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BIOCHEMICAL COMPOSITION AND *IN VITRO* DIGESTIBILITY OF COCOA (*THEOBROMA CACAO*) POD HUSK, CASSAVA (*MANIHOT ESCULENTA*) PEEL AND PLANTAIN (*MUSA PARADISIACA*) PEEL

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Abstract

The rational formulation of feeds using local agricultural by-products has been impossible owing to the paucity of research on the composition of such products. In this study, the composition and *in vitro* digestibility of three major plant by-products in the West African sub-region, namely cassava peel, plantain peel and cocoa pod husk have been analyzed to determine the content of protein, lipid, starch, soluble sugars and fibre. Cocoa pod husk contained the highest amount of protein (7.8 %) and fibre (26.9%) while cassava peel had the highest content of starch (9.1 %) and reducing sugars (3.8 %). The amount of lipid in plantain peel (6.0 %) exceeded those of the other two residues. Plantain peel and, to a lesser extent, cocoa pod husk contained substantial amounts of unsaturated fatty acids including linoleic acid, which is essential for pigs and poultry. Analysis of mineral elements showed high amounts of potassium were present in cocoa pod husk and plantain peel. Calcium levels were also high in cassava peel and cocoa pod husk. The rate of starch digestion in plantain peel was relatively high. However, in cocoa pod husk it was low and could have been caused by the high level of fibre. Treatment of residues with NaOH resulted in a marked increase in the rate of digestion of starch in cassava peels.

Résumé

ADAMAFIO, N. A., AFEKE, I. K., WEPEBA, J., ALI, E. K. & QUAYE, F. O.: *Composition biochimique et la digestibilité* in vitro *de l'ecale de la cosse de cacao* (Theobroma cacao) *la pelure de manioc* (Manihot esculenta) *et pelure de plantain* (Musa paradisiaca). La formulation rationnelle de rations utilisant les sous-produits agricoles locaux n'a pas été possible à cause de la disette de recherche sur les compositions de ces produits. Dans l'étude en question, la composition et la digestibilité *in vitro* de trois sous-produits majeurs de plante dans la sous-région de l'Afrique occidentale, à saviour, la pelure de manioc, la pelure de plantain et l'écale de la cosse de cacao ont été analysées pour déterminer le contenu de protéine, lipides, fécule, sucre soluble et fibre. L'écale de la cosse de cacao contient la quantité de protéine (7.8 %) la plus élevée et fibre (26.9 %) alors que la pelure de manioc avait le contenu de fécule (9.1 %) le plus élevé et les sucres réduisants (3.8 %). La quantité de lipide existante dans la pelure de plantain (6.0 %) excédait celle de deux autres résidus. La pelure de plantain et à un moindre degré l'écale de la cosse de cacao contenaient des quantités énormes d'acide gras non saturé y compris l'acide linoléique qui est essentiel pour les cochons et les volailles. Une analyse d'éléments minéraux montrait que des hautes quantités de potassium était présentes dans les écales de la cosse de cacao et la pelure de plantain. Les niveaux de calcium étaient également élevés dan la pelure de manioc et l'écale de la cosse de cacao. La proportion de digestion de fécule en pelure de plantain était relativement élevée. Toutefois, dans l'écale de la cosse de cacao elle était faible et pourrait être provoquée par la haute niveau de fibre. Le traitement de résidu avec NaOH aboutissait en une augmentation marquée dans la proportion de digestion de fécule dans les pelures de manioc.

Introduction

The development of feed resources from agricultural by-products generated in the West African sub-region would greatly enhance animal production and provide much needed animal protein for human consumption. Crop residues that abound in the region such as cassava (*Manihot esculenta*) peel, plantain (*Musa paradisiaca*) peel, and cocoa (*Theobroma cacao)* pod husk must be extensively evaluated to determine the type of processing or supplementation required to transform them into novel, inexpensive, highly nutritive feeds for non-ruminants as well as ruminants. Data on the biochemical composition of these crop residues are essential for such evaluation. Although proximate analysis is beneficial, studies on composition must be more extensive and must include quantitation of readily available nutrients such as sugars, starch and amino acids (Chang, Nagwani & Holtzapple, 1998). This is vital for the formulation of non-ruminant feedstuffs, because non-ruminants do not harbour cellulolytic micro-organisms and, therefore, depend on non-structural carbohydrates as a major source of energy. Furthermore, the non-existence of ruminal microbial synthesis of amino acids means that essential amino acids must be provided in their diets.

