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Volatiles Derived from Lipoxygenase-Catalysed Reactions in Winged Beans (*Psophocarpus tetragonolobus*)

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ABSTRACT

Volatiles formed by the decomposition of hydroperoxides generated by lipoxygenase-catalysed oxidation of linoleic acid contribute a beany flavour to legumes. Eleven volatiles have been identified after treatment of linoleic acid by lipoxygenase extracted from winged bean seeds. Five of these volatiles; namely, hexanal, 2-heptanone, 2-pentyl furan, undecane and tridecane, have been detected in the headspace of a sample of winged bean flour after storage. Moisture levels below 10% appear to inhibit lipoxygenase activity, since headspace samples from raw and heat treated samples had very similar composition, and the peroxide value remained low after 10 months' storage at 37°C. Incubation of a lipoxygenase extract with linoleic acid gave rise to a larger concentration of volatiles at pH 9 than at pH 6·5 due to the increased lipoxygenase activity at the higher pH.

INTRODUCTION

Winged beans are a promising agricultural crop for the humid tropics where soybeans do not flourish. They have a high protein content in the range of 30–38%, and a high oil content in the range of 15–21% (Spata, 1980). Several food products prepared from winged bean seeds have been reported including milk curd, *tempeh* and *miso* (Shurtleff, 1978), weaning

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foods (Varangoon *et al.*, 1981) and protein isolates (Dench, 1982). However, products prepared from winged beans often have a strong beany flavour which reduces their acceptability to consumers. Decomposition of hydroperoxides produced by lipoxygenase-catalysed oxidation of essential fatty acids, particularly linoleic acid, is known to be a major pathway contributing to the flavour of various legumes (Christopher & Axelrod, 1971). The formation of volatiles by winged bean lipoxygenase has been demonstrated (Van Den *et al.*, 1982). The present study comprises an investigation of the volatiles in winged bean seeds, identification of those formed by lipoxygenase-catalysed reactions and the effect of conditions on volatile formation.

MATERIALS AND METHODS

Linoleic acid (99%) and Tween 20 were purchased from Sigma Chemical Company Ltd. The winged bean seeds comprised a freshly harvested commercial seed mixture imported from Sri Lanka by Kins Plants Ltd.

The winged bean seeds were ground into a fine flour and defatted with hexane. Lipoxygenase was extracted from the defatted flour (10 g) by stirring with sodium phosphate buffer (0·1M, 150 ml, pH 6·5) at 0°C for 1 h. The slurry was filtered through four layers of cheese cloth and centrifuged at 12 000 *g* for 20 min at 4°C. The supernatant was treated with ammonium sulphate (30–40% saturation) and centrifuged at 4°C for a further period of 20 min at 12 000 *g*. The precipitate was collected, dissolved in sodium phosphate buffer (0·05M, pH 7·0), and diluted ten times with distilled water.

Lipoxygenase activity was assayed at 234 nm by a spectrophotometric procedure based on that of Ben-Aziz *et al.* (1970). Potassium hydroxide solution (1 ml, 1 M) was added to linoleic acid (0·4 ml) and Tween 20 (0·4 ml). Distilled water (3–5 ml) was added and the mixture was shaken at 60–70°C until clear. The linoleate solution was then diluted to 100 ml. The assay mixture comprised sodium phosphate buffer (2·0 ml, 0·05M, pH 9·0 unless otherwise specified), linoleate solution (0·4 ml) and enzyme extract (0·1 ml). A Perkin-Elmer 552 recording spectrophotometer was used for the assay. The lipoxygenase activity is quoted as 1000 × the rate of change of absorbance per millilitre of enzyme extract.

Lipoxygenase-generated volatiles were formed by mixing winged bean lipoxygenase extract (5 ml), linoleate solution (20 ml) and buffer (100 ml, 0·05M, pH 9·0 unless otherwise specified). A control sample was also prepared in which the lipoxygenase extract was replaced by distilled water. The sample was incubated for 1 h at 37°C, and then heated to 60°C. A sample of the headspace (5 ml) was removed with a gas syringe, and injected

into a capillary gas chromatograph. The volatiles were concentrated by immersing the initial section of the column in liquid nitrogen. Analysis was performed by GC–MS, using a Carlo Erba 2150 gas chromatograph containing a fused silica capillary column (25 m, OV1) with temperature programming from 50 to 220°C at 2°/min. Helium at 2 ml/min was used as carrier gas. The mass spectrometer was a Vg 70-70 F instrument, with a Vg 2235 data system. Ionisation was by electron impact with a 200 μA trap current.

