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EFFECTIVENESS OF CORN STALK ASH IN REDUCING TANNIN LEVELS AND IMPROVING *IN VITRO* ENZYMATIC DEGRADATION OF POLYSACCHARIDES IN CROP RESIDUES

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Abstract

The condensed tannin content of three major plant by-products in the West African sub-region, namely cassava peel (CP), plantain peel (PP) and cocoa pod husk (CPH) were measured. The values recorded for cassava peel and plantain peel were 2.0 and 1.8 per cent, respectively. Cocoa pod husk had the highest content (Amelonado–2.6 %; Tafo Hybrid–2.9 %; Amazonia–3.4 % dry wt). An aqueous extract of corn stalk ash, with a *p*H of 10.7, contained substantial amounts of potassium (51.0 ppm), sodium (16.4 ppm), chloride (22.4 ppm), and sulphate (9.5 ppm) ions. Treatment of the crop residues with corn stalk ash extract resulted in a time-dependent reduction of condensed tannin content of all crop residues (84 % reduction in cocoa pod husk after 48 h of treatment) and improved *in vitro* rates of degradation of cellulose (CPH–72.1%, CP–116.1%, PP–133.3 % after 48 h) and starch (CPH–107.5 %, CP–84.3 %, PP–82.2 % after 48 h). The findings suggest that treatment with corn stalk ash might be an effective means of detannifying and improving digestion of crop residues.

Résumé

ADAMAFIO, N. A., COOPER-AGGREY, E., QUAYE, F. O., LAARY, J. K. & QUAYE, J.: *Efficacité de la cendre de tige de maïs dans la réduction des niveaux de tannin et l'amélioration de la dégradation enzymatique in vitro de polysaccharides dans les résidus de culture.* Utilisation optimale des résidus de culture comme les ingrédients d'aliments exige une connaissance des niveaux des facteurs anti-nutritionnels tels que les tannins concentrés et les procédures simples pour leur élimination. Le contenu de tannin concentré de sous-produits de trois plantes majeures dans la sous-région de l'Afrique occidentale à savoir la pelure de manioc (PM), la pelure de plantain (PP) et l'écale de la cosse de cacao (ECC) ont été mésuré. Les valeurs obtenues pour la pelure de manioc et la pelure de banane plantain étaient respectivement 2.0 et 1.8 % l'écale de la cosse de cacao avait le contenu le plus élevé (Amelonado–2.6; Tafo Hybride–2.9; Amazonia–3.4 % poids du produit sec). Un extrait aqueux de la cendre de tige de maïs qui avait un *p*H de 10.7, contenait des quantités considérables d'ions de potassium (51.0 ppm), de sodium (16.4 ppm), de chlorure (22.4 ppm), et de sulfate (9.5 ppm). Le traitement des résidus de culture avec l'extrait de la cendre de tige de maïs menait à une réduction temps dépendante du contenu de tannin concentré de tous les résidus de culture (8.4 % de réduction en l'écale de cosse de cacao après 48 h de traitement) et améliorait les proportions in vitro de la dégradation de la cellulose (ECC–72.1, PM–116.1, PP–133.3 % après 48 h) et de la fécule (ECC–107.5, PM–84.3, PP–82.2 % après 48 h). Les résultats suggèrent que le traitement avec le cendre de tige de maïs pourrait être un moyen efficace de réaliser la détannification et la digestion améliorée des résidus de culture.

Introduction

The presence of condensed tannins, polyphenolic polymers that exert anti-nutritional effects (Kamalak *et al.*, 2004; Seresinhe & Iben, 2003; Rittner & Reed, 1992) in by-products of tanniferous plants, is a major limitation to their use as feed ingredients. Several factors contribute to the anti-nutritional effects of condensed tannins. First, the binding of the polyphenolic compounds to cell wall polysaccharides reduces the digestibility of the latter in ruminants (Schofield, Mbugua & Pell, 2001; Kumar & D'Mello, 1995). Furthermore, soluble tannins can form strong complexes with proteins which do not dissociate at physiological *p*H. Consequently, a variety of digestive enzymes, including cellulase and α-amylase, are inhibited (Kandra, 2004; Maitra & Ray, 2003; Quesada *et al.*, 1995; Petersen & Hill, 1991). In addition, condensed tannins impart an astringent taste and depress feed intake and use by animals (Brooker *et al.*, 1994), leading to growth depression. Therefore, information on the condensed tannin content of feed ingredients is essential, as is identification of simple inexpensive methods for removing tannins. Unfortunately, little is known about levels of condensed tannins in major crop residues generated in the West African sub-region.

