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# Extraction of Nitrogen from Palm Kernel Meal and Evaluation of Digestibility of Protein Isolate from the Meal by the in Vitro Method

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Techniques for maximizing the extraction of N from palm kernel meal have been investigated. Protein concentrate was also prepared from the meal and its digestibility evaluated by the in vitro method. Maximum extraction (97.78%) of N was obtained by using a meal to solvent ratio of 2 g/100 mL, 1 M NaOH solution, and an extraction time of 90 min. Other solvents such as sodium chloride and calcium hydroxide were not as efficient as NaOH. The protein concentrate was found to be more digestible than the original meal (77.1 vs. 55.2%).

The expansion of the local crushing industries has increased the availability of palm kernel meal (PKM) in Nigeria. Although palm kernel meal, with 19% protein, is cheaper than groundnut cake, which is commonly used in livestock feeds, its utilization by monogastric animals has been found to be impaired by its grittiness and high crude fiber content (Oyenuga, 1968; Fetuga, 1972; Owusu-Domfeh, 1967). Efforts have been made recently (Babatunde et al., 1975; Fetuga et al., 1977) to enhance the utilization of PKM by young and growing–fattening pigs. It was, however, felt that PKM could also be processed to yield a protein concentrate for food use. As a first step, the solubility of its nitrogenous constituents in various solvents has been studied to determine the most efficient procedure.

## EXPERIMENTAL SECTION

**Materials.** The palm kernel meal (PKM) samples used in the present study were obtained as commercial press cakes from vegetable oil (Nigeria), Ltd., Ikeja, Lagos. These were products of oil extraction by the mechanical screw press expeller method, after being heated in kettles up to 85 °C. The meal was ground into a fine powder of particle size ≤0.2 mm. The powdered material was stored at −5 °C. Its proximate analysis (on a dry matter basis) was 18.75% ($N \times 6.25$), 6.05% crude fiber, 6.39% fat, 4.43% ash, and 64.38% nitrogen-free extract.

**Extraction Procedure.** The extraction techniques employed in solubilizing N from the PKM were similar to those used by Kazazis and Kalaissakis (1979) with vetch seed using NaOH as the extractant. Other extractants [deionized water, NaCl, $Na_2HPO_4$, $KH_2PO_4$, $Na_2SO_4$, and $Ca(OH)_2$] were tested after establishing the optimum conditions for the extraction of N from the meal using NaOH. Extractions were carried out at ambient temperature (28 °C) by using a mechanical shaker. All suspensions were centrifuged at 1400*g* for 10 min, and duplicate aliquots of the supernatant were taken for N determination.

**Analyses.** Analyses for the proximate constituents of the seed were carried out by using official methods of the Association of Official Analytical Chemists (1975). True nitrogen in the supernatant solutions obtained by centrifuging the suspensions was measured by the method of Lowry et al. (1951).

**Protein Concentrate Preparation.** Ground PKM was vortexed for 20 min with 30 volumes of 1.0 M NaOH so-

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Table I. Extraction of N from Palm Kernel Meal Using NaOH at Different Meal/Solvent Ratios, Molarities of Solvent, and Shaking Time[a]

| | meal/solvent ratio (w/v) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2 g/100 mL | | | | 4 g/100 mL | | | |
| shaking time, min | 0.005 M NaOH | 0.10 M NaOH | 0.15 M NaOH | 0.20 M NaOH | 0.005 M NaOH | 0.10 M NaOH | 0.15 M NaOH | 0.20 M NaOH |
| 30 | 11.35 | 21.20 | 33.50 | 33.56 | 8.55 | 8.82 | 12.92 | 17.37 |
| 60 | 11.76 | 22.29 | 30.50 | 32.55 | 8.82 | 12.31 | 15.45 | 20.72 |
| 90 | 12.44 | 24.21 | 32.41 | 33.64 | 12.17 | 19.21 | 16.00 | 21.06 |
| 120 | 10.67 | 21.20 | 30.09 | 31.45 | 8.07 | 8.27 | 12.65 | 18.53 |

[a] Values are expressed as N extracted as a percentage of the total N in meal.

