers and many others (Abbasy *et al.*, 1981; Alu'datt *et al.*, 2013; Franzen and Kinsella 1976; Gherzova *et al.*, 2015; Ghodsvali *et al.*, 2005; Rodríguez-Ambriz *et al.*, 2005; Taha *et al.*, 1981; Tzeng *et al.*, 1990). In addition, this method is comparatively cheaper and straight forward, and has also been successfully used for extraction of protein from rapeseed (El-Nockrashy *et al.*, 1977) and almond (Sze-Tsao and Sathe, 2000). The isoelectric precipitation method consists of protein extraction from the cake using alkali solution, and followed by precipitation at isoelectric point. Isoelectric precipitation occurs when there is a change in pH in the solution.

The extraction of protein by alkali extraction is affected by many factors, such as meal to solvent ratio, protein solubility, temperature and time. It is then followed by precipitation of significant protein fractions at isoelectric point, which ranges from pH 3.0 to 5.0. Donna *et al.* (1997) developed the alkaline extraction technique to obtain the optimum protein extraction from canola meal. The extraction was carried out using sodium hydroxide (NaOH) (0.4%) and the sample to solvent ratio was 1 to 20. The

TABLE 2. AMINO ACID CONTENTS OF PALM KERNEL CAKE (g/16 g N)

Amino acid	%
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
Phenylalanine	3.96
Proline	3.31
Serine	4.11
Threonine	2.75
Tryptophan	1.06
Tyrosine	2.60
Valine	5.05

Source: Alimon (2004).

extraction was carried out at room temperature for 60 min. The protein obtained using the precipitation method at isoelectronic point (pH 4) was relatively high at 87.5%. A study by Liadakis *et al.* (1998) found that the yield of protein extract from tomato increased at a temperature between 30°C-50°C. Kwon *et al.* (1996) found that temperature of up to 60°C gave higher protein yield from coconut meal but the protein produced was denatured.

All oilseed meals have different isoelectric points. Generally, protein concentrates or isolates are precipitated at isoelectric point between pH 3.0-5.0. The pH adjustment of the extract supernatant is normally carried out using dilute acidic solutions. Previous studies have reported the adjustment of pH to 3.5 using acetic acid (Klockeman *et al.*, 1997) or hydrochloric acid (Tzeng *et al.*, 1990). The isoelectric points for soybeans (Franzen and Kinsella, 1976), canola (Klockeman *et al.*, 1997) and sunflower (Taha *et al.*, 1981) meals are at pH 4, 3.5 and 3.4, respectively. A similar study by Xu and Diosady (2003) found that the highest protein yield (50%) for rapeseed meals was obtained between pH 4.5 to 5.0. The isoelectric point for PKC is not yet known but based on the reference to oilseeds such as soybean and canola, it is deemed to be at pH 3.5. Thus, this article highlights on the optimisation of various parameters, such as pH, temperature, time, extractant and meal to solvent ratio on alkali extraction of PKC to produce high protein concentrate.

MATERIALS AND METHODS

Materials

Mechanical-extracted PKC was provided by a mill in Klang, Selangor, Malaysia. The cake was ground to pass through 80 mesh screen. AccQ Tag Chemistry packages for amino acid analysis consisting of 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AccQ) fluor reagent, AccQ Tag eluent and amino acid standard were obtained from Waters, USA. The internal standard, α amino butyric acid (AABA), was purchased from Sigma-Aldrich, USA. Hydrochloric acid and acetonitrile of high performance liquid chromatography (HPLC) grade were purchased from Merck, Germany. High purity water was supplied by Milli-Q purification system.

Proximate Analysis

Ash, moisture 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 the method described in AOAC (1995). Ammonia was distilled and collected in boric acid solution, which was then titrated using 0.1 N hydrochloric acid (HCl). Digestion and distillation were carried out using Kjeltac apparatus (Model 2200 Auto Distillation, Foss Tecator, Denmark).

Determination of Optimisation of Alkali Extraction

Protein concentrates were prepared from PKC by alkaline extraction using the method described by Oomah *et al.* (1994) as shown in Figure 1. The extraction was performed with several extractants at different conditions; NaOH concentration of 0.1-1.0 M; meal to solvent ratio of 0.5:50, 1:50, 1.5:50, 2.0:50, 3.0:50 v/w; pH 1-12; temperature of 30°C-70°C; and duration of 30-180 min. The slurry was then centrifuged at 10 000 g (Sorval RC-5C Plus, USA) for 10 min at 10°C and followed by filtration through Whatman No. 41 filter paper. The protein extracted was then precipitated by adjusting the pH to the isoelectric point and centrifuged at 5000 g for 10 min. The precipitated curd was then washed with distilled water and dried.