Corn stalk ash (CSA), used as a tenderizer by the Dagabas in the Upper West Region of Ghana, may render macromolecules like condensed tannins more susceptible to degradation. However, its composition and mode of action are yet to be studied.

This study aimed to measure condensed tannin levels in cocoa pod husk, cassava peel and plantain peel, to determine the composition of an aqueous extract of CSA, and to investigate the possibility that treating the crop residues with CSA extract might reduce tannin levels and improve *in vitro* rates of polysaccharide degradation.

Experimental

Dried pellets of cocoa pod (*Theobroma cacao*) husks (pericarp) of Amazonia, Amelonado and Tafo Hybrid varieties were collected from the Cocoa Research Institute, Tafo, Ghana. Cassava (*Manihot esculenta*) peels (cortex and periderm) and unripe plantain (*Musa paradisiaca*) peels (pericarp) of the Apemtu variety were collected from various locations in the Greater Accra Region, Ghana. They were sun-dried for 2-weeks, milled and stored at 4 °C. Cellulase (45 mU/mg) and α-amylase (25 IU/mg) were collected from Fluka Chemie, Buchs, Switzerland.

Preparation and analysis of corn stalk ash extract

Corn stalks were ashed at 600 °C. Corn stalk ash extract (CSAE) was prepared by suspending 39.2 g of ash in 100 ml deionized water and filtering first through cheese cloth and then Whatman No. 1 filter paper. The ion composition of CSAE was determined using a DX-120 Ion Chromatograph, a Perkin Elmer Atomic Absorption Spectrometer 3110, and an ATS 200M Flame Photometer.

Treatment of by-products with CSAE

One gram of each by-product was steeped in 7 ml of either water or CSAE (equivalent to 0.5 M NaOH) for 24 or 48 h at 37 °C. The mixtures were filtered and the residues dried at 35 °C.

Determination of condensed tannin content

The condensed tannin content of untreated, water- and CSAE-treated crop residues was determined using the vanillin method (Price *et al.*, 1978).

Determination of rates of degradation of cellulose and starch

Samples (0.2 g) of the untreated, water- and CSAE-treated by-products were then incubated with 0.25 U/ml cellulase in phosphate buffer, *p*H 5.0, or 1.3 U/ml α-amylase in phosphate buffer, *p*H 6.7, for 3 h at 37 °C. The incubation mixtures

were filtered and the amount of reducing sugars in each filtrate was determined using the Folin-Wu method (Kirk & Sawyer, 1991).

Statistical analysis

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

The levels of condensed tannins in untreated cassava peel, plantain peel, and cocoa pod husk (Tafo Hybrid) were 2.0, 1.8 and 2.9 per cent, respectively (Table 1). Waghorn & Jones (1989) reported that 0.17 per cent tannin reduced soluble protein and ammonia in the rumen of cattle. Also, at levels greater than 1.5 per cent dry matter, tannin-containing diets fed to ducks caused endogenous protein wastage and inhibited gastrointestinal enzyme activity (Brooker *et al.*, 1994). Furthermore, Wheeler, Norton & Shelton (1994) reported deleterious effects on organic matter digestibility caused by ingestion of 0.6 per cent tannin.

Based on these observations, it would seem that the condensed tannin levels of all three crop residues are high, and are likely to cause adverse effects if the residues are used as animal feed. Cocoa pod husk (Tafo Hybrid), whose use as a feed ingredient is currently being promoted in Ghana, had the highest condensed tannin content of 2.92 per cent dry wt. It was, therefore, of interest to determine whether the high level of condensed tannin was peculiar to this particular variety or common to other major varieties of cocoa.

