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Volatile Composition of White Bread Using Enzyme Active Soya Flour as Improver

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ABSTRACT

Soya flour is often used in the baking industry as a bread improver. This study deals with the influence of addition of enzyme active soya flour on the volatile composition of bread. These volatiles have been isolated by a dynamic headspace technique, analysed by gas chromatography and identified by combined gas chromatography/mass spectrometry. The chromatograms of bread with and without soya flour have been compared and the major differences quantified. Addition of enzyme active soya flour increases the concentrations of hexanal, 1-hexanol, 1-penten-3-ol, 1-pentanol and 2-heptanone, while 2-heptenal and 1-octen-3-ol have only been detected in bread containing soya.

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INTRODUCTION

Enzyme active soya flour is frequently used in the baking industry as a bread improver. This flour contains at least three lipoxygenase isoenzymes. Each of these isoenzymes, types 1, 2 and 3, is characterised by its own pH optimum (pH = 9·0, pH = 6·5 and pH = 6·0, respectively; Christopher *et al.*, 1970; Bild *et al.*, 1977; Gaillard & Chan, 1980; Engeseth *et al.*, 1987). Addition of a small amount of active soya flour to a dough considerably improves the quality of bread. Studies on the rheological effects of enzyme active soya flour in the présence of oxygen showed that rheological properties of dough improved, i.e. bread volume was higher and tolerance to overmixing had increased (Frazier *et al.*, 1973; 1977; Faubian & Hoseney, 1981; Frazier, 1983). Kieffer and Grosch (1980) demonstrated that lipoxygenase type-2 is probably responsible for the effects mentioned above. Bleaching of carotenoids in wheat flour has also been attributed to lipoxygenase activity (Faubian & Hoseney, 1981). Pigment bleaching is assumed to occur during lipoxygenase-mediated oxidation of unsaturated fatty acids (Weber *et al.*, 1974; Weber & Grosch, 1976; Grosch *et al.*, 1977; Grosch & Laskawy, 1979; Barimalaa & Gordon, 1988). Studies have shown that type-2 is a good pigment bleacher under aerobic conditions (Grosch & Laskawy, 1979; Klein *et al.*, 1984) but under anaerobic conditions type-1 seemed to be a stronger pigment bleaching agent (Klein *et al.*, 1984). These properties of soya lipoxygenase are very useful in the bread making industry. However, the amount of enzyme active soya flour is restricted because of the formation of volatiles causing off-flavours (Eskin *et al.* 1977).

Lipoxygenase catalyses the hydroperoxidation of linoleic acid and other polyunsatured lipids that contain a *cis,cis*-1,4-pentadiene binding. The hydroperoxides formed are assumed to be decomposed by homolytic cleavage via one electron transfer to produce various fragmentation products such as aldehydes, alcohols, ketones and hydrocarbons (Axelrod, 1974). These volatiles of secondary fat oxidation reactions can contribute to either the desirable fresh fruit and vegetable aromas or to the off-flavours occurring during storage or processing (Axelrod, 1974; Eskin, *et al.*, 1977). The development of off-flavours in legumes, in particular soya products, is well known (Cowan *et al.*, 1973; Axelrod, 1974; Eskin *et al.*, 1977; Sessa & Rackis, 1977; Sessa, 1979; Kinsella & Damodaran, 1980; Ames & MacLeod, 1984; Hildebrand & Kito, 1984). Currently also quality deterioriation in fish is associated with lipoxygenase activity (German & Kinsella, 1985; Hsieh & Kinsella, 1989).

In this study the influence of enzyme active soya flour on the volatile composition of white bread was investigated. The experiments included a control and the addition of 3 g soya flour to 100 g flour. A dynamic

headspace technique with Tenax TA as trapping material was used to collect the bread volatiles. The volatile components of the enzyme active soya flour itself were collected in the same way in order to investigate their influence on bread volatile composition.

MATERIALS AND METHODS

Sample preparation

The recipes for dough making of the two types of white bread are listed in Table 1. Bread with enzyme active soya flour contains 3 g soya on 100 g flour basis and the control contains no soya. Breadmaking was as follows: 100 g dough constituents were mixed for 8 min in a Spiral Mixer (25 Kg Kemper) at 26°C and 80% RH. Dough was divided into two parts and rounded. Second fermentation was carried out for 60 min at 26°C and 80% RH, followed by baking for 30 min in an oven (Rotary oven, Siemens) at 230°C. Total weight loss during baking was approximately 25%. Four loaves (400 g) of each dough (soya and control) were baked at the same time. After 1 h cooling, the loaves were cut into slices (approximately 90 g), packed in paper and aluminium foil and stored at −40°C until sampling of volatiles.