Protein Solubility

Protein solubility was measured as a function of pH (1-12.0). Twelve sets of 1 g sample each were dispersed in 50 ml of 1.0 M NaOH and each solution was adjusted to pH between 1 to 12 by adding either NaOH or HCl. The dispersion was agitated for 2 hr at 50°C and then centrifuged at 10 000 g for 15 min, followed by filtration through Whatman No. 1 filter paper. Nitrogen (N) content in the supernatant was determined by Kjeldhal method. The percentage of soluble protein was calculated as follows:

$$\text{Protein solubility (\%)} = \frac{\text{amount of N in the supernatant}}{\text{amount of N in the PKC}} \times 100$$

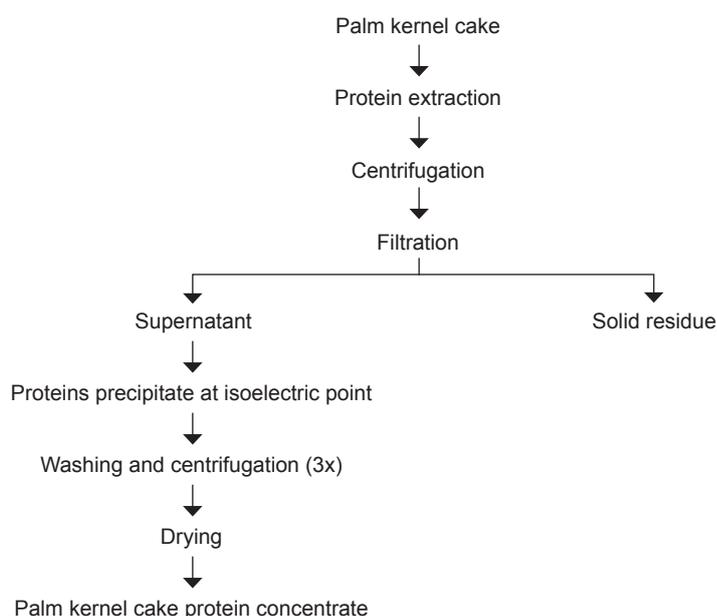


Figure 1. Process flow of alkali precipitation for palm kernel cake protein extraction.

Determination of Isoelectric Point

Four sets of PKC extracted with NaOH (100 ml each) were placed in 250 ml flasks. The extracts were tested at different pH of 3.0, 3.5, 4.0 and 4.5. The protein curd formed was then separated by centrifugation for 15 min at 5000 g. The percentage of protein yield was plotted against pH to determine the isoelectric point. Isoelectric point was indicated by maximum PKC protein yield.

Amino Acid Determination

A reverse-phase HPLC method using AccQ Tag RP-18 column (3.9x150 mm) was used for the determination of amino acids in PKC and its protein concentrate. The method was conducted based on Waters AccQ Tag method that is capable of analysing 21 amino acids in 40 min (Cohen and Michaud, 1993). The detection was carried out by Waters 2475 Multi-Fluorescence Detector with excitation at 250 nm and emission at 395 nm (Waters Breeze HPLC System, USA).

RESULTS AND DISCUSSION

Proximate Analysis

Analysis showed that the mechanical-extracted PKC contained 14.35% protein, 13.67% oil, 13.26% shell, 4.28% ash and 8.75% moisture. The amount of protein obtained in this study was almost similar to that reported by Alimon (2004) and Nuzul Amri (2013), while other components varied depending on the sources and efficiency of the oil extraction operation.

Protein Solubility

Figure 2 shows the solubility of PKC protein in the pH range of 1 to 12. Protein solubility was the