Experiments conducted to measure the condensed tannin content of pod husks from Amelonado and Amazonia varieties showed that both had relatively high levels (Amelonado, 2.64 %; Amazonia, 3.40 %), suggesting that a high per cent level may be common to all varieties of cocoa. The differences between the three varieties were small but statistically significant ($P< 0.05$). The findings collectively indicate that detannification of the crop residues investigated, particularly cocoa pod husk, is desirable if they are to be used as feed ingredients.

The possibility that corn stalk ash might act as an effective detannification agent was also investigated. An aqueous solution prepared from the ash was found to be strongly alkaline, *p*H 10.7, and ion analysis showed that it contained substantial amounts of potassium, sodium, chloride and sulphate (Table 2). Treatment of the crop residues with CSAE for 48 h resulted in a dramatic reduction in tannin content. The magnitude of the detannification effect was greatest in cocoa pod husk (84 % reduction). Less

TABLE 1
Condensed tannin content of selected plant by-products

By-product	*Condensed tannin content (% dry wt)*
Cassava peel	20.00 + 0.06
Plantain peel	1.83 + 0.07
Cocoa pod husk (Tafo Hybrid)	2.92 + 0.02

Results are expressed as mean values and standard errors of at least three determinations

TABLE 2
Ion composition of corn stalk ash extract

Cation	*Concentration (ppm)*	*Anion*	*Concentration (ppm)*
Na^+	16.4	Cl^-	22.4
K^+	51.0	NO_3^-	0.4
Ca^+	0.3	PO_4^{3-}	2.5
Mg^{2+}	1.0	SO_4^{2-}	9.5
Zn^{2+}	0.04	F^-	1.6
Fe^{2+}	0.3		

Results are expressed as mean values of at least three determinations.

pronounced effects were observed when the residues were treated for 24 h (Fig.1), indicating that the action of the extract was time-dependent.

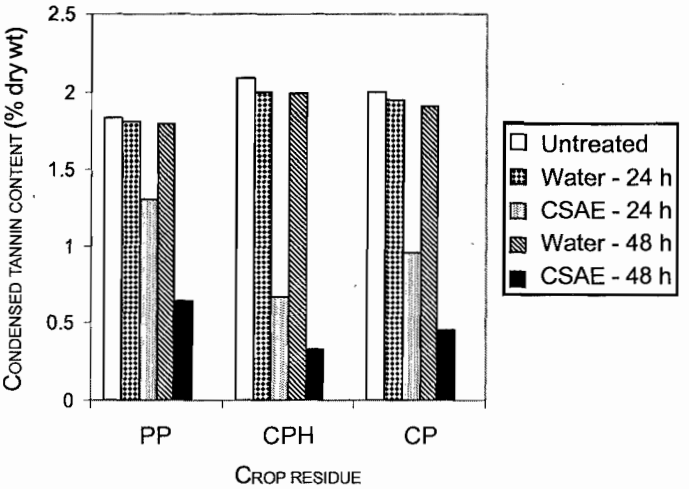

Fig. 1. Condensed tannin levels in untreated, water- and CSAE-treated crop residues.

Dried ground crop residues were pretreated with water or corn stalk ash extract for 24 or 48 h as described in the methods section. Condensed tannin levels of untreated and treated crop residues were determined using the Vanillin-HCl method.

For instance, the condensed tannin content of cassava peel decreased by 52.0 per cent after 24 h and 77.5 per cent after 48 h of treatment. The observed reduction in tannin levels may be due to alkaline hydrolysis and solubilization of tannins, as these polyhydroxylated compounds are thought to undergo alkali-induced deprotonation and delocalization of their *pi* electrons, rendering the carbon-carbon bonds linking their monomers susceptible to attack and cleavage by alkali (Kratzer & Singleton, 1969).