Isolation and analysis of volatiles

The extraction of the bread volatiles was performed on the Aroma Isolation Apparatus of MacLeod and Ames (1986) with two large sample flasks

TABLE 1
Ingredients used for Dough Making of White Bread With and Without Enzyme Active Soya Flour

Ingredient	*Quantities on flour basis (g)*	
	Control dough	*Soya dough*
Regular bread flour	100	100
Salt	2	2
Yeast	2·5	2·5
Total mix[a]	2·4	2·4
Water	52	52
Soya flour	0	3

[a] The total mix contains several other agents for bread improvement.

(550 cm^3). Each flask was connected to one glass sampling tube filled with 100 mg Tenax TA (60–80 mesh, Chrompack).

A frozen slice (90 g) of each kind of bread was cut into pieces and brought into a sample flask. Purified nitrogen was passed through the bread stack at 20 cm^3 min^{-1} for 7 h at room temperature and the volatiles were trapped on Tenax TA. A blank of the system is made under identical headspace conditions.

Collection of volatiles of enzyme active soya flour was carried out with 10 g of the flour (corresponds with the quantity of soya flour in the bread samples) in a small sample flask (40 cm^3) with the same experimental conditions as mentioned before.

Volatiles were thermally desorbed (10 min at 180°C) from Tenax by the Chrompack Thermal Desorption Cold Trap Injector (Schaeffer, 1989). A Carlo Erba GC 6000 vega with capillary column (60 m × 0·25 i.d., Supelcowax 10) and a flame ionisation detector (250°C) was used for gas chromatography. Column temperature was programmed: 4 min 40°C; 2·5°C/min to 100°C; 4°C/min to 250°C. Peak areas of the three bread types (six replicates) were compared and statistically evaluated by the student *t*-test.

Compounds were identified by GC/MS using identical thermodesorption and chromatographic conditions as for gas chromatography analyses. The capillary column was connected to a VG MM7070F mass spectrometer. Spectra were obtained at 70 eV EI.

Quantification of volatiles

Quantification experiments were carried out with the Aroma Isolation Apparatus mentioned above. Hexanal, and 2-heptanone were obtained from Alltech Europe (Belgium) and 1-pentanol, hexanol, 1-octen-3-ol and 1-penten-3-ol were obtained from Janssen Chimica (Belgium). Solutions containing 50 μl standard/10 ml pentane were prepared. Mixtures of three standards were made containing 100 μl of each standard solution. Ten microlitre mixture was injected into the inlet of the sampling flasks with a 100 μl Hamilton syringe. GC and desorption conditions were identical to the ones used for the bread samples. Peak areas of bread volatiles were correlated to the peak areas of the corresponding standards.

RESULTS AND DISCUSSION

The compounds identified in the headspace samples of the control and soya-bread are listed in Table 2. Nine components have not been detected before

TABLE 2
Volatiles Identified (GC/MS) in the Headspace of White Bread With and Without Enzyme Active Soya Flour Addition