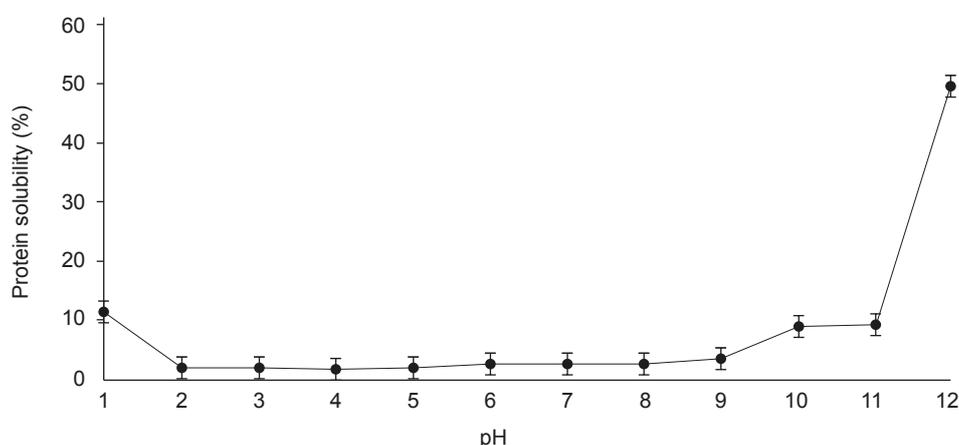


Figure 2. The solubility of palm kernel cake protein as a function of pH.

highest (49.45%) at pH 12. At pH 1, solubility of PKC protein was 10.54%, while minimum solubility occurred at pH 2 to pH 9 (less than 3%). The results suggested that there was some portion of the protein not affected by NaOH solution. This finding is in agreement with that of Osman (2002) who reported on protein entrapped in a hard cell structure in the PKC as the protein of PKC was fully protected by tough cell walls. With an increase in pH, the trapped protein would be freed and unattached, thus, increasing protein concentrate yield.

Isoelectric Point

Figure 3 shows the isoelectric point for PKC. The samples were tested at different pH of 3.0, 3.5, 4.0 and 4.5. The highest protein yield (81%) was obtained at pH 3.5. The results showed that the PKC has its isoelectric point at pH 3.5. Oilseed meals have isoelectric points at pH 4 for soybean (Franzen and Kinsella, 1976) and pH 4.5 for canola (Klockeman *et al.*, 1997). The isoelectric point ascertains the level of minimum solubility of protein owing to protein-protein interactions being favoured over protein-water interactions (Luft *et al.*, 2011). Solubility refers to the amount of protein in a sample that dissolves into solution (Zayas, 1997). This means that protein in PKC is soluble in solution with pH of 3.5.

Effect of Different Extractants on PKC Protein Extraction

NaOH is the most efficient solvent for the extraction of protein from PKC with the highest N extractability (83%). This is also true for other oilseed meals. Wolf (1970) and Donna (1997) found that NaOH extracted the highest yield of protein from soybean and canola meal. Terry *et al.* (1992) found that 76.8% protein could be extracted from Nigeria seed using 0.1 M NaOH. Alkali solution is effective in solubilising rice bran proteins because NaOH can break hydrogen, amide, and disulphide

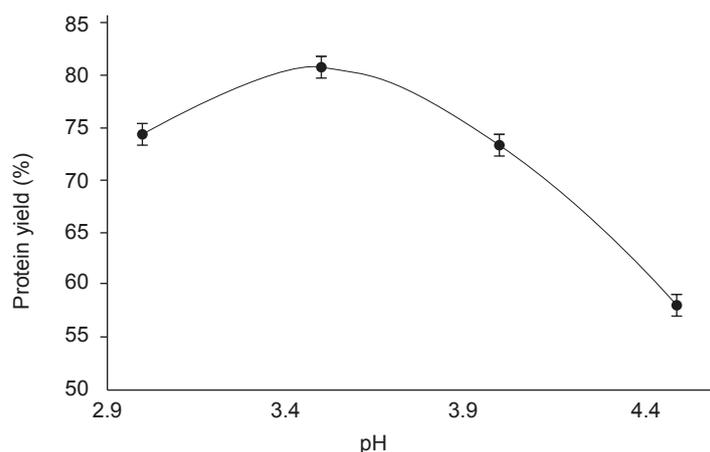


Figure 3. Protein yield of palm kernel cake at different pH.

bonds in proteins (Hamada, 1997). Results showed that sodium chloride (NaCl) solution was less effective for PKC protein extraction. According to Cui *et al.* (2017), presence of hydrophobic groups and disulphide bonds between protein molecules hinders the solubility of protein in water. Thus, this prompts for the use of aqueous solution such as salt, acid and alkali solution, which is beneficial to extract protein due to its large solubility and protein stability. This explains the efficiency of NaOH in extracting the highest protein from PKC among other extractants. Table 3 shows the effect of different extractants on the extraction of protein from PKC.

