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On the Determination of Papaya Seed Adulteration of Black Pepper

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ABSTRACT

Determination of the amount of benzyl glucosinolate (glucotropaeolin) in black pepper seed and products affords a sensitive method of determining the degree of adulteration with papaya seed. The glucotropaeolin may be estimated directly using gas chromatography, or indirectly by measuring the glucose which is quantitatively released following enzymic treatment. No attempt has been made to optimise the conditions of the extraction since, as described, the method allows detection of 0·1% papaya seed contamination/adulteration.

INTRODUCTION

Pepper (*Piper* spp.) is widely grown in India and South East Asia and is the most important of the oriental spices. Govindarajan (1977) reported that over a quarter of the total spice trade was associated with pepper. The annual trade in pepper is valued at around £21 million (Pruthi, 1980). Because of its commercial value, pepper seed is liable to adulteration with cheaper plant materials of similar shape, size and colour (Sreedharan *et al.*, 1981). In particular, seeds of the black pepper (*P. nigrum* L.) may be adulterated with seeds of papaya (*Carica papaya* L.). Gross contamination of this kind may be detected by alcohol flotation or microscopic examination (Pruthi & Kulkarni, 1969) or by taking advantage of the different chromatographic behaviour and UV

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characteristics of the phenolics isolated from these seeds (Hartman *et al.*, 1973). Recently, Sreedharen *et al.* (1981) have described a detection method based upon the staining of seed sections with a solution of potassium iodide/iodine, pepper giving a blue coloration (due to starch) whilst the papaya sections were stained pale red (characteristic of medium length dextrins).

It has been reported (Ettlinger & Hodgkins, 1956; Tang, 1970; Chan *et al.*, 1978) that papaya seed contains benzyl glucosinolate (glucotropaeolin). Glucosinolates, of which almost a hundred are now known (Fenwick *et al.*, 1983), are of well defined botanical origin, being confined to families of dicotyledonous angiosperms (Kjaer, 1974). As such they would not be expected to occur in the monocotyledonous Piperaceae. Sensitive techniques have recently been developed for the analysis of glucosinolates (Olsen & Sørensen, 1981; McGregor *et al.*, 1983) and have, for example, been applied in a study of charlock (wild mustard, *Sinapis arvensis* L.) contamination of rapeseed (Fenwick *et al.*, 1981). It was thought that a similar method might be appropriate for the determination of papaya seed adulteration and the results of the subsequent study are presented here.

EXPERIMENTAL

Materials

Samples of black pepper seed and ground pepper were obtained from local shops and supermarkets. Papaya seed was obtained from India, Malaysia and from Singapore, as well as from fruit grown in Hawaii. Adulterated pepper samples (AP 1–4) were supplied by Dr H. Narasima. Laboratory adulteration of pepper (LAP 1–7) was carried out by adding the requisite amount of ground papaya seed to the pepper seed or powder. 2-Propenyl glucosinolate (sinigrin) was isolated from brown mustard (*Brassica juncea* L.) as described by Hanley *et al.* (1983).

Methods

Ground seed samples and pepper powder were extracted according to Thies (1976) with isooctane:isopropanol (9:1, 2 × 10 ml per gram of seeds). The air dried product was assayed for glucotropaeolin by gas chromatographic and glucose release methods.

Gas chromatographic method

A hundred milligrams of the defatted product were extracted with boiling water (2 ml) for 15 min, cooled, centrifuged and the solids extracted with a further 1 ml of boiling water for 5 min. The combined supernatants were diluted with 1 ml of sinigrin solution and made up to 4·0 ml with water, 2 ml of this solution was further diluted with water (3 ml) and treated with a barium acetate (0·5 M) and lead acetate (0·5 M) mixture (1 : 1 by volume, 200 μl). After centrifugation a sample of the supernatant (2 ml) was applied to a microcolumn of DEAE Sephadex A-25 (20 mg) (Thies, 1977) and washed with pyridine acetate (0·02 M, 2 × 1 ml). The glucosinolates were subsequently desulphated (Thies, 1979) and eluted, derivatised and analysed as described by Heaney & Fenwick (1980).

Glucose release

A hundred milligrams of the defatted product were extracted as above. The combined supernatants were made up to 4 ml and 2 ml of this was applied to the microcolumn of DEAE Sephadex A-25 (20 mg). In the case of the samples LAP 1–2, all of the sample was applied to the column. The columns were washed with water (300 μl) and then filled up and allowed to drain. Glucose release analysis was then carried out according to the method of Heaney & Fenwick (1981), the glucose being estimated using a Sigma glucose kit No. 115 (Sigma Chem. Co.).

RESULTS AND DISCUSSION

The papaya seeds examined in the present study contained between 1·1 and 3·0 g glucotropaeolin per 100 g of defatted seed, as determined by gas chromatography (Heaney & Fenwick, 1980). Previous workers (Ettlinger & Hodgkins, 1956; Tang, 1970; Chan *et al.*, 1978) had found lower levels (0·7–1·1 g/100 g) using analysis based upon measurement of the benzyl isothiocyanate released from glucotropaeolin on treatment with myrosinase. In agreement with these earlier reports, no additional glucosinolates were found in papaya seed. This enables the glucose released after myrosinase treatment and estimated spectrophotometrically (Heaney & Fenwick, 1981) to be expressed in terms of glucotropaeolin content. As was expected, none of the black pepper samples examined contained glucosinolates.