Evaluation of the digestibility of by-products is also necessary, because crop residues are often lignified (Arosemena, DePeters & Fadel, 1995; DePeters, Fadel & Arosemena, 1997) and vary greatly in their rates of digestion. Where digestibility is found to be extremely low, it may be enhanced by treating crop residues with alkali. However, the effectiveness of such treatment depends on the unique chemical characteristics of a given by-product (Meeske, Meissner & Pienaar, 1993; Snyman & Joubert, 2002). Unfortunately, there is a paucity of research directed toward comprehensive analysis of tropical residues.

This study was, therefore, undertaken to provide detailed baseline data on important nutritional parameters as well as *in vitro* digestibility of three major crop residues in the sub-region, namely cocoa pod husk, cassava peel and plantain peel. The data generated should facilitate feed resource development, especially for non-ruminants.

Experimental

Dried pellets of cocoa (*Theobroma cacao*) pod husks (pericarp) were collected from the Cocoa Research Institute, Tafo, Ghana. Cassava (*Manihot esculenta*) peels (cortex and periderm) and unripe plantain (*Musa paradisiaca*) peels (pericarp) were collected from various locations in the Greater Accra Region, Ghana. They were sun-dried for 2 weeks, milled and stored at 4 °C. Alpha-amylase (25 $U.mg^{-1}$) was purchased from BDH Chemicals Ltd, England.

Sugars and starch were determined, using the Luff-Schoorl method (Kirk & Sawyer, 1991). Protein was measured as Kjeldahl nitrogen (Pomeranz & Meleon, 1977), and crude fibre was analyzed by a gravimetric method (Bennick, 1994). Amino acids were quantitatively analysed by an automated HPLC Waters 717 - Millenium 2010 system, using norleucine (Nor) as an internal control. Lipid content was determined by 4 h soxhlet extraction using petroleum ether, and fatty acids were extracted, methylated and then analyzed by gas-liquid chromatography (Christie, 1993). Mineral elements were determined using atomic absorption spectrometry and flame photometry (Na and K) after wet digestion of the samples (Christian & O'reilly, 1986).

In vitro digestibility

Ground crop residues were steeped in 2.5 % NaOH or distilled water for 24 h. Test samples were then neutralized with HCl. Samples were filtered, washed with distilled water, and oven-dried at 37 °C. All samples were then incubated in 1.3 $U.ml^{-1}$ α-amylase in 1*M* $NaHPO_4$ buffer, *p*H 6.7 at 37 °C for 4 h. At the end of the incubation period, 1% (w/v) NaOH was added and the suspensions were filtered through Whatman

No.1 filter paper. The amount of reducing sugars in each filtrate was determined using the Folin-Wu method (Kirk & Sawyer, 1991). The Student's t-test was used for statistical analysis. Differences were considered significant if the value of P was less than 0.05.

Results and discussion

Biochemical composition

The protein values (Table 1) recorded for all the three crop residues fell below the maintenance requirements for ruminants (10 %), poultry (14-18 %), and pigs (13-26 %) (Campbell, Taverner & Raynor, 1988; Boyd *et al.*, 1991). Amino acids were analysed because essential amino acids must be included in the diets of non-ruminants and young ruminants. The results showed the presence of 13 amino acids in the three crop residues (Fig. 1a, b, c). The absence of tryptophan may have been due to its destruction during acid hydrolysis of the proteins. Five of the amino acids–arginine, threonine, valine, leucine and phenylalanine–are considered to be essential for young pigs (Campbell *et al.*, 1988), chickens (Waldroup *et al.*, 1976), sheep and goats (Payne & Wilson, 1999). The peak areas show a relatively high level of arginine in cassava peels while the levels of the remaining four essential amino acids were highest in cocoa pod husk.

TABLE 1
Selected constituents of crop residues

Constituent	*CPH*	*CP (% dry wt)*	*PP*
Protein	7.84	5.02	6.76
Starch	2.73	9.05	2.48 [a]
Red. sugars	2.28	3.82	1.40 [a]
Lipids	2.04	1.90	5.99 [b]
Fibre	26.86	7.96	6.96 [c]

Results are expressed as mean values of at least three determinations. [a] Each mean value is significantly different ($P < 0.05$) from the other two. [b] Difference between the values for CPH and CP is not statistically significant. [c] Difference between the values for PP and CP is not statistically significant.