TABLE 3. EFFECT OF DIFFERENT EXTRACTANTS ON PALM KERNEL CAKE PROTEIN EXTRACTION

Extractant	Protein extracted (%)
1.0 M potassium hydroxide (KOH)	71.35 ± 1.36
1.0 M sodium hydroxide (NaOH)	83.66 ± 1.80
1.0 M sodium chloride (NaCl)	50.88 ± 0.21
Water (H ₂ O)	1.46 ± 0.15

Note: Temperature - 50°C; extraction time - 2 hr; meal to solvent ratio - 1:50 (w/v).

Effect of Different NaOH Concentrations on PKC Protein Extraction

Increasing the concentration of NaOH resulted in high protein yield (Table 4). The protein yield was low (1.20%-6.36%) for 0.01-0.5 M NaOH. Increasing the molarity of NaOH to 1.0 M increased the protein yield to 41.16%. Donna *et al.* (1997) used 0.04 M NaOH to obtain optimum protein extraction of 90% from canola meal. While protein extracted using 0.5 M NaOH resulted in less than 10% yield. Results showed that protein extraction from PKC needed high NaOH concentration. However, high alkaline concentration facilitates the breakdown of hydrogen bonds and dissociates hydrogen (Cui *et al.*, 2017).

TABLE 4. EFFECT OF SODIUM HYDROXIDE (NaOH) CONCENTRATIONS ON PALM KERNEL CAKE PROTEIN EXTRACTION

Molarity of NaOH (M)	Protein extracted (%)
0.01	1.20 ± 0.28
0.05	1.90 ± 0.21
0.10	2.94 ± 0.45
0.25	3.13 ± 0.33
0.50	6.36 ± 1.05
1.00	43.71 ± 1.22

Note: Temperature - 50°C; extraction time - 1 hr; extraction - NaOH; meal to solvent ratio - 1:50 (w/v).

Effect of Meal to Solvent Ratio on PKC Protein Extraction

Table 5 shows the protein yield extracted at various ratios of meal to solvent. Other process parameters were fixed at 50°C, 1 hr extraction and 50 ml of NaOH (1.0 M). Results showed that low meal to solvent ratio resulted in high protein yield. This study is in agreement with Taha *et al.* (1987) who successfully extracted 91.4% protein from sesame seed using 0.4 M NaOH to sample ratio of 1:25. Increasing the meal to solvent ratio resulted in decrease in N extractability at any NaOH concentration. Meal to solvent ratio of 0.5:50 and 1.0:50 (w/v) did not have significant effect on protein yield. Thus, meal to solvent ratio of 1:50 (w/v) was found to be the satisfactory condition for PKC extraction.

Effect of Time on PKC Protein Extraction

Extraction time is one of the factors that also influences protein yield. However, time factor has less significance on the extraction yield. Results showed that the longer the extractability duration,

TABLE 5. EFFECT OF MEAL TO SOLVENT RATIO ON PALM KERNEL CAKE PROTEIN EXTRACTION

Sample (g)	Protein extracted (%)
0.5	87.53 ± 2.43 ^a
1.0	87.87 ± 2.97 ^a
1.5	85.16 ± 1.64 ^b
2.0	74.92 ± 3.23 ^c
2.5	69.84 ± 1.86 ^d
3.0	64.91 ± 4.19 ^e

Note: Temperature - 50°C; extraction time - 2 hr; extractant - 1.0 M sodium hydroxide (NaOH); volume of NaOH - 50 ml.

Means with the same letters are not significant different ($p > 0.05$).

the higher the protein yield until it reached a plateau at 120 min (Table 6). Cui *et al.* (2017) also revealed that long extraction time improved protein extracted from tea. Thus, the optimum extraction time that can produce the highest protein yield from PKC was 120 min.

Effect of Temperature on PKC Protein Extraction

Study on the effect of temperature on protein yield was carried out at 30°C, 40°C, 50°C, 60°C and 70°C, and the process parameters were fixed at 2 hr extraction time and meal to NaOH (1.0 M) ratio of 1:50 (w/v) as shown in Table 7. Protein extraction was the lowest (61.19%) at 30°C. Increasing the temperature resulted in protein yield increment. High temperature stress may trigger changes in plant tissue affecting physiological processes (Lurie, 2006). Zhang *et al.* (2009) emphasised that increasing temperature could induce mass transfer and solubility, reduce viscosity of the solution and thus, increase the extraction rate. However, the extraction should not be performed at high temperatures due to the possibility of protein denaturation. According

TABLE 6. EFFECT OF TIME ON PALM KERNEL CAKE PROTEIN EXTRACTION

Time (min)	Protein extracted (%)
30	61.38 ± 3.20 ^c
60	76.47 ± 2.63 ^b
90	80.72 ± 3.11 ^{a,b}
120	82.19 ± 1.57 ^{a,b}
150	82.75 ± 2.72 ^a
180	83.15 ± 5.11 ^a

Note: Temperature - 50°C; meal to solvent ratio - 1:50 (w/v); extractant - 1.0 M sodium hydroxide (NaOH).