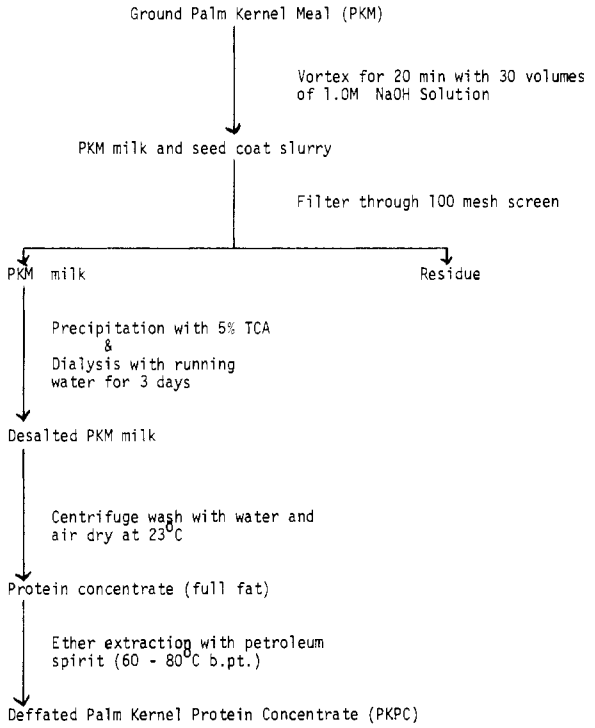


**Figure 1.** Preparation of protein concentrate from palm kernel meal.

lution and the suspension obtained subjected to screen filtration similar to that described by Lo and Hill (1971). This was followed by the addition of 5% trichloroacetic acid to the milk obtained after the removal of the residue by filtration. The milky solution was dialyzed under running tap water for 24 h and centrifuged, and the "cake" obtained was thoroughly washed with water and dried with atomized air dryer at ambient temperature (23 °C). At no time did the drying temperature exceed 25 °C. The process for the preparation of the palm kernel protein concentrate (PKPC) is outlined in Figure 1.

**Assay of Protein Digestibility.** The digestibility of the protein of the PKPC was assayed by using the new in vitro method described by Furuya et al. (1979). A 0.5-g sample of PKM or PKPC was placed in a 100-mL Erlenmeyer flask, 20 mg of pepsin (EC 3.4.4.2) in 10 mL of 0.075 M hydrochloric acid was added, and the mixture was incubated for 4 h at 37 °C. Then, after neutralization with 0.2 M sodium hydroxide, 10 mL of pig's intestinal fluid was added and incubated for an additional 4 h at 37 °C. This fluid was obtained from a pig immediately after it was slaughtered (University of Ibadan Teaching and Research Farm, Ibadan, Nigeria). After the two-stage incubations, the contents of the flask was centrifuged for 10 min at 1250*g*, and the residue was transferred to preweighed filter paper for dry matter (DM) and crude protein (nitrogen × 6.25; CP) determinations. The in vitro CP digestibility was calculated on the basis of original CP contents of the PKM and PKPC: CP digestibility = $(1 - R/S) \times 100$ where $R$ is the percent CP of the oven-dry sample residue and $S$ is the percent CP of the original meal or protein concentrate.

Table II. Comparative Extraction of N from Palm Kernel Meal by Different Dispersing Agents at Fixed Solvent Concentration (0.01 M) Using Meal to Solvent Ratios of 2 and 4 g/100 mL (w/v)[a]

| | water/solvent ratio (w/v) | |
|---|---|---|
| solvent | 2 g/100 mL | 4 g/100 mL |
| NaOH | 24.48 | 19.83 |
| $Na_2HPO_4$ | 17.37 | 7.93 |
| $KH_2PO_4$ | 16.68 | 7.32 |
| $Na_2SO_4$ | 14.77 | 7.18 |
| NaCl | 12.72 | 6.77 |
| $Ca(OH)_2$ | 10.12 | 5.47 |
| distilled water | 9.30 | 4.79 |

[a] Values are expressed as N extracted as percentage of the total N in meal.

