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Effect of irradiation on volatile oils of black pepper

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Ionising radiation is a potential method for microbial decontamination of spices, and a method for the detection of irradiated spices is required. The steam-distilled volatile oils of black pepper from three sources, untreated and treated with 10, 20 and 30 kGy doses of gamma radiation, were compared. The effect of post-irradiation storage times of 1, 30 and 90 days at 24°C was also determined. The composition of the volatile oils was analysed by capillary gas chromatography. No systematic change was observed in the volatile oil content with radiation dose or storage time. Principal components analysis of the GC data showed clear differentiation between control and irradiated samples of two peppers immediately after treatment. Upon storage, the differences became smaller. The third pepper sample showed few changes due to radiation.

INTRODUCTION

Pepper (*Piper nigrum* L.) is the most widely used of all condiments. Black peppercorns are the whole, dried berries of the pepper vine, and are the form traditionally traded, black pepper alone accounting for about 25% of the total spice trade. Black pepper is normally marketed on the basis of geographical origin or port of shipment, designated types differing in physicochemical and sensory properties (Farrell, 1985). The components contributing to its value are the alkaloids, of which piperine is the most important, for pungency, and the volatile (essential) oil for odour and flavour. The steam-volatile oil can vary quantitatively and qualitatively owing to cultivar, maturity at harvest, processing method, and storage conditions. It consists primarily of 70–80% monoterpene hydrocarbons (Debrauwere & Verzele, 1975), 20–30% sesquiterpene hydrocarbons and less than 4% oxygenated compounds (acids, esters, carbonyl compounds, alcohols and oxides). The chief components of the monoterpene fraction are α- and β-pinene, sabinene, δ-3-carene and limonene, while β-caryophyllene is the major sesquiterpene (Lawrence, 1981).

Whereas many spices are highly contaminated with microorganisms, it is of growing importance that the microbial contamination level should not exceed an acceptable limit of 10^4 organisms g^{-1} (Farkas, 1984). In general, black pepper is among the most highly contaminated spices, containing viable counts reaching levels of 10^6–10^8 g^{-1} (Eiss, 1984). Decontamination treatment with ionising radiation has been applied with considerable success, the more penetrating X-rays and gamma rays being preferred for bulky materials. The ions produced decompose rapidly to form free radicals which react with each other and with unchanged molecules, ultimately to produce stable chemical substances, the radiolytic products. It is the reactions of free radicals that produce the desired technological effects. A dose between 10 and 20 kGy is required to achieve a total viable count of less than 10 g^{-1} in natural spices (radappertisation), while doses of 3–10 kGy (radicidation) can reduce the viable cell count to an acceptable level. This is approximately equal in microbiocidal effect to fumigation (Eiss, 1984).

Since irradiation has achieved limited regulatory approval, qualitative and quantitative tests should be available to enforce restrictions and labelling regulations. Irradiation of black pepper at doses up to 30 kGy was found to produce free radicals detectable by electron spin resonance (Shieh & Wierbicki, 1985), but the radicals were not long-lived. Heide & Bögl (1987) showed that irradiated ground black pepper could be identified up to 14 days after treatment by chemiluminescence, and up to 120 days by thermoluminescence. Sanderson *et al.* (1989) refined the method and showed that thermoluminescence, while a reliable detection method for most irradiated herbs and spices, was due to slight mineral contamination.

The aim of this study was to investigate the effects of gamma irradiation on the volatile components of black pepper, and to assess the possibility that multiple

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minor changes might form the basis of a detection method.