It is reasonable to expect that a decrease in condensed tannin content would be associated with a corresponding increase in the digestibility of crop residues, as they inhibit the activities of digestive enzymes (Maitra & Ray, 2003). For this reason, the *in vitro* rates of degradation of cellulose and starch after treating residues with the CSAE were also investigated. Fig. 2 shows that treatment of the by-products with CSAE resulted in marked increases in the subsequent rate of degradation by exogenous cellulose (CPH–72.1, CP–116.1, PP–133.3 % after 48 h). This improved *in vitro* digestibility may be partly attributed to the CSAE-induced decrease in condensed tannin levels and disruption of non-covalent interactions between cellulose and tannins, as tannins bind to cellulose and inhibit cellulase activity (Schofield *et al.*, 2001; Kumar & D'Mello, 1995). The disruption of lignocellulosic bonds by the alkaline CSAE may also have contributed to the enhanced rate of cellulose degradation (Hartley & Jones, 1978; Sobamiwa & Longe, 1994). Fig. 3 depicts the effect of CSAE on degradation of starch, which was also impressive (CPH–107.5, CP–84.3, PP–82.2 % after 48 h).

The treatment of crop residues, particularly cocoa pod husk, with corn stalk ash extract is an effective means of reducing con-densed tannin content and upgrading polysac-charide digestibility. As cost effectiveness is a major objective in the use of crop residues as feed ingredients, any detannification procedure used must be relatively inexpensive. Fortunately, preparation of the ash extract does not require sophisticated techniques, and corn stalks are available in most parts of the West African sub-region at little or no cost.

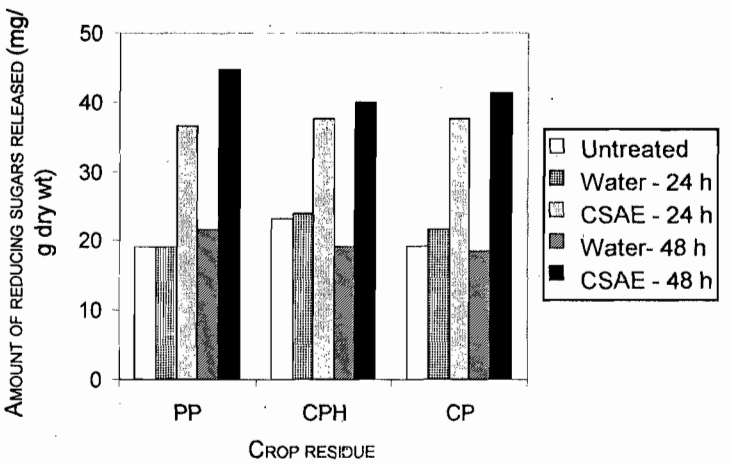

Fig. 2. Effect of CSAE treatment on cellulose degradation.

Dried ground crop residues were pretreated with water or corn stalk ash extract for 24 or 48 h as described in the methods section. Untreated and treated crop residues were subsequently incubated with 0.25 U/ml cellulase in phosphate buffer, *p*H 5.0, for 3 h at 37 °C. The amount of reducing sugars released into the incubation medium was measured spectrophotometrically.

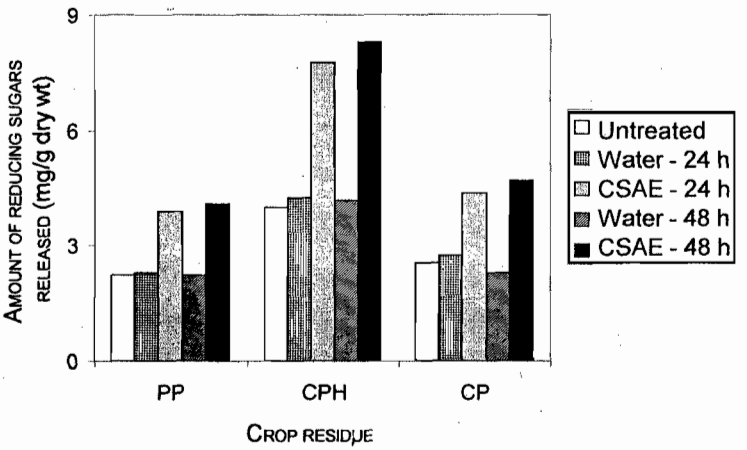

Fig. 3. Effect of CSAE treatment on rate of starch degradation.

Dried ground crop residues were pretreated with water or corn stalk ash extract for 24 or 48 h as described in the methods section. Untreated and treated crop residues were subsequently incubated with 1.3 U/ml α-amylase, *p*H 6.7, for 3 h at 37 °C. The amount of reducing sugars released into the incubation medium was measured spectrophotometrically.

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