## RESULTS AND DISCUSSION

Table I shows the effect of meal to solvent ratio, concentration of NaOH, and extraction time on N solubility. In all the molarities and meal to solvent ratio tested, optimum N extractability was obtained by using a 90-min extraction time. Similar observation has been reported for vetch seed (Kazazis and Kalaissakis, 1979) and for West African locust bean seed (Balogun and Odutuga, 1981). The 0.20 M NaOH extracted more N (about 33%) than any other concentration of NaOH. The N solubility at this concentration was, however, lower than those reported for leguminous seeds (Kazazis and Kalaissakis, 1979; Balogun and Odutuga, 1981) with lower concentration (0.01 M). This may be attributable to the fact that most of the proteins are bound to cellulose, the hardness of the kernel, or the presence of phenolic compounds in the meal. Williams and McEwin (1967) reported that kernel hardness already affected the efficiency of protein extraction. The work of Merellith and Wren (1956) on the nitrogen solubility of cocoa meal indicates a substantial decrease in N solubility due to the presence of polyphenols. Increasing the meal to solvent ratio resulted in decreased N extractability at any concentration of NaOH.

The effect of dispersing agents at fixed solvent concentration (0.01 M) on N solubility of PKM is presented in Table II. NaOH gave the highest N extractability of 24.48 and 19.83% for meal/solvent ratios of 2 and 4 g/100 mL, respectively. Distilled water was the least effective in extracting N from PKM.

Table III shows the effect of the meal to solvent ratio on N solubility of PKM by using a fixed solvent concentration. There was a decrease in N extractability as the meal to solvent ratio increased with all solvents. A further increase in N solubility was observed when 0.05 M NaOH

Table III. Effect of Meal to Solvent Ratio on the Extractability of N from Palm Kernel Meal Using a Fixed Solvent Concentration[a]

| meal/solvent ratio (w/v), g/100 mL | 0.05 M NaOH | 0.05 M $Na_2SO_4$ | 0.005 M $Ca(OH)_2$ | 0.025 M $Ca(OH)_2$ | 0.005 M $Na_2HPO_4$ | 0.01 M $KH_2PO_4$ | distilled water |
|---|---|---|---|---|---|---|---|
| 2 | 50.19 | 9.85 | 11.35 | 9.30 | 11.62 | 16.53 | 9.30 |
| 4 | 43.42 | 11.56 | 8.62 | 9.16 | 9.09 | 7.18 | 4.79 |
| 6 | 32.73 | 9.34 | 7.65 | 7.20 | 8.07 | 5.52 | 6.29 |
| 8 | 30.05 | 9.13 | 8.03 | 6.94 | 7.04 | 4.96 | 7.01 |
| 12 | 20.97 | 7.79 | 7.41 | 6.22 | 6.15 | 5.47 | 6.61 |

[a] Values are expressed as N extracted as a percentage of the total N in meal.

Table IV. Solubilization of N from Palm Kernel Meal with Sodium Hydroxide at Different Concentrations

| | meal/solvent ratio (w/v) | | | |
|---|---|---|---|---|
| | 2 g/100 mL | | 4 g/100 mL | |
| molarity, M | pH of dispersion | N extracted, % of total N in meal | pH of dispersion | N extracted, % of total N in meal |
| 0.05 | 11.95 | 50.19 | 11.60 | 43.42 |
| 0.075 | 12.30 | 56.21 | 12.10 | 44.65 |
| 0.10 | 12.40 | 58.53 | 12.20 | 49.57 |
| 0.20 | 12.80 | 60.25 | 12.65 | 51.97 |
| 0.40 | 12.80 | 66.32 | 12.75 | 56.41 |
| 0.60 | 12.85 | 78.63 | 12.80 | 64.96 |
| 0.80 | 12.95 | 88.21 | 12.90 | 73.85 |
| 1.00 | 12.80 | 97.78 | 12.75 | 81.37 |

Table V. Effects of Varying the Meal to Solvent Ratio and Solvent Concentration on the Extraction of N from Palm Kernel Meal by Aqueous NaCl[a]

| meal/solvent ratio (w/v), g/100 mL | NaCl molarity | | | | |
|---|---|---|---|---|---|
| | 0.20 M | 0.40 M | 0.60 M | 0.80 M | 1.00 M |
| 2 | 11.08 | 13.40 | 9.98 | 14.22 | 13.95 |
| 4 | 9.16 | 11.35 | 10.74 | 10.60 | 11.56 |

[a] Values are expressed as N extracted as a percentage of the total N in meal.

was used (33.64 vs. 50.19%, Table I).