MATERIALS AND METHODS

Materials

Whole black peppercorns were obtained from three suppliers: British Pepper & Spice Co. Ltd, Northampton, UK, Sarawak pepper (BS); Pepper Marketing Board, Malaysia, Sarawak FAQ Grade (PMB); TRS Brand, Indian pepper (TRS) (purchased locally). Samples (24 × 200 g) of each were packed in polyethylene bags and heat-sealed, representing three storage times (1, 30 and 90 days) and unirradiated control plus three treatments in duplicate. Gamma irradiation was carried out at the Scottish Universities Research and Reactor Centre, East Kilbride, Scotland, using a cobalt-60 source of activity, 22·2 × 10^{13} Bq (6 kCi). The doses were 10, 20 and 30 kGy ± 15% at a rate of 2·5 kGy h^{-1} at approximately 15°C. These doses were chosen to achieve radicidation and radappertisation of the spices. After irradiation, the samples were immediately stored at 24°C in the dark. Internal standards were octane (BDH, Poole, UK) and tridecane (Sigma, Poole, UK).

Chemical analysis

Whole black pepper was ground in a sample mill to a particle size of approximately 1 mm (Cyclotec 1093, Tecator AB, Höganäs, Sweden) for subsequent analysis. Moisture content was determined on duplicate samples by the entrainment method (ISO 939: 1980). The pooled standard deviation of all determinations was 2 ml kg^{-1}. Volatile oil content was determined on triplicate samples by steam distillation (British Pharmacopoeia, 1980). Freshly ground black pepper (40 g) and de-ionised water (400 ml) were distilled at 2–3 ml min^{-1} for 4 h and the volume of oil collected noted. Distillation was continued until two consecutive readings taken at 1 h intervals showed no change. The oil was separated from the aqueous layer into glass vials and stored under nitrogen at −20°C. The pooled standard deviation was 0·8 ml kg^{-1}.

The volatile oil components were analysed in duplicate with a GCD gas chromatograph fitted with a fused silica WCOT column (25 m × 0·22 mm i.d.) coated with OV101 (Pye Unicam, Cambridge, UK). The carrier was helium at 1 ml min^{-1}; injector 200°C; column temperature 60–200°C at 4°C min^{-1}; flame ionisation detector at 250°C; hydrogen 40 ml min^{-1}; air 600 ml min^{-1}; make-up gas nitrogen at 29 ml min^{-1}. Internal standards were added to all samples (0·8 μl in 400 μl pepper oil), and 0·1 μl was injected at a split ratio of 70 : 1. Peaks were integrated with a TRIO computing integrator (Trivector Systems International Ltd, Bedfordshire, UK), interfaced with an Apricot XEN microcomputer for data collection, matched by retention time and expressed in the form of relative peak areas of the volatile oil constituents, omitting those less than 0·1%.

Sensory analysis

Triangle tests were used to determine if there was a detectable difference between control and irradiated samples. Pepper oil was diluted in de-ionised water at 10–150 μl $litre^{-1}$ (Uchman *et al.*, 1983), and a small group of assessors found 50 μl $litre^{-1}$ to be of moderate intensity. Water was used as it was found to be the best dispersal medium for reflecting odour quality (Pangborn *et al.*, 1970). A panel of 18 assessors, comprising students and staff of the Department, nosed 15 ml of the 50 μl $litre^{-1}$ pepper oil dispersion in 28 ml bottles with polypropylene screw caps. This study was carried out in three sessions over 3 days (one session per type of pepper). Each assessor nosed 12 triangles during each session, being four replicates of the comparison of the control with each irradiated sample.

Data analysis

GC data were also analysed by principal components analysis (PCA; Piggott & Sharman, 1986). PCA calculates linear combinations of variables (components) describing as much of the variance of the original data as possible. This allows the original multidimensional matrix to be simplified without substantial loss of information, and so eases interpretation of complex data matrices. The results of PCA can be graphically displayed as two set of plots. In the first the correlations of the variables with successive components can be plotted, to aid interpretation of the components; in the second the sample scores can be plotted to show relationships between samples.