Retention time (min)	*Component*	*Retention time (min)*	*Component*
	Hydrocarbons		*Ketones*
12·59	Toluene	6·36	2-Butanone
21·55	Limonene	9·76	2-Pentanone
	Alcohol	9·76	2,3-Butanedione
		21·18	2-Heptanone[c]
7·85	Ethanol	18·45	3-Penten-2-one
12·59	1-Propanol	28·45	3-Hydroxy-2-butanone
16·33	2-Methyl-1-propanol	29·31	1-Hydroxy-2-propanone
18·19	1-Methoxy-2-propanol	44·39	Butyrolactan[a]
19·03	1-Butanol		*Esters*
19·99	1-Penten-3-ol[ac]	4·32	Methyl acetate[a]
23·47	2-Methyl-1-butanol	5·95	Ethyl acetate
23·47	3-Methyl-1-butanol	9·09	Isobutyl formate
25·62	1-Pentanol[c]	11·58	Isobutyl acetate[a]
31·51	1-Hexanol[c]	13·02	Ethyl butyrate[a]
35·54	1-Octen-3-ol[bc]	14·43	Pentyl formate[a]
40·29	2,3-Butanediol	14·52	Butyl acetate[a]
53·51	2-Phenylethanol	17·30	Isopentyl acetate
	Aldehydes	31·13	Ethyl lactate[a]
3·81	2-Methylpropanal		*Bases*
7·07	2-Methylbutanal	22·46	Pyridine
7·07	3-Methylbutanal	23·47	Pyrazine
9·76	Pentanal	26·73	Methylpyrazine
15·26	Hexanal[c]	30·22	2,5-Dimethylpyrazine
20·89	Heptanal	30·61	2-Ethylpyrazine
27·31	Octanal	32·04	2,3-Dimethylpyrazine
29·66	2-Heptenal[b]		*Furans*
33·73	Nonanal	26·59	2-Methyltetrahydro-furan-3-one
38·57	Decanal	37·34	Furfural
39·93	Benzaldehyde	39·05	2-Acetylfuran
	Acids	41·94	5-Methyl-2-furfural
36·75	Acetic acid	44·82	2-Furfurylalcohol
40·71	Pentanoic acid		*Sulfur Compounds*
41·41	2-Methylpropanoic acid	33·05	Methyltrisulfide
45·29	2-Methylbutanoic acid	36·15	3-Butenylisothio-cyanate[a]
45·29	3-Methylbutanoic acid		

[a] Volatile, which was not detected before in white bread.
[b] Volatile, which was only detected in bread with soya flour added.
[c] Volatile, which was confirmed both by GC/MS and retention time of standards.

