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Technical Note

Preliminary Study of Enzymic Solubilization of Nitrogenous Constituents of Palm Kernel Cake

Commercial palm kernel cake, the residue from oil expression, contains about 13% crude fibre and 19% virtually insoluble protein. Eight proteases, two cellulases and a pectinase were tested for their ability to solubilize the denatured protein in the cake. The proteolytic enzymes used (except papain) increased the protein (nitrogen) solubility by 34–62%. Cellulases also achieved a considerable level (35–37%) of protein solubilization.

INTRODUCTION

The aim of the work reported in this Note was to investigate the use of commercial enzyme preparations for the dissolution of the nitrogenous constituents of commercial palm kernel cake. This would aid the isolation of palm kernel protein products of reduced fibre content and increased nutritional value.

EXPERIMENTAL

Materials

Palm kernel meal

Screw-pressed palm kernel meal was obtained from BOCM Silcock, London, Great Britain. It was exhaustively defatted with petroleum ether (boiling point, 40–60 °C) in a Soxhlet extractor before use.

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Enzymes
All the enzymes used were obtained from the Sigma Chemical Company, Poole, Dorset, Great Britain.

Enzymic solubilization technique

A known mass of enzyme and a 5-g sample of defatted palm kernel meal were shaken with 100 ml of distilled water at the desired temperature for 18 h. Manufacturer's incubation conditions for optimum activity of enzymes were utilized. One millilitre of 0·1 % ethyl mercurithiosalicylate (thimerosol) was added to the suspension to prevent microbial growth. Shaking was accomplished in a Gallenkamp shaker bath set at forty oscillations a minute. After shaking, the mixture was centrifuged for 30 min at 4 °C and 2500 × *g* in an MSE Mistral 6L refrigerated centrifuge. The supernatant was decanted through Whatman No. 1 filter paper and the crude protein determined.

Analytical methods

Moisture content was determined by heating a 5-g portion of the meal at 100 °C for 5 h in an electric oven fitted with a fan. The ether extract was determined by extracting the meal exhaustively with petroleum ether (boiling point, 40–60 °C) in a Bolton extractor. Nitrogen and crude protein were determined by the micro-Kjeldhal method (Pearson, 1976); the protein was calculated as N × 6·25. Ash content was determined by incineration at 600 °C (Pearson, 1976). Crude fibre was also determined (Pearson, 1976).

RESULTS AND DISCUSSION

N-solubility in water

The proximate composition of palm kernel cake (PKC), Table 1, shows a protein content of about 19 %, which is comparable with published results (Evans, 1960), and over 40 % carbohydrate. The high fibre content (13 %) is responsible for the grittiness and low digestibility of PKC. Table 2 shows the nitrogen solubility of PKC in water at various pH values. Only low amounts (about 4–8 % of the PKC nitrogen) were soluble at pH

TABLE 1
Composition of Palm Kernel Cake (Percentage by Weight)

Constituent	%
Moisture	12·5 ± 0·0
Crude protein (N × 6·25)	18·8 ± 0·0
Ether extract	7·7 ± 0·1
Ash	3·7 ± 0·1
Crude fibre	13·1 ± 0'4
Carbohydrates (by difference)	41·2 ± 0·5

values ranging from 2 to 8·5. The data of Aghazu *et al.* (1979) for solvent extracted palm kernel meal show nitrogen solubilities ranging from 10% to 50% in the same pH range, suggesting that the screw pressing process has denatured the PKC protein, rendering it insoluble.

Enzymic solubilization

The conditions used for incubation with each enzyme and the corresponding proportion of PKC nitrogen solubilized are given in Table 3. The concentration of enzyme in each medium was dictated by the activity of the preparation used.

The nitrogen contents of the enzyme preparations were unknown and, in subtracting the nitrogen due to the enzyme from the total in the filtrate,

TABLE 2
Solubilization of Nitrogenous Constituents of Palm Kernel Cake[a] in Water

Conditions	*Nitrogen solubility* (%)
At 37°C:	
pH 2·0	5·3 ± 0·6
5·2	4·3 ± 0·7
8·5	6·7 ± 0·5
At 50°C:	
pH 4·5	4·2 ± 0·4
6·5	8·4 ± 0·5

[a] 5% w/v dispersion.