Starch and sugars constitute an important source of readily available energy for non-ruminants. The quantity of starch and sugars in each crop residue was, therefore, determined. The starch content of cassava peel was markedly greater (about 3-fold) than those of the other residues. Moreover, the amount of reducing sugars in cassava peel was much higher than the levels in the other two residues. These findings (Table 1) indicate that of the three residues, cassava peel may provide the largest amount of energy derived from non-structural carbohydrates for use by non-ruminants.

The term fibre in this context refers to a combination of cellulose, hemicellulose and lignin. It has been reported that high levels of fibre produce adverse effects on the growth performance of non-ruminants (Longe & Ogedegbe, 1989). Furthermore, studies on laying hens have shown an inverse relationship between egg size and fibre content of the diet (Longe, 1984). In this study, the amount of fibre in cocoa pod husks was found to be extremely high. This should be factored into the formulation of cocoa pod husk-based diets to avoid any negative influences, especially on non-ruminants.

Lipids serve as energy reserves for animals. In addition, the amount of lipid in the diet affects milk production, egg yolk composition, and appearance. Determination of lipid content is, therefore, an important aspect of feed analysis. The lipid content of plantain peels was 3-fold those of the other two residues (Table 1). In addition, plantain peel contained fairly high levels of monounsaturated and polyunsaturated fatty acids (Table 2). Cocoa pod husk was also found to contain substantial amounts of unsaturated fatty acids. High levels of unsaturated fatty acids raise the possibility of undesirable oxidative