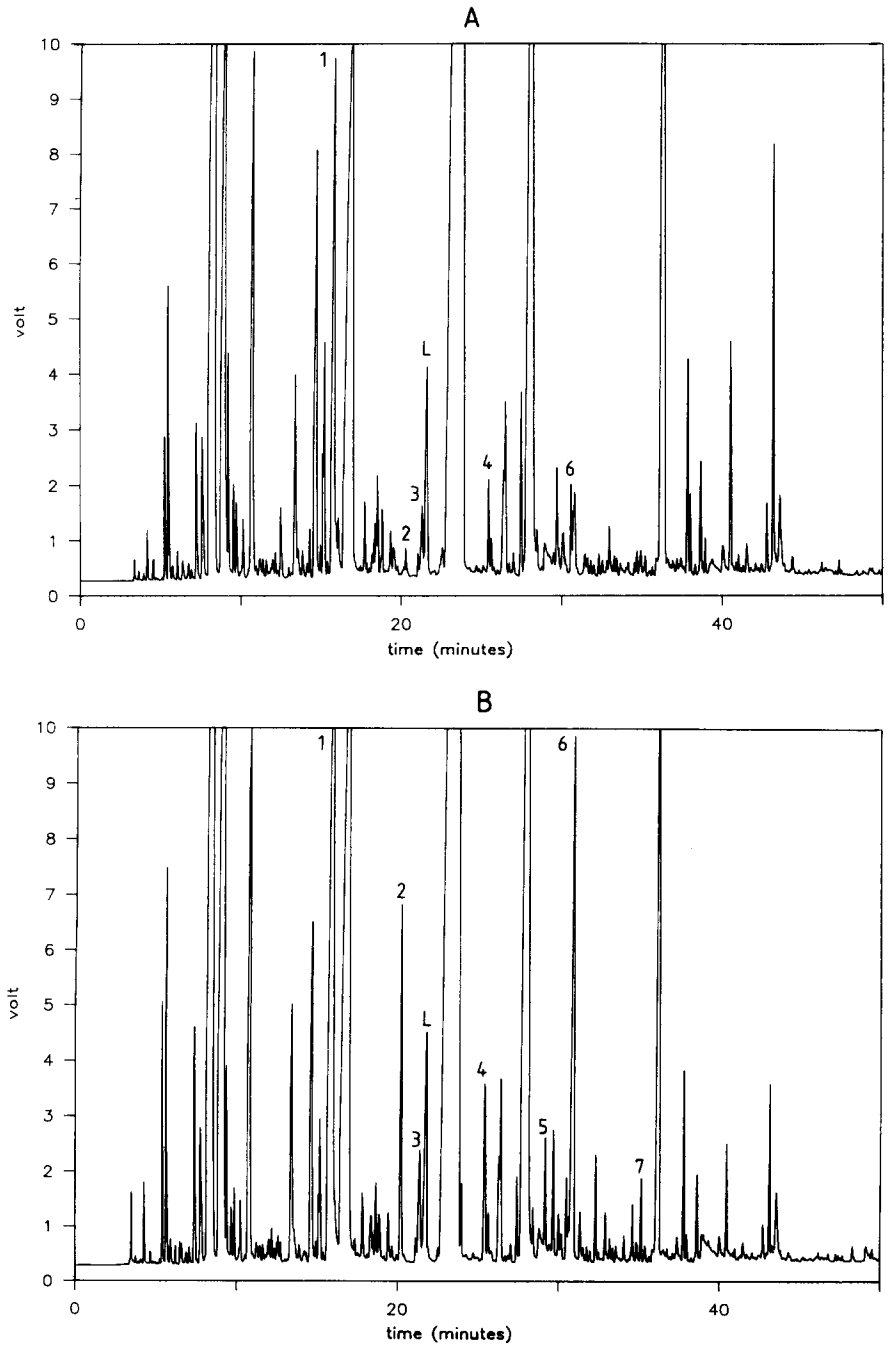

Fig. 1. Chromatograms of a dynamic headspace sample of respectively (*A*) the control white bread and (*B*) bread with enzyme active soya flour added. Peak L = limonene and peak 1–7 refer to Table 3.

in the volatile composition of white bread according to the data of van Straten & Maarse (1989).

Figure 1 presents GC-chromatograms characteristic for bread with and without enzyme active soya flour added. Comparison of both chromatograms shows differences in both peak areas and component composition. The qualitative results of GC/MS are listed in Table 2. As compared with the control, 2-heptenal and 1-octen-3-ol were only detected in soya-bread. Figure 1 shows that the peak areas of hexanal, 1-penten-3-ol, 2-heptanone, 1-pentanol and hexanol are much larger in soya added bread. It was shown statistically that the concentration of these volatiles was significantly higher in soya added bread as compared with the control bread ($p < 0{\cdot}05$). These components are quantified and the results listed in Table 3. The differences in quantity obtained could be caused either by the addition of the soya flour itself or by lipoxygenase activity of soya flour during processing. The content of volatiles of enzyme active soya flour was investigated to study the contribution of its own volatiles to the bread ones. Figure 2 presents a typical chromatogram of the volatiles of soya flour, of which the GC/MS identified compounds are presented in Table 4.

The chromatogram of soy flour (Fig. 2) shows that limonene has the largest peak area while the limonene peak in the chromatogram of soya bread is not much larger than in the control (Fig. 1). The concentrations of hexanal, 1-hexanol and 1-pentanol are very low in the headspace samples of 10 g soya flour and very high in soya bread. These results indicate that volatiles of soya flour hardly influence the volatile composition of bread. It was ensured that the chromatogram of the blank run of the system contained no peaks.

TABLE 3
Quantified Volatiles from Dynamic Headspace Samples of Bread With and Without Soya Flour

Peak no.	*Component*	*Control bread (ng/g)*	*Stand. dev. (n = 6)*	*Soya bread (ng/g)*	*Stand. dev. (n = 6)*	*Ratio soya control*
1	Hexanal	0·078	0·014	0·173	0·023	2·22
2	1-Penten-3-ol	0·004	0·001 5	0·026	0·002	6·50
3	2-Heptanone	0·006	0·001 4	0·010	0·001 9	1·67
4	1-Pentanol	0·006	0·001 5	0·014	0·002	2·33
5	2-Heptenal[a]	—	—	0·014	0·001 9	—
6	1-Hexanol	0·009	0·002	0·061	0·011	6·78
7	1-Octen-3-ol	—	—	0·003	0·000 6	—

[a] 2-heptenal is correlated to the peak area of hexanal because this standard was not available.

