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The Polyphenol Content of the Outercoat of the Mature Fruit of *Treculia africana*

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ABSTRACT

The polyphenol content of the outercoat of the mature fruit of T. africana *has been determined, as a preliminary part of a study to ascertain the basis of toxicity of the outercoat to animals. The fruits of* T. africana *are not susceptible to bird or animal damage due to the high level of polyphenolic material in the outercoat.*

Mature fruits of T. africana *obtained from trees cultivated on low-nutrient soils in the rain forest—location I (Ishara-Remo) and location IV (Ijebu-Ode)—contain significantly higher concentrations of phenolic compounds than those found in similar rain forest vegetation on nutrient-rich soils—location II (Ibadan) and location III (Gambari Forest reserve).*

The significance of these results is discussed.

INTRODUCTION

Bird damage poses a serious problem to the fruit-growing industry in Nigeria, but the level of damage observed varies from fruit species to species and from variety to variety. However, the fruit of *T. africana* has been found not to be susceptible to animal or bird damage. An investigation into the reason for this has shown that the outercoat of the fruit *T. africana* contains high levels of polyphenolic compounds.

Secondary plant metabolites play an important role in determining the palatability of plant tissues to herbivores (Harborne, 1982) and, in

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particular, plant materials containing high levels of polyphenolic substances (>0·2%) are not acceptable to most herbivores (Ariga *et al.*, 1981; Reed *et al.*, 1982). The Colobus monkey avoids the mature leaves of the plants in favour of young growing leaves containing relatively low levels of polyphenols (Oates *et al.*, 1977).

The so-called bird-resistant varieties of sorghum grain contain higher concentrations of polyphenols than susceptible varieties (Green, 1974; Bullard *et al.*, 1981).

The polyphenol content of plant material varies with the stage of development of the plant, and the conditions under which it is grown. Bullard *et al.* (1981) reported that the polyphenolic content of sorghum grains varies during its development due to both the non-enzymic, spontaneous condensation of the flavonoid units and polyphenol oxidase (PPO)- and peroxidase (POD)-mediated reactions.

Plants grown under poor soil conditions exhibit higher polyphenol concentrations than the same varieties grown in soil with a better nutrient content; thus, soil conditions could be implicated in food selection (McKey *et al.*, 1978; Gartland *et al.*, 1980). This study was carried out to determine the polyphenol content of the outercoat of the fruits of *T. africana* obtained from different locations in Nigeria.

EXPERIMENTAL

Materials and methods

Sample collection

The mature fruits of *T. africana* were collected from different locations in Nigeria: (i) Ishara-Remo, Ogun State; (ii) Ibadan, Oyo State; (iii) Gambari Forest reserve; and (iv) Ijebu-Ode. The fruits were weighed.

Sample preparation

The outercoat of the fruit of *T. africana* was removed using a sharp knife and then dried in a current of air at about 50°C for 3 days. The material was then weighed, ground to a fine powder using a hammer mill and stored desiccated at −20°C until required for analysis.

The dried material (50 mg) was extracted overnight at 4°C in methanol (5 ml). Water (5 ml) was added and the material extracted for a further overnight period. The extract was centrifuged at 8000 *g* for 30 min and an aliquot (2·5 ml) of the supernatant was retained for subsequent analysis. The sample extract was assayed by two chemical methods; namely, the Folin–Denis (F/D) and vanillin (VAN) techniques.

Folin–Denis assay. This is based on the non-stoichiometric oxidation of the molecules containing a phenolic hydroxyl group.

The reagent was prepared by the method of the AOAC (1975). Stock reagent (15 ml) was diluted with water (85 ml) just prior to use. Plant extract (50 μl) was added to the diluted F/D reagent (2·5 ml) in a cuvette and mixed.

Exactly 90 s later, a saturated solution of sodium carbonate (100 μl) was added and the mixture incubated at room temperature for a further 18·5 min. The absorbance at 725 nm was determined against a reagent blank and quantified against 50 μl aliquots of a standard curve of 0–1 mg/ml tannic acid solution assayed in the same manner as the samples. The results of this assay are expressed as milligrams of tannic acid equivalents per gram dry weight of plant material.