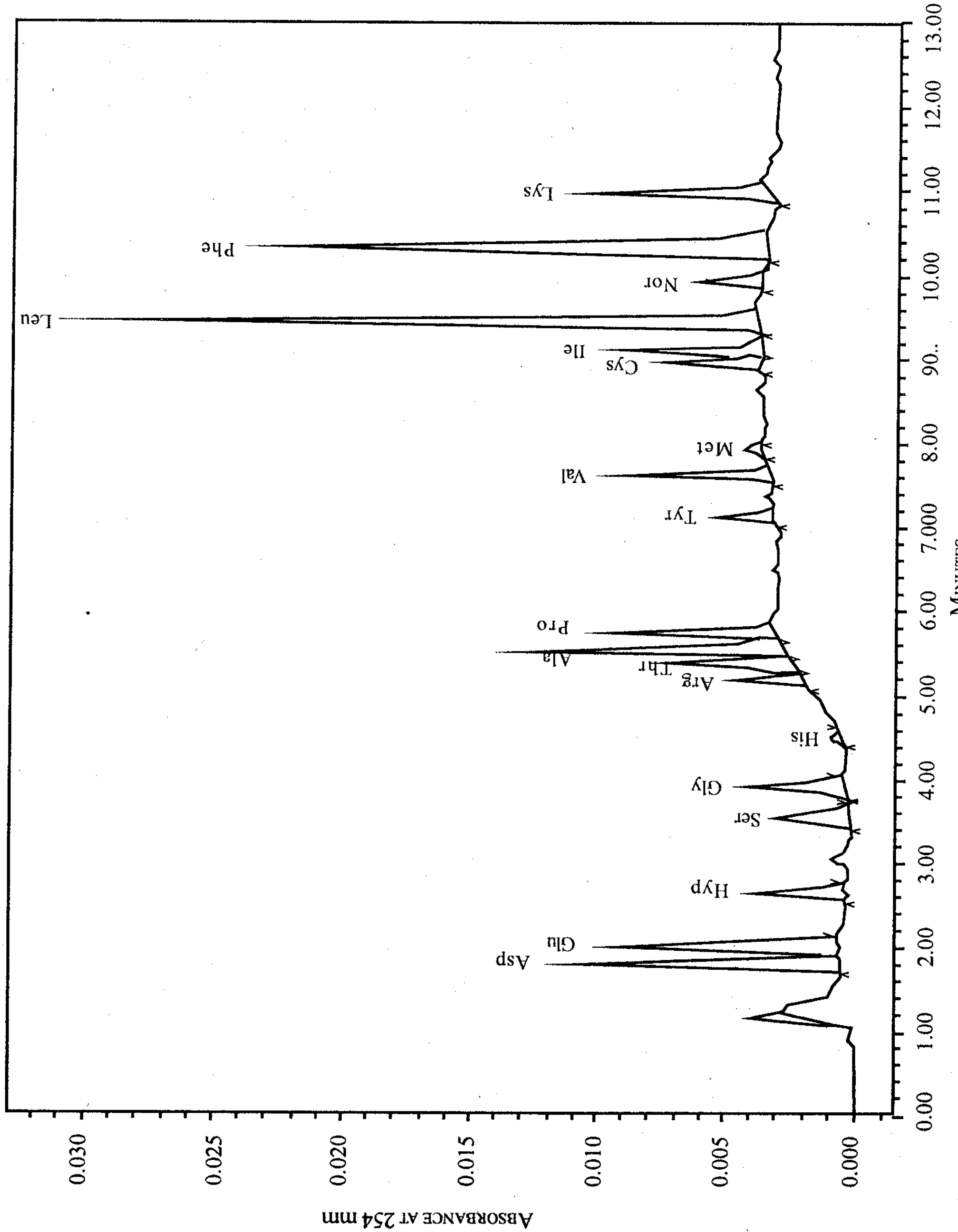

Fig. 1a. Chromatograms of derivatised amino acids from cocoa pod husk separated by HPLC, showing peaks of 13 standard amino acids and an internal standard, norleucine (Nor).