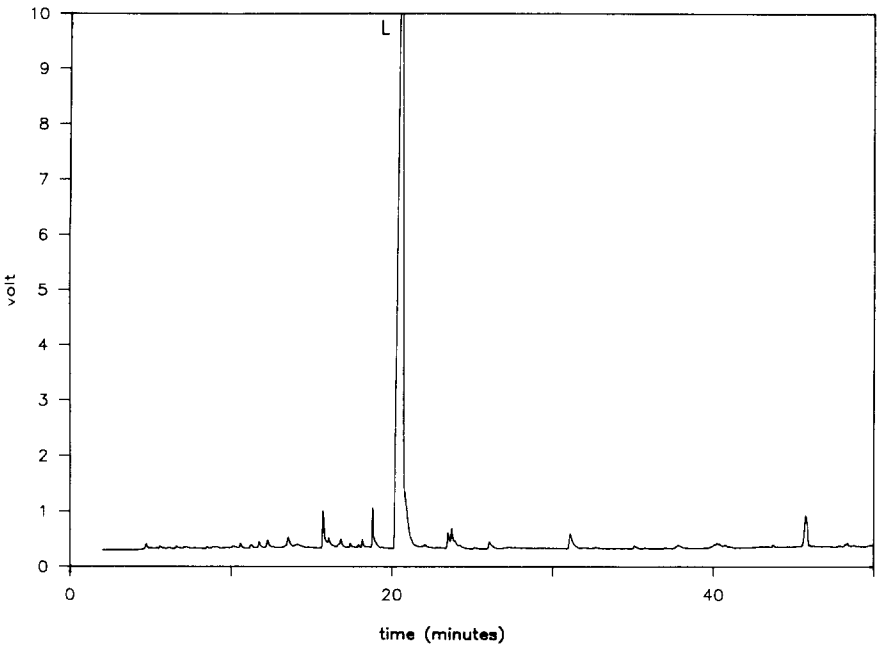

Fig. 2. Chromatogram of a dynamic headspace sample of enzyme active soya flour. Peak L = limonene.

Another explanation for the increase of the components mentioned in Table 3 could be the production of secondary lipid oxidation products from hydroperoxides initiated by soya lipoxygenase. Several studies prove that lipid oxidation increases in doughs containing soya flour (Smith & Andrews, 1957; Tsen & Hlynka, 1962; Morrison & Panpaprai, 1975). Other studies give the identity of volatiles originated by soya lipoxygenase (Grosch & Schwencke, 1969; Arai *et al.*, 1970; Leu, 1974; Grosch & Laskawy, 1975;

TABLE 4
Compounds Identified (GC/MS) in Headspace Samples of 10 g Enzyme Active Soya Flour

Retention time (min)	*Component*	*Retention time (min)*	*Component*
10·00	α-Pinene	17·15	Myrcene
11·41	Toluene	19·13	Limonene
13·80	Hexanal	22·27	2-Methylbutanol
14·17	2Methyl-1-propanol	22·53	3-Methylbutanol
15·62	Ethylbenzene	25·09	1-Pentanol
16·46	1-Methoxy-2-propanol	30·57	1-Hexanol

Ames & MacLeod, 1984). The results in Table 3 are in agreement with the studies mentioned except that 1-penten-3-ol has not been detected before as a possible secondary lipid oxidation product from soya lipoxygenase. However, soya flour contains small amounts of alcohol dehydrogenase (Leblová & Perglerová, 1976) which might reduce 1-penten-3-al.

From this study it is not clear which isozymes of lipoxygenase could be responsible for the increase of the volatiles listed in Table 3. Probably lipoxygenase type 2 and/or 3 are active during dough making because the pH varies in the range 5·1–5·8 during kneading and rising and this is near the optimum pH of lipoxygenase-2 and -3. Some work has been done with lipoxygenase-deficient mutant seeds to investigate the role of the isozymes in off-flavour formation (Matoba *et al.*, 1985). They suggested that isozyme-2 is responsible for *n*-hexanal formation in soybean homogenate at 25°C and 70°C. Sensory evaluation of soya flour and soya milk samples indicates that genetic removal of the isozyme-2 may reduce off-flavours in soy products (Davies *et al.*, 1987).

CONCLUSION

This study shows that enzyme active soya flour influences both the volatile composition and the concentrations of several volatile components in bread. 2-Heptenal and 1-octen-3-ol were only detected in bread containing soya. Addition of enzyme active soya flour increases the concentrations of hexanal, hexanol, 1-penten-3-ol, 1-pentanol and 2-heptanone. Probably these components originate from the action of soya lipoxygenase during dough making and the initial stage of bread baking.

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