Volatiles from undefatted winged bean flour were adsorbed on to activated charcoal traps using a closed loop stripping apparatus as described by Grob & Zürcher (1976). Volatiles were isolated from winged bean flour (19 g) for 3 h at 55°C by trapping on activated charcoal (5 mg). The trapped volatiles were extracted with carbon disulphide (50 μl) and analysed by GC–MS using a WCOT fused silica capillary column (50 m, SP1000) with temperature programming from 55°C to 220°C at 2°C/min. The run was continued for 25 min after the last identified peak.

GLC analysis to determine the effect of pH on volatile formation was performed on a Perkin-Elmer Sigma 3B Gas chromatograph equipped with a glass column (2 m × 3 mm id) packed with Carbowax 20 M (10% on Chromosorb W). Integration was performed with a Hewlett-Packard integrator, model 3390 A.

The peroxide value was determined by the IUPAC method (IUPAC, 1975).

RESULTS AND DISCUSSION

GLC analysis of the volatiles generated by incubation of extracted winged bean lipoxygenase with linoleic acid revealed the presence of at least eighteen components present at significant concentrations (Fig. 1). Eleven components, which represented approximately 90% of the total volatiles, were identified by GC–MS (Table 1). The major components detected were hexanal and 2-pentyl furan. Most of the volatiles are commonly observed in products derived from lipoxygenase-catalysed oxidation of linoleic acid. The presence of both 9- and 13-hydroperoxyoctadecadienoic acid isomers was suggested by the presence of 2-pentyl furan and pentane, respectively. Whilst hexanal may be formed from both isomers (Schieberle & Grosch, 1981), pentane is produced via a pentyl radical which can only be derived from the 13-hydroperoxide (Grosch, 1982). The absence of unsaturated aldehydes is rather surprising, since these have been reported in volatiles derived from soybean lipoxygenase (Fischer & Grosch, 1977). The presence of heptane, nonane, undecane and tridecane is also difficult to explain on a simple mechanistic basis. However, agreement of the experimental mass