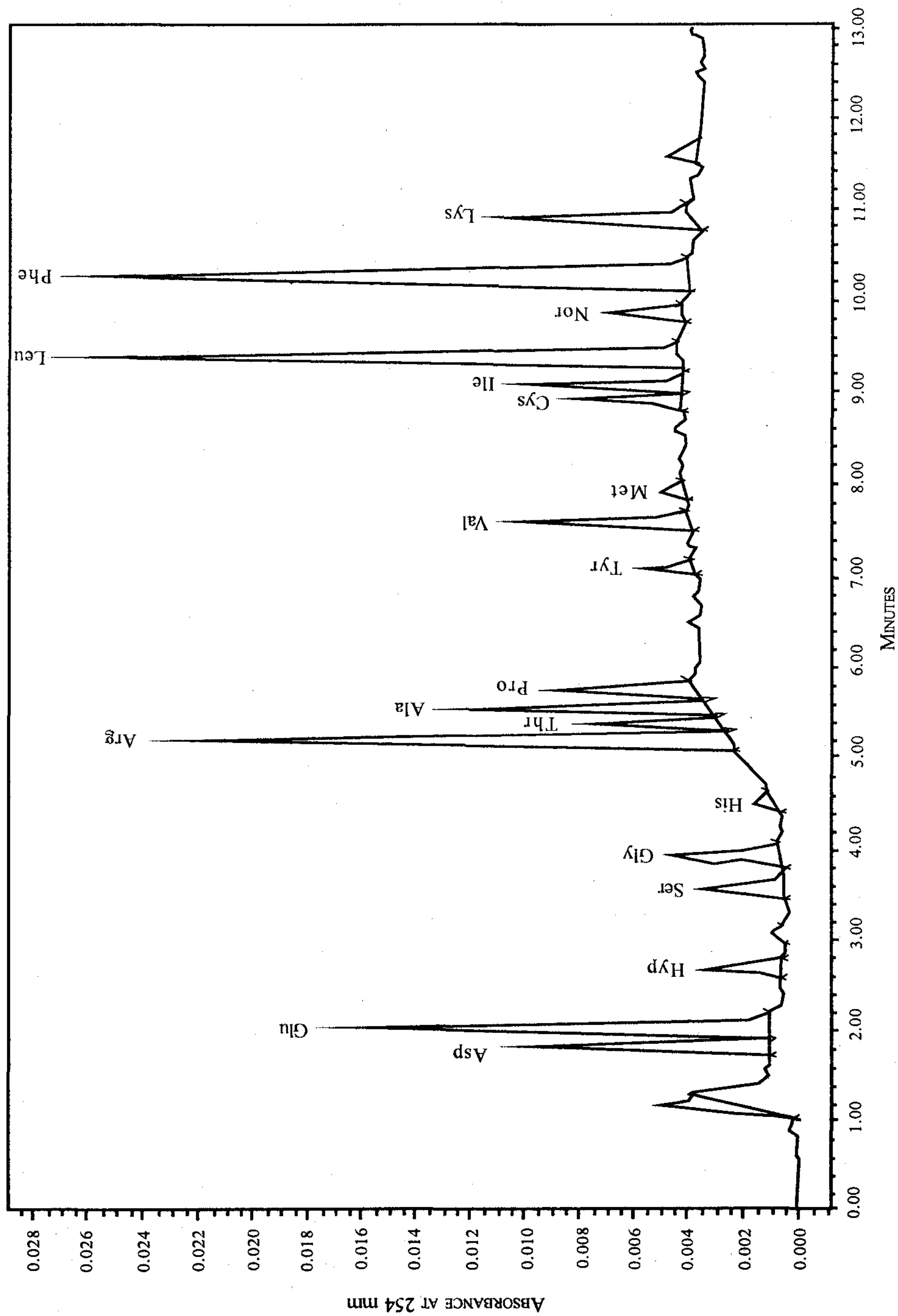

Fig. 1b. Chromatograms of derivatised amino acids from cassava peel separated by HPLC, showing peaks of 13 standard amino acids and an internal standard, norleucine (Nor).