Results from Tables I–III indicate that N extractability increased with increasing concentration of NaOH. It was, however, felt necessary to determine the concentration of NaOH that would give optimum N solubility. Increasing the molarity of NaOH from 0.05 to 1.0 M increased the effectiveness of NaOH in extracting N from PKM at all meal to solvent ratios (Table IV). For example, with a meal to solvent ratio of 2 g/100 mL, the N solubility was increased from 50.19 to 97.78%, while with a meal to solvent ratio of 4 g/100 mL, the N solubility was increased from 43.4 to 81.4%. It appears that the N solubility of PKM depends on the pH of the dispersing medium. The maximum N solubility of 97.78% was obtained when the pH of dispersion was increased to 12.95.

The use of NaCl in extracting proteins, particularly the globulins from legume seeds, is well-known. Balogun and Odutuga (1981) have shown that maximum solubility of N (81%) could be obtained from West African locust bean seed by using 0.75 M NaCl and a meal to solvent ratio of 2 g/100 mL. Increasing the molarity of NaCl from 0.20 to 1.00 M (Table V) did not give any appreciable N extractability (11.08 vs. 13.95%) from PKM. This suggests that NaCl solution is an ineffective solvent for the extraction of N from PKM. Perhaps a pretreatment of the meal with cellulase to free the protein from the cellulose would enhance the effectiveness of NaCl when compared

Table VI. Nitrogen Solubility Profiles of Palm Kernel Meal at Varied pH Values in Meal to Solvent Ratios of 2 and 4 g/100 mL (w/v)

| | meal/solvent ratio (w/v) | | | |
|---|---|---|---|---|
| | 2 g/100 mL | | 4 g/100 mL | |
| pH of 0.05 M | pH of dispersion | N solubility[a] | pH of dispersion | N solubility[a] |
| 10.0 | 6.40 | 8.21 | 6.05 | 7.52 |
| 11.0 | 6.45 | 9.44 | 6.15 | 9.09 |
| 11.5 | 9.40 | 12.44 | 7.50 | 10.19 |
| 12.0 | 11.25 | 21.61 | 10.10 | 16.96 |
| 12.6 | 12.35 | 62.91 | 12.10 | 42.39 |
| 13.0 | 12.85 | 77.95 | 12.80 | 58.12 |

[a] Values are expressed as N extracted as a percentage of the total N in meal.

Table VI. Apparent Digestibility of Proteins from Palm Kernel Meal and Protein Concentrate from It

| sample | % apparent digestibility[a] |
|---|---|
| palm kernel meal (PKM) | 55.2 ± 0.8 |
| palm kernel protein concentrate (PKPC) | 77.1 ± 1.3 |

[a] Mean ± standard error of three determinations.

to 1.0 M NaOH. The economic benefit of this would have to be determined.

For determination of the effect of pH on the extractability of N from PKM, 0.05 M NaOH was prepared and the required pH values (10–13) were obtained by adjusting the original pH (11.3) of the NaOH solution with 1.0 M HCl and concentrated NaOH solution. The pH of the suspension was measured prior to centrifugation. Table VI shows that with a meal to solvent ratio of either 2 or 4 g/100 mL, N solubility from PKM was minimal at a pH of dispersion of 6.40–11.25. However, N extractability increased as the pH of the dispersion was increased from 11.25 to 12.85 (21.61 to 77.95%) corresponding to increasing the pH of 0.05 M NaOH solution from 12 to 13. Similar N solubility of about 78% was obtained by using 0.60 M NaOH when the pH of dispersion was 12.85 (Table IV). This therefore suggests that 0.05 M NaOH (pH 13.0) would be effective and economical in solubilizing the N from PKM. From these results it appears that a meal to solvent ratio of 2 g/100 mL and 0.05 M NaOH (pH 13.0) and a 90-min extraction time would be an effective and economic technique for the extraction of N from this locally available protein source.