Means with the same letters are not significant different ($p > 0.05$).

to Lee *et al.* (2003) who examined the impact of temperature on protein yield in soybean, soy protein meal denatured at 60°C. Thus, 50°C was found to be the satisfactory temperature for PKC protein extraction.

Protein Extraction at Optimum Condition

After the optimum conditions were established, the protein concentrates extracted from PKC was carried out at 50°C and stirred continuously at 200 rpm for 2 hr using meal to NaOH (1.0 M) ratio of 1:50 (w/v). The suspension was then centrifuged at 10 000 g for 10 min at 10°C, followed by filtration through Whatman No. 41 filter paper. Extracted protein in the supernatant was precipitated by adjusting the pH to isoelectric point at pH 3.5 with 1.0 M HCl and then separated by centrifugation at 5000 g for 10 min. The precipitated curds were then washed with distilled water and dried. The protein recovery was 80% to 86%. Optimisation of protein extraction will enhance its nutritive values for animal feed application. Protein deficiency may reduce body protein deposition and animal performance, where part of the essential amino acids diverts to non-essential amino acids synthesis due to lack of non-specific nitrogen for this process (Dean *et al.*, 2006).

Amino Acid Composition

Amino acid is an important measure of protein quality (Bryden and Li, 2010). Amino acid for feed is crucial in improving the efficiency of utilising protein in animal feed (Toride, 2000). Seventeen amino acids were separated and detected in PKC and its protein concentrate, namely glutamic acid, aspartic acid, alanine, arginine, cysteine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine and valine. Tryptophan was destroyed by acid hydrolysis; this is not shown in the chromatogram. There was also loss of cysteine and methionine in very low analytical values.

TABLE 7. EFFECT OF TEMPERATURE ON PALM KERNEL CAKE PROTEIN EXTRACTION

Temperature (°C)	Protein extracted (%)
30	68.19 ± 2.09 ^c
40	75.00 ± 1.78 ^b
50	86.45 ± 2.52 ^a
60	86.58 ± 1.41 ^a
70	85.21 ± 2.37 ^a

Note: Extraction time - 2 hr; meal to solvent ratio - 1:50 (w/v); extractant - 1.0 M sodium hydroxide (NaOH).

Means with the same letters are not significant different ($p > 0.05$).

The amino acid compositions and HPLC chromatogram of PKC and its protein concentrate are presented in Figures 4, 5 and Table 8, respectively. Acid composition in PKC is similar as reported by Alimon (2004). Glutamic acid was the most abundant amino acid, while lysine was the least abundant amino acid in PKC. PKC protein concentrate was found to be rich in arginine, glutamic acid and phenylalanine.

to solvent ratio of 1:50 (w/v), pH 12 and 120 min time reaction. The extracted protein was isolated by isoelectric precipitate at pH 3.5 using 1.0 M HCl acid. The percentage of protein recovery was 80%-86%. Total recovered protein content was between 45%-50%. Analysis by HPLC showed that arginine, glutamic acid, phenylalanine and leucine were the most amino acids present in PKC protein concentrates.

CONCLUSION

NaOH was found to be the most suitable solvent for protein extraction. The optimum condition for protein extraction was 1.0 M NaOH, 50°C, meal