TABLE 3
Solubilization of Palm Kernel Cake Nitrogen by Enzymes

Enzyme	*Incubation conditions*			*Nitrogen solubilized* (%)
	Enzyme conc. (% *w/v*)	*pH*	*Temperature* (°*C*)	
Protease (*S. griseus*)	0·030	7·5	37	54·0
Protease (*A. oryzae*)	0·137	7·5	37	46·0
Protease (*B. subtilis*)	0·007	7·5	37	62·0
Protease (*S. caespitosus*)	0·256	7·5	37	46·7
Papain	0·176	6·5	50	5·6
Pepsin	0·180	2·0	37	34·0
Trypsin	0·180	8·5	37	53·3
Bromelain	0·129	4·5	50	42·5
Bromelain and cellulase (*A. niger*)	0·135 0·183	4·5	50	40·5
Pepsin and pectinase (*A. niger*)	0·180 0·099	2·4	37	46·4
Cellulase (*A. niger*) and pectinase (*A. niger*)	0·180 0·104	4·5	37	46·5
Cellulase (*T. viride*)	0·292	5·0	37	15·8

it was assumed that the enzyme preparations were 100% protein and contained 16% nitrogen. However, the nitrogen content of commercial enzymes would be less than this value. Therefore, it would be expected that the proportion of PKC nitrogenous constituents solubilized by enzyme would be greater than that shown in Table 3. All the enzymes increased the solubilization of PKC nitrogen. The percentage increases ranged from 6% for papain to 62% of a protease from *B. subtilis*. All the enzymes studied except papain showed a greater ability to solubilize PKC nitrogen than the carbohydrases. However, the low figures for the latter could be due partly to the assumption in the calculation that the enzyme preparations were 100% protein. Relatively, larger amounts of low activity carbohydrase preparations were used.

A combination of bromelain, a proteolytic enzyme, and a cellulase from *A. niger*, did not increase the efficiency of solubilization over that attainable by bromelain alone. A pepsin/pectinase mixture slightly improved (by 16%) the solubility (34%) achieved by pepsin alone.

The result of a further experiment with cellulases is shown in Table 4. The nitrogen contents of the enzyme preparations were determined as 9%

TABLE 4
Solubilization of Palm Kernel Cake (PKC) Nitrogen by Cellulase

Enzyme[a]	*N-content of enzyme*	*PKC nitrogen solubilized* (%)
Cellulase Type I (*Aspergillus niger*)	9·28	34·8
Cellulase Type IV (*Trichoderma viride*)	4·12	37·0

[a] Incubation conditions: 5% w/v PKC dispersion, 100 activity units of enzyme, pH 5·2, 37°C, 21 h.

and 4% by weight for cellulase Types I and IV, respectively. The mass of each enzyme used was equivalent to 100 units of activity, as defined by the manufacturer. Cellulase Type I (from *Aspergillus niger*) and Type IV (from *Trichoderma viride*) solubilized 35% and 37% PKC nitrogen, respectively.

It has been suggested (Childs *et al.*, 1977) that the reduced solubility of protein from heated cottonseed products is the result of cytoplasmic proteins 'gluing' together with the cytoplasmic granules containing storage proteins so that they cannot disperse in the solubilizing medium. Aghazu *et al.* (1979) reported that the palm kernel consists of microscopic cellulosic sacks containing fat in which are embedded small granules of both protein (15–20 m microns in size) and carbohydrates (up to 60 microns). It is therefore likely that the insolubilization of PKC protein could be partly due to the binding or entrapment of proteins or storage granules by polysaccharides under the influence of the heat and pressure of oil-extraction processes. The increased enzymic solubilization of PKC nitrogen may be attributed to the hydrolysis of cytoplasmic proteins (proteases) or polysaccharides (cellulases) responsible for insolubilization. The cellulases would be expected to solubilize PKC nitrogen as intact proteins, not as peptides or amino acids.

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