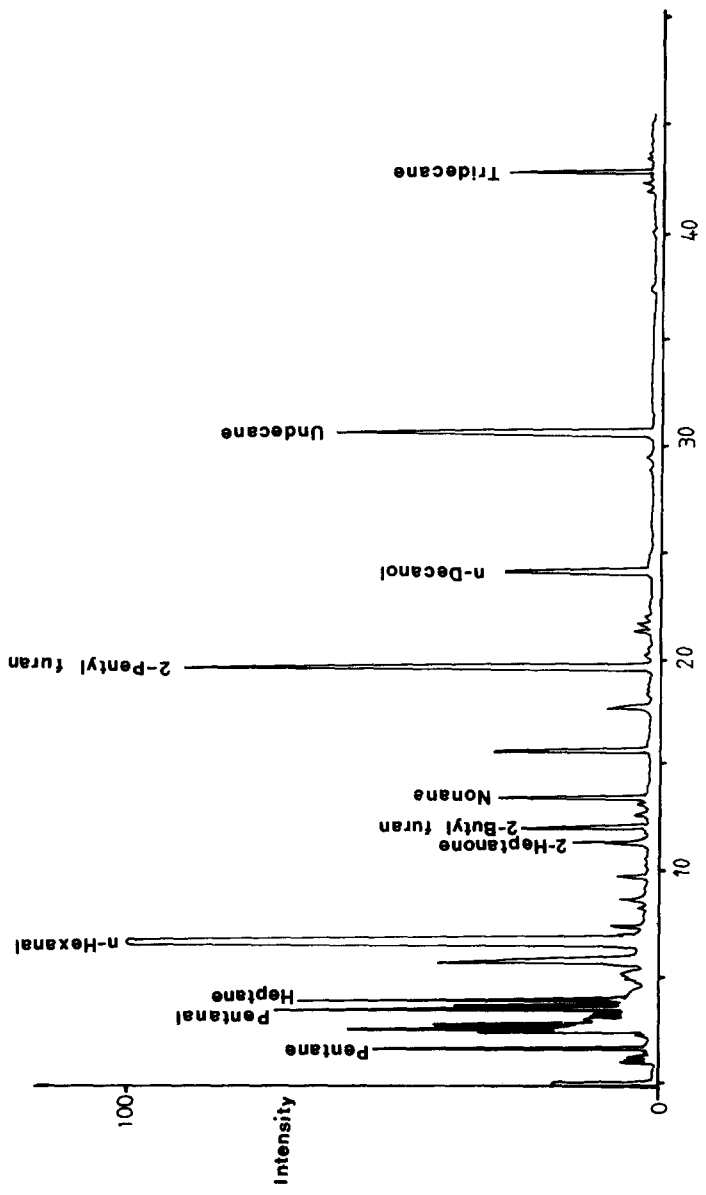

Fig. 1. Gas chromatogram of the volatiles formed by incubation of winged bean lipoxygenase extract and linoleic acid at pH 9.

TABLE 1
Volatiles Generated from Linoleic Acid on Incubation at 37°C with Lipoxygenase at pH 9

Compound	*Per cent composition*[a]
n-Pentane	6·5
Pentanal	6·4
Heptane	5·9
n-Hexanal	28·0
2-Heptanone	1·0
2-Butyl furan	3·4
Nonane	3·9
2-Pentyl furan	13·0
n-Decanol	7·4
Undecane	12·0
Tridecane	3·1

[a] Per cent composition is based on areas of GLC peaks, without correction for detector response.

spectra with the literature (Eight Peak Index, 1983) was good, and the identity of these components does not appear to be in doubt.

Incubation of the samples at pH 9 appeared to give rise to considerably more volatiles (about six times) than incubation at pH 6·5, as assessed by the total integrated areas of the volatile peaks, although the same components appeared to be present at both pH values. This difference is mainly due to the greater activity of the winged bean lipoxygenase at pH 9. The winged bean lipoxygenase was observed to be more active at pH 9 than at pH 7, with an optimum difference in activity of 7·5:1 at 1·7 mM linoleic acid concentration. The characteristics of winged bean lipoxygenase will be reported in more detail in a separate publication.

The volatiles of a sample of winged bean flour with a lipoxygenase activity of 4700 units were analysed by GC–MS after the flour had been stored for 10 months at 37°C. A complex mixture of volatiles was detected (Fig. 2). Hexanal was the major component present (Table 2) and other volatiles which had been detected in the headspace of the lipoxygenase extract incubated with linoleic acid comprised 2-heptanone, 2-pentyl furan, undecane and tridecane. Several components co-eluted with unidentified compounds, and the identification of some of the volatiles remains tentative due to the complex mass spectra.