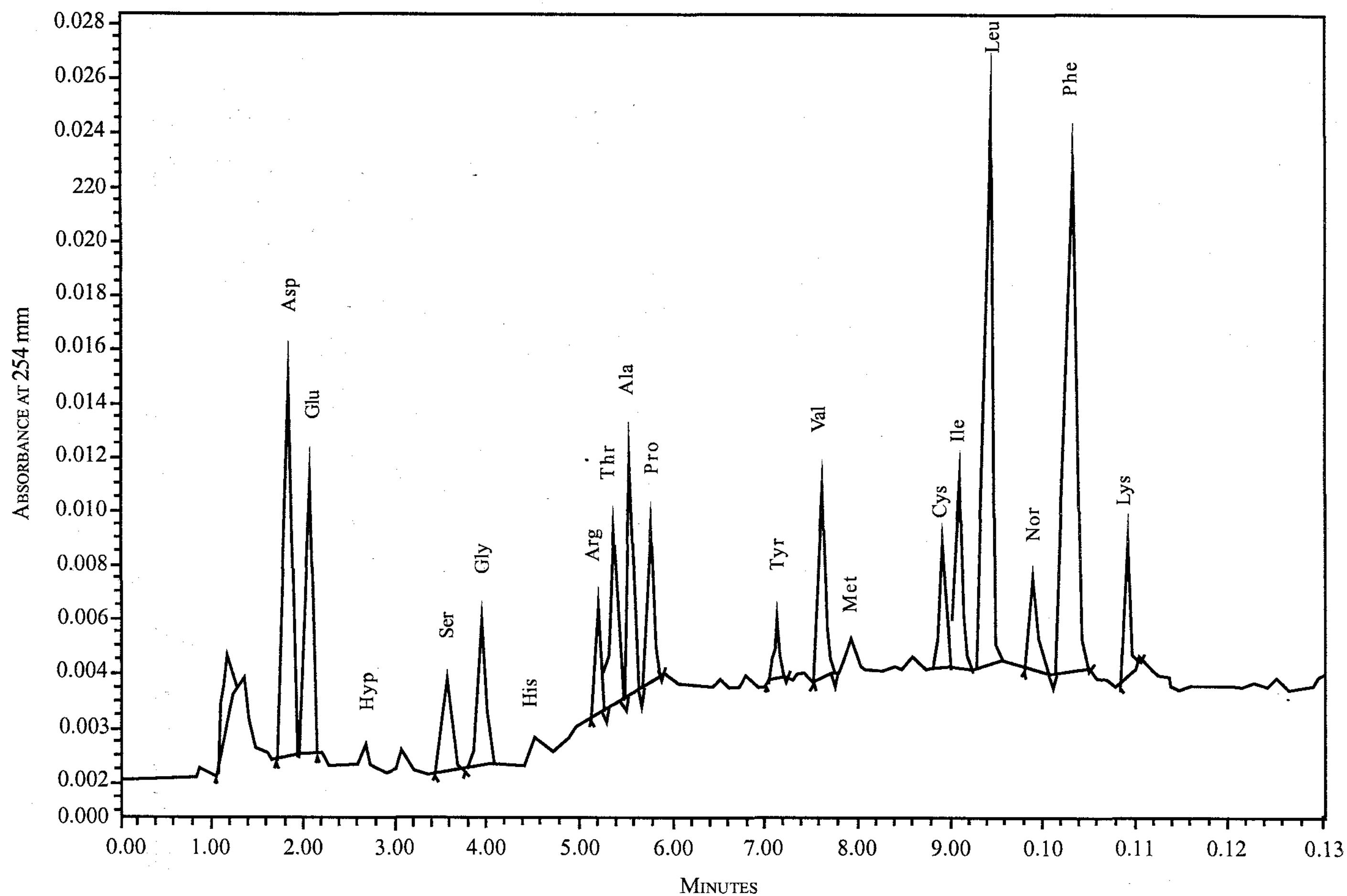

Fig. 1c. Chromatograms of derivatised amino acids from plantain peel separated by HPLC, showing peaks of 13 standard amino acids and an internal standard, norleucine (Nor).

rancidity in plantain peel-based feeds. This could lead to unpleasant taste and, consequently, poor feed use. Both cocoa pod husk and plantain peel contained linoleic acid, an essential fatty acid for poultry and pigs. Analysis of fatty acids isolated from cassava peel showed several peaks that could not be identified.

Since mineral elements perform critical functions in animals, the determination of mineral elements is an indispensable component of feed analysis. Inadequate as well as excessive amounts are associated with innumerable developmental and health-related problems. Studies were, therefore, conducted to investigate the mineral profile of the crop residues. The values (Table 3) for potassium in plantain peel and cocoa pod husk were far in excess of the amounts recommended for poultry (2.5 $mg.g^{-1}$) (BOA, 1994) and for pigs (1.7-3.0 $mg.g^{-1}$) (BOA, 1998). Similarly, the levels of calcium in cocoa pod husk and cassava peel were also high and

TABLE 2
Major fatty acids identified by gas-liquid chromatography

Fatty acid		*CPH*	*CP** *% total FA*	*PP*
12:0	lauric	5.7	-	3.9
16:0	palmitic	20.14	18.59	30.72
18:0	stearic	30.85	3.76	4.10
18:1	oleic	29.80	1.77	24.12
18:2	linoleic	9.99	-	14.07
18:3	linolenic	-	-	11.61
20:1	eicosenoic	-	-	10.46

Results are mean values from representative chromatograms. *Several peaks did not correspond with those of standard fatty acids.

TABLE 3
Analysis of mineral elements by atomic absorption spectrometry

Element	*CPH*	*CP* *($mg\ g^{-1}$)*	*PP*
Na	0.13	0.20	0.20 [a]
K	3.22	0.72	4.20
Zn	0.06	0.07	0.03
Mn	0.15	0.08	0.06 [b]
Fe	0.29	0.91	0.36 [c]
Ca	4.47	3.16	0.78 [b]
Mg	0.66	1.63	0.69 [b]
Cu	0.02	0.01	0.01
P	0.11	0.19	0.68

Results are expressed as mean values of at least three determinations.
[a] The differences between the values for all three residues are not statistically significant.
[b] The difference between the values for CP and PP is not statistically significant.
[c] The difference between the values for CPH and PP is not statistically significant.

may be of particular benefit to layers. The iron content of cassava peel was high. To avoid a surfeit of potassium and calcium in cocoa pod husk-based and cassava peel-based diets formulated for different animals, the mineral profile of each residue as well as the specific requirements of different animals should be considered during mineral supplementation.

In vitro digestibility

The digestibility of a crop residue is of utmost importance in determining the extent to which an animal would derive nutrients from it. Various approaches have been used in studying the digestibility of plant by-products. In this study, the rate of starch digestion was chosen as an index of *in vitro* digestibility. Starch is hydrolyzed by α-amylase to yield disaccharides and oligosaccharides, which act as reducing sugars. Fig. 2 presents the rate of release of reducing sugars by α-amylase from each of the crop residues. A comparison of the curves observed over a 4-h period for the untreated crop residues showed that cassava peel and cocoa pod husk had relatively low rates of digestion. In contrast, the rate of digestion of starch in untreated plantain peels was about 4-fold the rates observed for cassava peels and cocoa pod husks.