Table 1 contains the results of the analysis of the laboratory

TABLE 1
Analysis[a] of Laboratory Adulterated Black Pepper

Sample	*Calculated glucotropaeolin*	*GC*	*Glucose release*
LAP 1	1·6 mg[b]	1·3 ± 0·3[b]	1·4 ± 0·7[bc]
LAP 2	2·4 mg	2·8 ± 0·3	2·8 ± 0·6[c]
LAP 3	5·6 mg	5·2 ± 0·4	5·9 ± 0·4
LAP 4	10·7 mg	11·1 ± 0·5	10·4 ± 0·5
LAP 5	20·6 mg	20·4 ± 0·3	21·3 ± 0·9
LAP 6	40·4 mg	40·0 ± 0·2	41·0 ± 0·6
LAP 7	72·3 mg	71·9 ± 0·6	72·8 ± 0·3

[a] In triplicate.

[b] Glucotropaeolin content expressed as mg/100 g defatted meal.

[c] Four millilitres of original extract taken for glucose release.

adulterated samples (LAP 1–7), using gas chromatographic and glucose release methods. At the lowest levels (LAP 1–3, corresponding to papaya adulteration of <0·5%) the glucose release method was less reproducible than the gas chromatographic method due mainly to the low level of glucose released and the consequent low absorption of the colour produced by the glucose-detection reagent.

However, in all cases, glucose was released, even when an additional sample containing 0·8 mg glucotropaeolin/100 g meal was examined (in a single assay). Since the flotation of adulterated black pepper seed had previously been shown to concentrate papaya seeds (Pruthi & Kulkarni, 1969) this was applied to a number of pepper seed/papaya seed mixtures, glucose release figures, with and without the flotation procedure, being shown in Table 2. In all cases the 'floaters' contained the expected papaya seed as well as light pepper seeds. The reproducibility was improved, as can be seen in the table, for the analysis of the trace adulteration (seed samples 4 and 5). The lack of agreement between the glucose release figures at the highest level of adulteration (sample 1) may be ascribed to different weights of individual papaya seeds and also to the variation in glucotropaeolin content within individual seeds.

Assuming an average glucotropaeolin content in papaya seed of 1 g/100 g then the 5 mg level noted in Tables 1 and 2 corresponds to 0·5% adulteration. It should be emphasised here that little attempt was made to optimise the conditions of the analysis; for example, by varying the

TABLE 2
Analysis[a] of Papaya–Black Pepper Seed Mixtures

Sample	*Papaya/pepper ratio*	*Glucose release*	*Flotation glucose release*
1	1:50	25·6 ± 1·8[b]	27·6 ± 0·9[b]
2	1:100	15·7 ± 1·3	16·9 ± 0·8
3	1:200	7·3 ± 0·9	6·6 ± 0·3
4	1:400	3·9 ± 1·2	3·0 ± 0·4
5	1:600	2·4 ± 0·9	1·9 ± 0·4

[a] In triplicate.
[b] Glucotropaeolin content expressed as mg/100 g defatted meal.

amount of extract passed through the A25 column, or by increasing the amount of seed or powder analysed. However, the flotation of samples prior to defatting and analysis would allow a considerable degree of adulterant concentration.

Table 3 shows the results of the analysis of contaminated pepper samples, using both gas chromatographic and glucose release methods. Four samples, AP1–4, were found to contain glucotropaeolin (and therefore were presumed to have been contaminated with papaya seed). AP5, which was also thought to be adulterated with papaya seed, was found to be free of glucotropaeolin. These samples were also examined by the method of Sreedharan *et al.* (1981), which enabled the samples AP1–5 to be ranked according to the degree of papaya contamination. Whilst this was not put on a quantitative basis, it will be seen that there is agreement between this ranking and the glucotropaeolin contents found.

TABLE 3
Analysis[a] of Adulterated Black Pepper

Sample	*GC*	*Glucotropaeolin content*[b]	*KI/Iodine*
AP1	8·6 ± 0·3	8·1 ± 0·4	+
AP2	7·3 ± 0·3	7·9 ± 0·6	+ +
AP3	18·1 ± 0·9	17·4 ± 0·8	+ + + +
AP4	11·6 ± 0·7	11·9 ± 1·0	+ + +
AP5	—	—	+

[a] In triplicate.
[b] mg/100 g defatted seed.

The glucosinolate content of papaya seed is likely to vary with the cultivar type, conditions of growth and maturity of the seed, all of which factors have been shown to have a profound effect in other glucosinolate containing plants (Fenwick *et al.*, 1983); thus, it is difficult to be precise about the degree of papaya seed contamination *per se*; this being best expressed indirectly in terms of milligrams of glucotropaeolin per 100 g seed (meal).

Whilst papaya seed contamination of pepper seed may be estimated by other techniques, the present methods are equally applicable to seed and powdered material and appear suitable for the determination of $>0{\cdot}1\,\%$ adulteration of pepper seed by papaya seed. The gas chromatographic method is limited by the need for specialised equipment and thus may be better suited to a quality control laboratory. The microcolumn method, originally developed for the rapid analysis of rapeseed and *Brassica* vegetable extracts, would thus seem more appropriate for routine screening purposes.

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