Heating a sample of winged bean flour in an oven for 30 min at 100°C

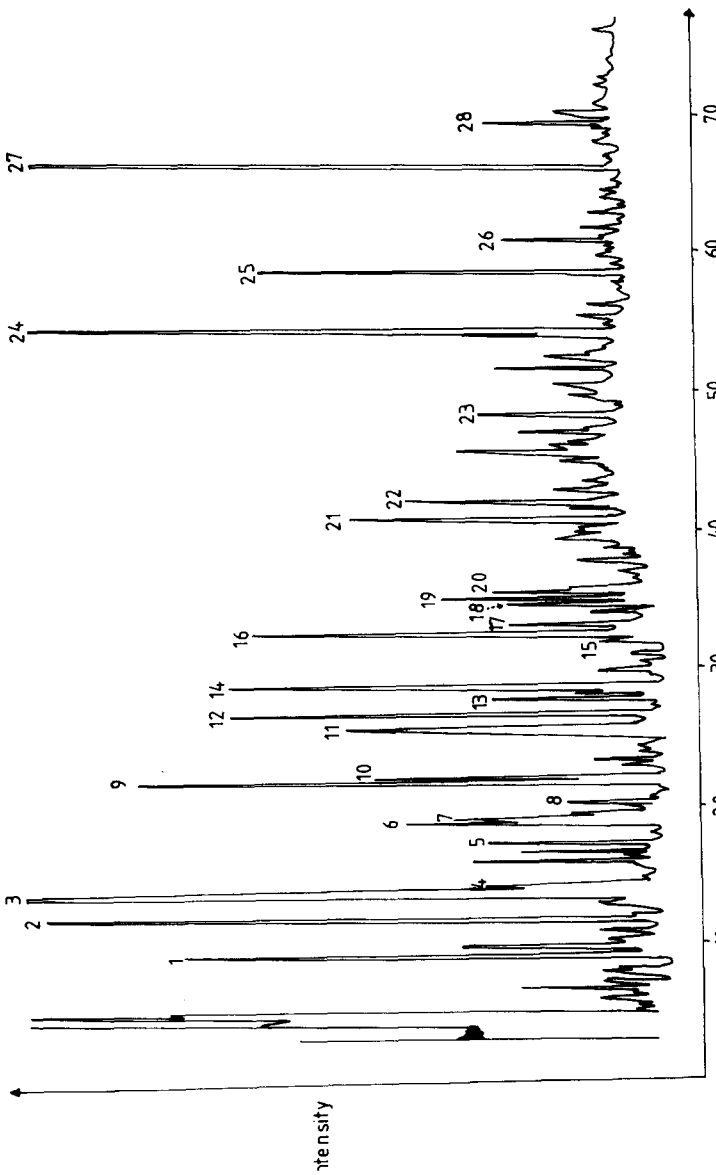

Fig. 2. Gas chromatogram of the volatiles isolated from the headspace of stored winged bean flour.

TABLE 2
Major Volatile Compounds Generated from Winged Bean Flours

Peak number	*Compound*	*Percent composition in (flour)*	
		Raw	*Heated*
1	Decane	1·9	—
2	Toluene	2·6	1·7
3	Hexanal	13·6	9·7
4	Undecane	0·5	0·8
5	Monoterpene	—	0·5
6	Heptanone	1·4	0·9
7	Heptanal	—	0·7
8	Isoamyl alcohol	0·7	0·8
9	2-Pentyl furan	2·1	1·3
10	Styrene	—	0·2
11	Octanal	3·2	2·8
12	3(4-methyl-3-pentyl) furan	2·2	2·5
13	2-Methoxymethyl ether	?	1·3
14	Hexanol[a]	1·8	0·3
15	Tridecane	0·2	—
16	Nonanal	2·6	3·6
17	3-Octen-2-one	0·4	—
18	Acetic acid	—	0·6
19	2-Ethyl hexanol	1·4	0·4
20	Heptanol	0·9	—
21	Benzaldehyde	2·1	1·7
22	$C_{15}H_{28}O$ or alcohol[a]	1·4	—
23	2- and 3-Methyl butanoic acid	0·6	—
24	Naphthalene	3·3	2·8
25	Hexanoic acid[a]	1·3	0·2
26	Benzyl alcohol[a]	0·3	—
27	Benzothiazole[a]	5·5	5·2
28	Dimethyl naphthalene and octanoic acid[a]	0·7	0·5

[a] Mass spectrum complex due to co-elution with contaminant. Identification tentative.

before storage reduced the lipoxygenase activity from 4700 units to 1000 units but the volatiles present in the headspace of the two samples after 10 months' storage were remarkably similar (Table 2). The samples had been dried to a moisture content of less than 10% before storage (9·8% and 9·1% for the raw and heat-treated samples, respectively) and it appears that this moisture content is sufficiently low to minimise the formation of lipoxygenase-derived volatiles. Most of the hydroperoxides which are the precursors of the volatiles were generated prior to drying of the flour. The

peroxide values of the raw and heat-treated flours were 7·5 and 3·2 meq/kg oil initially and only 7·4 and 3·7 meq/kg oil after 10 months' storage at 37°C.

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