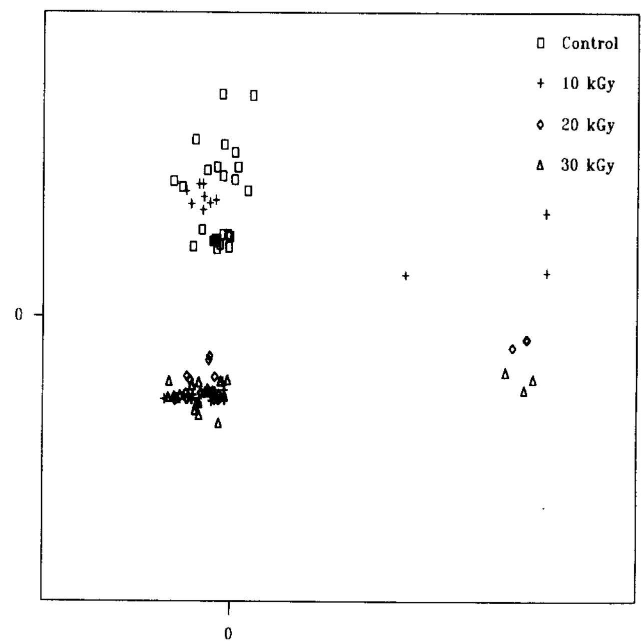

Fig. 1. Scores of control and irradiated BS pepper samples 1, 30 and 90 days after irradiation on first (horizontal) and second (vertical) components from principal components analysis of 125 normalised GC peaks. Samples 1 day after irradiation are on the right of the figure.

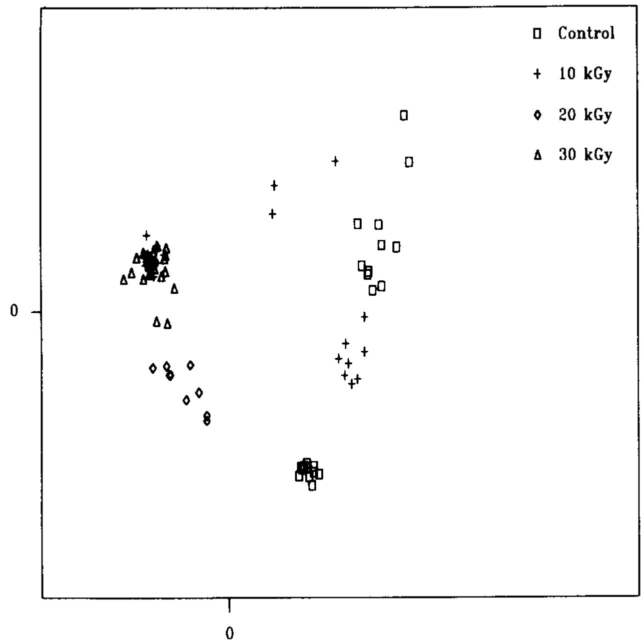

Fig. 2. Scores of control and irradiated BS pepper samples 1, 30 and 90 days after irradiation on second (horizontal) and third (vertical) components from principal components analysis of 125 normalised GC peaks.