The results of the in vitro digestibility of the PKM and PKPC are shown in Table VII. Protein digestibility of PKPC was significantly ($P < 0.05$) higher than that of PKM, being 77.1 and 55.2%, respectively. The apparent digestibility of protein for the PKM was inferior to those reported for cooked and autoclaved conophor seed meals, soybean meal, and groundnut meal (Fetuga, 1972). How-

ever, the apparent protein digestibility of 77.1% for PKPC compares favorably with those earlier reported for most conventional urorthodox Nigerian plant protein sources (Fetuga, 1972). It is envisaged that adequate heat treatment of the protein concentrate from PKM might inactivate any residual antinutritional factors present in the protein concentrate, improve the digestibility of its protein, and enhance a better utilization of the protein by monogastric animals. A comprehensive evaluation of the nutritive potential of the protein isolate from PKM is currently being investigated.

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# Isolation of Yeast Protein with Reduced Nucleic Acid Level Using Reversible Acylating Reagents: Some Properties of the Isolated Protein

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Yeast proteins isolated by isoelectric precipitation, by alcohol precipitation, or by ammonium sulfate precipitation contained high levels of contaminant nucleic acids (>22 g of nucleic acid/100 g of protein). Gel filtration of these yeast proteins on a Sepharose 6B column indicated that yeast proteins and nucleic acids were present as a nucleoprotein complex. Chemical modification of the $\epsilon$-$NH_2$ group of lysine in the yeast proteins by maleic or citraconic anhydride destabilized the nucleoprotein complex and facilitated the separation of proteins and nucleic acids at pH 4.0–4.2. Subsequent incubation of the maleylated or citraconylated yeast proteins resulted in deacylation of the modifying groups. Some functional properties and the in vitro digestibility of the yeast proteins isolated by this method are reported.

In previous papers the problem of nucleic acids contamination in yeast proteins was reviewed, and the preparation of yeast protein isolate with a reduced nucleic acid level by succinylation of the $\epsilon$-$NH_2$ group of lysine residues in the yeast protein was described (Kinsella and Shetty, 1979; Shetty and Kinsella, 1979a,b). This method offered some advantages over conventional methods for reducing nucleic acid levels in the yeast protein isolate. These include enhanced extraction of proteins, inhibition of proteolytic enzymes during the isolation, and improvement in the functional properties: i.e., solubility, emulsification, and foaming properties of the isolated proteins (Kinsella and Shetty, 1979). The disadvantages of the succinylation procedure is that the final product is succinylated protein. Because the succinyl group cannot be removed from the succinylated proteins under mild conditions (Means and Feeney, 1971), it is unlikely that succinylated yeast protein can be used as a practical source of dietary protein. However, we have demonstrated the feasibility of using a reversible modifying agent, citraconic anhydride, with $\beta$-lactoglobulin and soy protein and determined optimum conditions for deacylation while minimizing alteration of the protein (Brinegar and Kinsella, 1980, 1981). Palacian et al. (1981) used dimethylmaleic anhydride for dissociation of protein components from chromatin. In this paper, we report the successful use of reversible modifying reagents (maleic and citraconic anhydride) to isolate yeast proteins with low nucleic acid levels. Optimum conditions for removal of modifying groups were determined; some functional properties and the in vitro digestibility of the protein are described.

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## EXPERIMENTAL SECTION

**Materials.** Brewer's yeast (*Saccharomyces carlsbergensis*) was obtained from Genesee Brewing Co., Rochester, NY, washed 3 times with distilled water, and freeze-dried. Bovine serum albumin (Cohn fraction V), pancreatin trichloroacetic acid, and orcinol were purchased from Sigma Chemical Co. (St. Louis, MO). Soy protein isolate was obtained from Ralston Purina Co. (St. Louis, MO), and 11S and 7S soy protein fractions were isolated according to the method described by Thanh and Shibasaki (1976). 2,4,6-Trinitrobenzenesulfonic acid (TNBS) and maleic and citraconic anhydride were purchased from Eastman Kodak Co. (Rochester, NY). Pepsin was obtained from Nutritional Biochemicals Corp. (Cleveland, OH). All other reagents were of reagent grade. Doubly distilled deionized water was used in the preparation of all solutions.

**Methods.** *Alkaline Extraction of Proteins from Disrupted Yeast Cells.* Yeast cells were disrupted according to the procedure described earlier (Shetty and Kinsella,

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