No apparent correlation was observed between starch content and the rate of starch digestion. The lignocellulosic bonds that are resistant to enzymatic attack increase indigestibility of plant by-products (DePeters *et al.*, 1997). Thus, the relatively low rate of starch digestion in untreated cocoa pod husk is attributable, at least in part, to its unusually high fibre content. However, the results for untreated cassava peel cannot be explained by fibre content, because the amount of fibre in cassava peel was similar to that in plantain peel. The anti-nutritional factors that inhibit the activity of digestive enzymes (Schofield, Mbugua & Pell, 2001) is a distinct possibility. Alkali-treatment of plant by-products has been extensively studied and is reported to

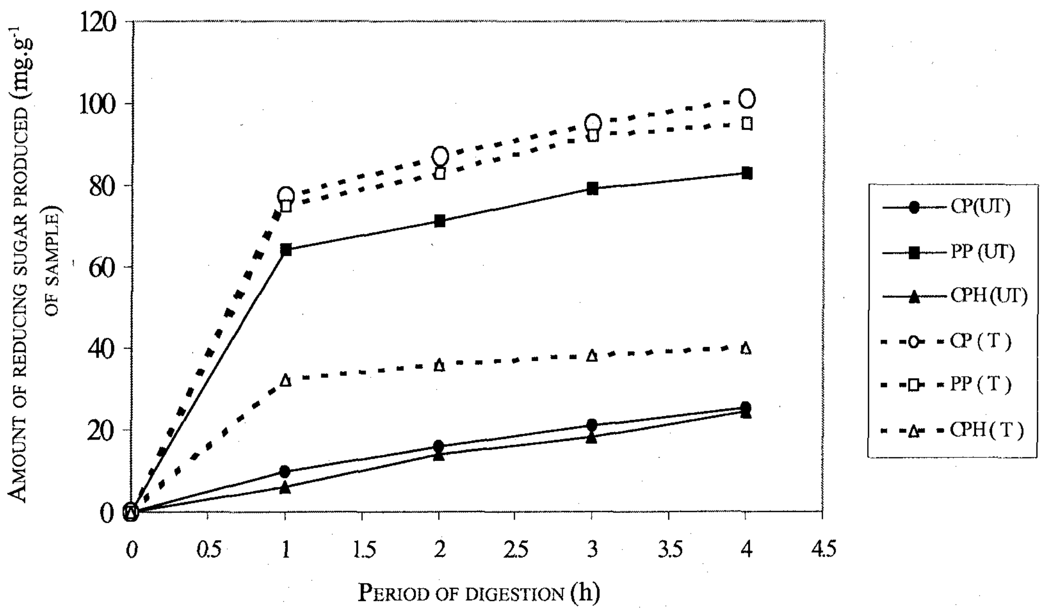

Fig. 2. Rate of *in vitro* starch digestion in untreated (——) and alkali-treated (-------) crop residues.

increase digestibility and improve nutritive value (Meeske *et al.*, 1993; Sobamiwa & Longe, 1994). Studies were, therefore, conducted to determine the extent to which treatment with NaOH would enhance *in vitro* starch digestibility of the three crop residues. The action of NaOH on the selected crop residues was not uniform. Treatment with alkali produced a dramatic improvement in the rate of starch digestion in cassava peels. However, the effects on the other residues were only marginal. Interestingly, the rate of digestion of alkali-treated cocoa pod husk was about 50 per cent that of untreated plantain peel. Since alkali treatment is reported to disrupt linkages between lignin and carbohydrate polymers (Sundstol, 1988; Sobamiwa & Longe, 1994), the results probably reflect differences in the degree of association between carbohydrate and lignin in the crop residues.

Conclusion

This study suggests that the high fibre content of cocoa pod husk may render it less digestible than other crop residues. The dramatic improvement in the digestibility of cassava peels after alkali treatment suggests that the use of alkali-treated cassava peels should be preferred to the untreated peel. Precautions must be taken to prevent or minimize undesirable oxidative rancidity during the production of plantain peel-based feedstuffs. The information generated in the study provides a basis for rational decisions concerning the type and extent of supplementation required in formulating feedstuffs for non-ruminants and ruminants using cassava peel, plantain peel, and cocoa pod husk. This should ease the formulation of highly nutritive indigenous feeds for use in the West African sub-region.

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