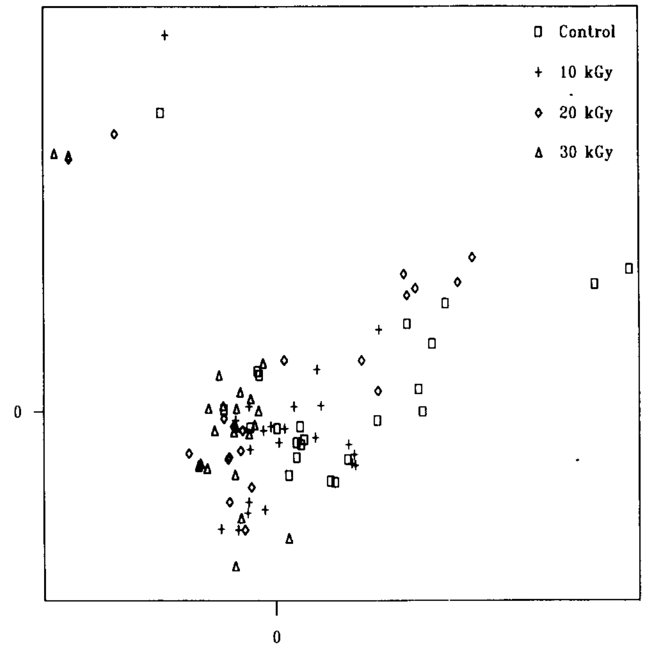

Fig. 4. Scores of control and irradiated PMB pepper samples 1, 30 and 90 days after irradiation on second (horizontal) and third (vertical) components from principal components analysis of 79 normalised GC peaks.

RESULTS

The Sarawak peppers had a higher ($p < 0{\cdot}01$) original moisture content (124 g kg^{-1}) than the Indian pepper (105 g kg^{-1}), probably due to conditions prior to shipment. Irradiation had no significant effect, but during storage the moisture content of the Sarawak peppers decreased ($p < 0{\cdot}01$) to an average of 117 g kg^{-1},

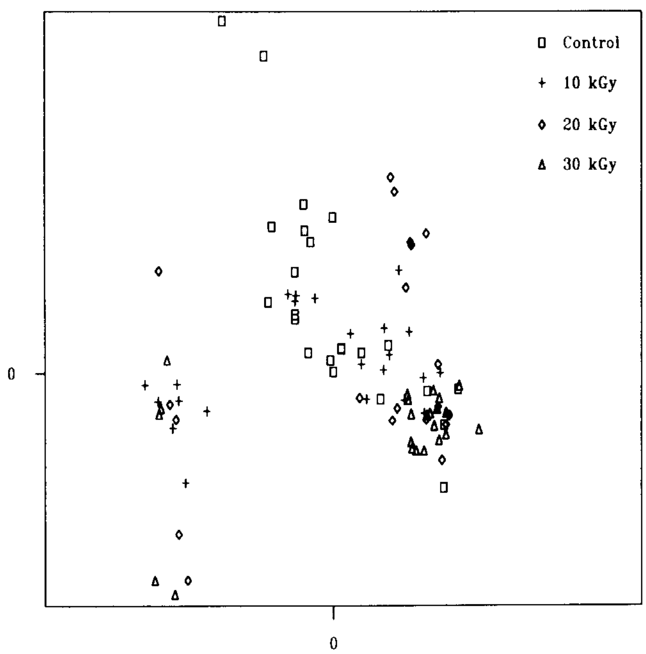

Fig. 3. Scores of control and irradiated PMB pepper samples 1, 30 and 90 days after irradiation on first (horizontal) and second (vertical) components from principal components analysis of 79 normalised GC peaks. Samples 1 day after irradiation are on the left of the figure.

probably due to the lower relative humidity of the experimental storage compared with conditions in the exporting country. Neither irradiation nor storage time had any significant effect on yield of volatile oil, which was slightly lower ($p < 0{\cdot}01$) for the Indian pepper (28·2 ml kg^{-1}) than the Sarawak peppers (29·5 ml kg^{-1}).

The chromatograms of oils of the Sarawak peppers were visually quite similar but differed from the Indian pepper, presumably due to geographical and varietal variation. To reduce the complexity of the data and aid interpretation, PCA was carried out of the GC data for the three different types of pepper at three storage times, after anomalous and outlying replicates were discarded. Plots of the sample scores on the first three components for BS are shown in Figs 1 and 2; the components accounted for 22, 18 and 7% of variance respectively. Sample scores for PMB are shown in Figs 3 and 4, where the components accounted for 35, 15 and 10% of variance. The TRS sample showed no clear separation into control and irradiated groups.

None of the 27 triangle tests was statistically significant, except for the 20 kGy treated TRS sample after 90 days' storage ($p < 0{\cdot}05$). This was likely due to random variation.