Vanillin (VAN) assay. This is specific for dihydroxy phenols and is particularly sensitive for *meta*-substituted di- and tri-hydroxybenzene-containing molecules.

Vanillin (1 g) was dissolved in 12·8 M H_2SO_4 (100 ml) ensuring that the temperature did not rise above 40°C. Sample extract (25 μl) was added to the reagent (2·5 ml) in a cuvette and the mixture incubated at room temperature for exactly 15 min. The absorbance of the red carbonium adduct produced was measured at 500 nm and quantified by reference to a standard curve of 0 to 1·25 mg per millilitre phloroglucinol treated in the same manner as the samples. The results are expressed as milligrams of phloroglucinol equivalents per gram dry weight of plant material.

RESULTS AND DISCUSSION

Table 1 shows the level of phenolic substances in the outercoat of the fruit of *T. africana* as determined by (i) the Folin–Denis assay of phenolic hydroxyl groups present in the extract, and (ii) the vanillin assay of *meta*-substituted di- and tri-phenols present in the extract. The results are expressed as milligrams per gram dry weight tannic acid equivalents and phloroglucinol equivalent.

The level of total phenolic material, as determined by the Folin-Denis assay, is significantly higher than the vanillin assay for all the different localities. The F/D assay is based on a non-stoichiometric oxidation of any phenolic hydroxyl groups in the outercoat so the values obtained in Table 1 represent the level of simple phenols and more complex molecules in the extracts. The vanillin reagent is more specific than the F/D reagent because it reacts most strongly with *meta*-substituted di- and tri-phenols such as phloroglucinol (1,3,5-trihydroxybenzene). Under acidic

TABLE 1
Level[a] of Phenolic Substance in the Outercoat of the Mature Fruits of *T. africana* from Different Locations in Nigeria

Sample of outercoat of fruit of T. africana *from*	*Folin–Denis (F/D) assay (milligrams per gram dry weight tannic acid equivalent)*	*Vanillin (VAN) assay (milligrams per gram phloroglucinol equivalent)*
Location I (Ishara-Remo)	95·0 ± 0·9	45 ± 0·7
Location II (Ibadan)	76 ± 0·8	36 ± 0·6
Location III (Gambari Forest reserve)	70 ± 1·2	34 ± 0·5
Location IV (Ijebu-Ode)	89·5 ± 1·1	43·5 ± 0·9

[a] Mean value ± standard error of four determinations.
Results are expressed as milligrams per gram dry weight tannic acid equivalent and phloroglucinol equivalents.

conditions, vanillin undergoes an electrophilic substitution (condensation with the phenol), a reaction which is favoured with *meta*-substitution of the latter but which will take place at a slower rate with *ortho*-substituted molecules such as catechol (1,2-dihydroxybenzene). Thus, the decrease in apparent vanillin-positive material may reflect an increased degree of polymerisation of polyphenol content. Such an increase would not be reflected by the more non-specific F/D assay.

With all results (F/D and VAN assays) mean values obtained for locations I and IV were significantly greater than those for locations II and III. These findings agree with the views expressed by Janzen (1974) that vegetation growing on poor nutrient soils would be found to contain greater concentrations of herbivore-deterrent toxic, secondary compounds (such as tannins, saponins and alkaloids) than would vegetation growing on more nutrient-rich soils.

In locations I and IV, the relative poverty of the soils of these sites is reflected in the low rates of regeneration after clearing and the poor performance of farms and gardens on these soils.

Polyphenols constitute the most widespread major class of ecologically important secondary compounds. However, the absence of any general role for most polyphenols in the internal economy of plants (Levin, 1971; Swain, 1977) supports the view that the polyphenols owe their wide distribution and their often high concentrations to selective advantages conferred by their deterrence of herbivores and pathogens.

O-Diphenols found in plant materials readily undergo enzymic or spontaneous oxidation to *O*-semiquinone radicals or to *O*-quinones on rupture of cells. These products can couple covalently with various functional groups found in proteins such as amines, thiol, indole and imidazole. These covalent and non-covalent interactions would affect the digestibility and nutritive value of proteins (Synge, 1975; Van Sumere *et al.*, 1975).