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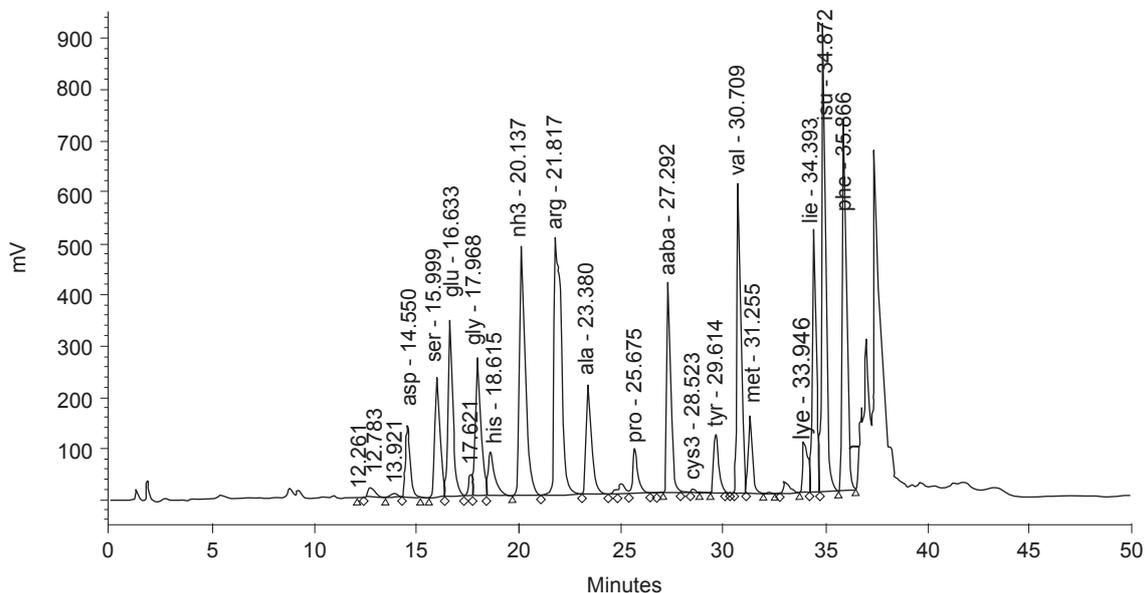


Figure 4. Amino acid chromatogram of palm kernel cake.

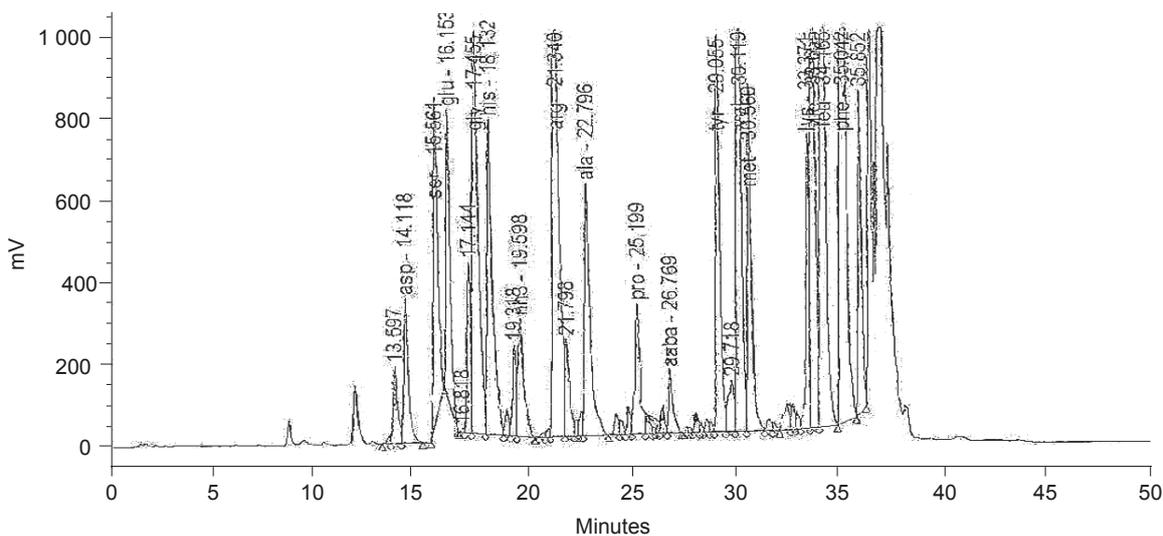


Figure 5. Amino acid chromatogram of palm kernel cake protein concentrates.

TABLE 8. AMINO ACID COMPOSITIONS IN PALM KERNEL CAKE (PKC) AND ITS PROTEIN CONCENTRATE

Amino acid	PKC (g/16 g N)	Protein concentrate (g/100 g protein)
Alanine	0.92	1.19
Arginine	2.18	4.75
Aspartic acid	1.55	3.71
Cystine	0.20	0.25
Glutamic acid	3.01	6.96
Glycine	0.83	1.14
Histidine	0.29	2.49
Isoleucine	0.62	1.99
Leucine	1.11	3.45
Lysine	0.59	1.46
Methionine	0.30	1.87
Phenylalanine	0.73	6.46
Proline	0.63	1.32
Serine	0.69	0.89
Theorinne	0.55	2.41
Tyrosine	0.38	1.87
Valine	0.90	2.40
Total	14.35	44.61

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