DISCUSSION

The yield of volatile oil obtained from TRS pepper was slightly lower than from the others, presumably due to the factors discussed above. The BS and PMB peppers both originated from Sarawak, and were probably the same cultivar (Farrell, 1985). Gamma irradiation produced no significant effect on the steam-distilled oil content of black pepper, as reported by, for example, Zehnder *et al.* (1979). In contrast, a number of reports have recorded a decrease in oil yield at doses from 5 kGy to 60 kGy (Bachman & Gieszczynska, 1973; Uchman *et al.*, 1983). Storage time had no significant effect on oil yield, as has been reported for whole black pepper (Bahari *et al.*, 1983).

Principal component analyses were carried out to summarise the detailed GC data, and to test whether the combined effect of all the minor changes in the chromatograms was to distinguish between control and irradiated samples. For the BS pepper, the first component clearly separated the freshly-irradiated samples from the others (Fig. 1), and the second and third components separated these samples into treatment groups. The drift of the control samples on the third component (Fig. 2) appears to reflect a change in the control samples during storage. The combined effect of the three components was clearly to separate the irradiated samples from the controls, and showed some sign of a dose-dependent separation. For the PMB pepper, the first component again separated the freshly-irradiated samples (Fig. 3), and the second and third components showed a separation, though less clearly, into irradiated and control samples (Fig. 4). In contrast, the TRS pepper showed no clear and consistent separation of control and irradiated samples.

A complex series of inter-related changes seems to have been occurring in these samples; since the oil constituents were not identified it was not possible to determine which were affected by the radiation. Previous studies have not shown consistent results, possibly because different types of pepper have been used. Bachman & Gieszczynska (1973) found no substantial changes in the volatile oil content in most spices treated at 10–15 kGy, and suggested that most of the volatile compounds in black pepper, such as terpene hydrocarbons, are resistant to iradiation at such levels. However, the contents of aromatic compounds, carbohydrates and sulphur compounds were shown to decrease, while the volatile carbonyl compounds increased in irradiated pepper at the dose level of 10–60 kGy (Uchman *et al.*, 1983). Gruiz & Biacs (1978) found a very slight decrease in C_{15}–C_{18} hydrocarbons on irradiation up to 45 kGy and 3 months storage. Hewamanna & Boteju (1985) showed that the yield of terpinolene, humulene and bisabolene decreased appreciably on irradiation at 7 kGy, while the yield of β-caryophyllene increased and β-elimine and terpene-4-ol remained unchanged.

While none of the triangle tests gave a significant result, it is still possible that small sensory differences were detected by some assessors. Frijters (1988) has shown how stimuli may be sensorily discriminated, despite a statistical test showing a non-significant result. Doses of 15–20 kGy produced slight or noticeable alterations in the flavour characteristics of some spices, and 12·5 kGy has been reported as the threshold dose for sensory effects in black pepper (Farkas, 1984). Bahari *et al.* (1983) showed no detectable changes in odour quality of irradiated black pepper, and Eiss (1984) reported no differences in the aroma attributes of black pepper irradiated up to 10 kGy when applied in three types of food. However, Bachman & Gieszczynska (1973) found that the flavour of black pepper changed appreciably at doses of more than 10–12·5 kGy, and Uchman *et al.* (1983) showed a very significant influence of radiation dose on the detection and recognition thresholds of pepper. Given the variation in the changes in volatile oil composition found in this study, it is possible that some peppers will show significant sensory changes on irradiation.

CONCLUSIONS

Gamma irradiation at up to 30 kGy and subsequent storage for 90 days had no significant effect on the steam-distilled volatile oil content of three black peppers. The sensory quality, determined by triangle tests, was not significantly affected by irradiation. Principal components analysis of compositional data showed clear differentiation between unirradiated and irradiated samples immediately after treatment and during storage, and some variations were also observed between samples irradiated at different dose levels. This multivariate statistical approach to discrimination of irradiated pepper showed promise.

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