The exact mechanisms by which polyphenols influence the behaviour of a herbivore are unknown, but, in human terms, this group of compounds is known to affect the taste of plant materials and products, such as wine and beer, derived from them. In addition, the presence of high concentrations of polyphenolic material may adversely affect the nutritional quality of plant material.

Drying and storage of plant material can result in qualitative and quantitative changes in polyphenolic compounds (Harborne, 1982). In this study, due to the use of oven drying, no attempt has been made to distinguish between the various polymer sizes within the total polyphenol extracts. As this factor plays an important role in determining the organoleptic properties of the material, future experiments are planned using freeze-dried material and HPLC to separate and quantify the constituent polyphenol groups. Whether polyphenol composition alone is important in ascertaining the toxicity of the outercoat or whether other factors, such as the presence or absence of other chemicals in the outercoat, have a part to play will also be investigated.

CONCLUSIONS

(1) Our study provides empirical support for the hypothesis that fruits cultivated on soils of low nutrient-supplying capacity contain higher concentrations of polyphenols than those cultivated on soils of rich nutrient-supplying capacity.

(2) Our data suggest that high contents of polyphenol compounds in the outercoat of the fruit of *T. africana* may explain the general avoidance of the outercoat of fruits by birds and animals.

REFERENCES

AOAC (1975). *Official methods of analysis of the Association of Official Analytical Chemists* (12th edn) (Horwitz, W. (Ed.)), AOAC, Washington, DC, 164–5.

Ariga, T., Assao, Y., Sugimoto, H. & Yokotsuke, T. (1981). The occurrence of astringent oligomeric proanthocyanidins in legume seeds. *Agric. Biol. Chem.*, **45**, 2705–8.

Bullard, R. W., York, J. O. & Kilburn, S. R. (1981). Polyphenolic changes in ripening bird-resistant sorghum. *J. Agric. Fd Chem.*, **29**, 973–81.

Gartland, J. S., McKey, D. B., Waterman, P. G., Mbi, C. N. & Struhsaker, T. T. (1980). A comparative study of phytochemistry of two African rain forests. *Biochem. System. Ecolog.*, **8**, 401–22.

Goldstein, J. L. & Swain, T. (1963). Changes in tannins in ripening fruits. *Phytochemistry*, **2**, 371–83.

Green, V. E. (1974). Yield and digestibility of bird resistant and non-bird resistant grain sorghum. *Proc. Soil Crop. Sci. Soc. Florida*, **33**, 13–16.

Harborne, J. B. (1982). *Introduction to ecological biochemistry* (2nd edn), Academic Press, 155–77.

Janzen, D. H. (1974). *Biotropica*, **6**, 69. Cited by McKey *et al.* (1978).

Levin, D. (1971). Plant phenolics: an ecological perspective. *Am. Nat.*, **105**, 157–81.

McKey, D. B., Waterman, P. G., Mbi, C. W., Gartland, J. S. M. & Struhsaker, T. T. (1978). The phenolic contents of vegetation in two African rain forests. Ecological implications. *Science*, **202**, 61–3.

Oates, J. F., Swain, T. & Zantovska, J. (1977). Secondary compounds and food selection by Colobus monkeys. *Biochem. System. Ecol.*, **5**, 317–21.

Reed, J. D., McDowell, R. E., Van Soest, P. J. & Horvath, P. J. (1982). Condensed tannins: A factor limiting the use of Cassava forage. *J. Sci. Fd Agric.*, **33**, 213–20.

Swain, T. (1977). Secondary compounds as protective agents. *Ann. Rev. Plant Physiol.*, **28**, 479–501.

Synge, R. L. M. (1975). Interactions of polyphenols with proteins in plants and plant products. *Qual. Plant.–Plant Foods Hum. Nutr.*, **24**, 337–50.

Van Sumere, C. F., Albrecht, J., Dedonder, A., De Pooten, H. & Irma, P. E. (1975). Plant proteins and phenolics. In *The chemistry and biochemistry of plant proteins* (Harborne, J. B. & Van Sumere, C. V. (Eds)), Academic Press, New York, 211–64.