Nash, R. G.; Beall, M. L., Jr. *J. Agric. Food Chem.* **1980**, *28*, 614.
Nestrick, T. J.; Lamparski, L. L. *Anal. Chem.* **1982**, *54*, 2292.
Quensen, J. F.; Matsumura, F. *Environ. Toxicol. Chem.* **1983**, *2*, 261.
Sethunathan, N. *Residue Rev.* **1973**, *47*, 143.
Smith, A. E. *Weed Sci.* **1971**, *19*, 536.
Smith, A. E.; Muir, D. C. G. *J. Agric. Food Chem.* **1984**, *32*, 588.
Stalling, D. L.; Smith, LM.; Petty, J. D.; Hogan, D. L.; Rappe, C.; Buser, H. R. In "Human and Environmental Risks of Chlorinated Dioxins and Related Compounds"; Tucker, R. E., Young, A. L., Gray, A. P., Eds.; Plenum Press: New York, 1983; p 221.
Stumm, W.; Morgan, J. J. "Aquatic Chemistry", 2nd ed.; Wiley-Interscience: New York, 1981.
Tamagishi, T.; Miyazaki, T.; Akiyama, K.; Morita, M.; Nakagawa, J.; Horil, S.; Kaneko, S. *Chemosphere* **1981**, *10*, 1137.
Taylor, A. W. *J. Air Pollut. Control Assoc.* **1978**, *28*, 922.
Tsushimoto, G.; Matsumura, F., Sago, R. *Environ. Toxicol. Chem.* **1982**, *1*, 61.
Tucker, E. S.; Saeger, V. W.; Hicks, O. *Bull. Environ. Contam. Toxicol.* **1975**, *14*, 705.
Ward, C. T.; Matsumura, F. *Arch. Environ. Contam. Toxicol.* **1978**, *7*, 349.

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Effect of Antioxidants on the Volatiles of Roasted Sesame Seeds

Mervat A. Soliman,* Abd Alla A. El-Sawy, Hoda M. Fadel, and F. Osman

The aroma concentrates of roasted sesame seeds free from antioxidants (sesamin–sesamolin) as well as of roasted defatted sesame seeds mixed with sesamin–sesamolin were fractionated into neutral–acidic and basic fractions. The volatile components of each fraction were identified by the retention times of the authentic samples in gas–liquid chromatography–mass spectrometry. The major changes in components of the neutral–acidic fraction of the sesamin–sesamolin free sample were the amounts of 2,4-undecadienal (32.59%) and 2,4,6-dodecatrienal (18.61%) compared to those of the whole seeds (49.44% and 6.9%, respectively). However, irregular changes were observed for the pyrazine derivatives of the basic fraction. The predominance of the nutty flavor, thus, might be due to the increase of some individual pyrazine derivatives at the expense of some furan derivatives. However, the aroma components of the defatted white sesame seeds either with or without sesamin–sesamolin did not follow a definite pattern regarding the pyrazine derivatives.

INTRODUCTION

Animal and vegetable lipids contain many minor constituents which may be quite important to the odor and flavor characteristics of foods, although their total concentration provides less than 1% of total lipids present; the most important of these is the antioxidants.

It is known that the resistance of vegetable fats to oxidation rested on the presence of antioxidants which occur naturally in the tissue and which are present in the oil when it is pressed. These antioxidants inhibit the effect of prooxidants which accelerate the onset of rancidity. The uptake of oxygen and the onset of rancidity seems to be related to the unsaturation of the fat. The oxidation is not, however, a simple oxidation of the double bond.

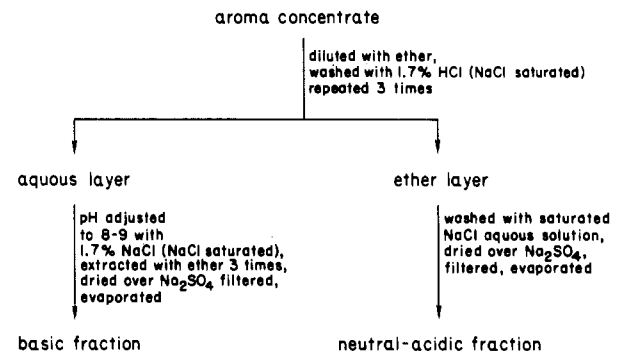
Analysis of the developed flavor of rancid fat shows that a very complex mixture of compounds is formed. Among them, heptanealdehyde formed the largest amount (Meyer, 1964).

On the other hand, these antioxidants undergo reactions with the amino acids present in the seed. This reaction is considered one of the most nonenzymatic browning reactions of food products. In neutral medium these reactions are stronger than those between glucose and amino acids (Segal et al., 1971).

Sesame seeds contain approximately 50% of edible oil which contains its characteristic antioxidant compounds (sesamin–sesamolin). These compounds are responsible for its stability and good quality.

The aim of the present work is to judge the role of the antioxidant (sesamin–sesamolin) on the development and changes of flavor compounds of roasted white sesame seeds.

Scheme I



MATERIALS AND METHODS

Materials. Local sesame seeds (*Sesamum indicum*) variety Giza-33 were obtained from the development of

Fats and Oils Laboratory, National Research Centre, Cairo (M.A.S., H.M.F., and F.O.), and Faculty of Science, Benha University, Benha, Egypt (A.E.-S.).

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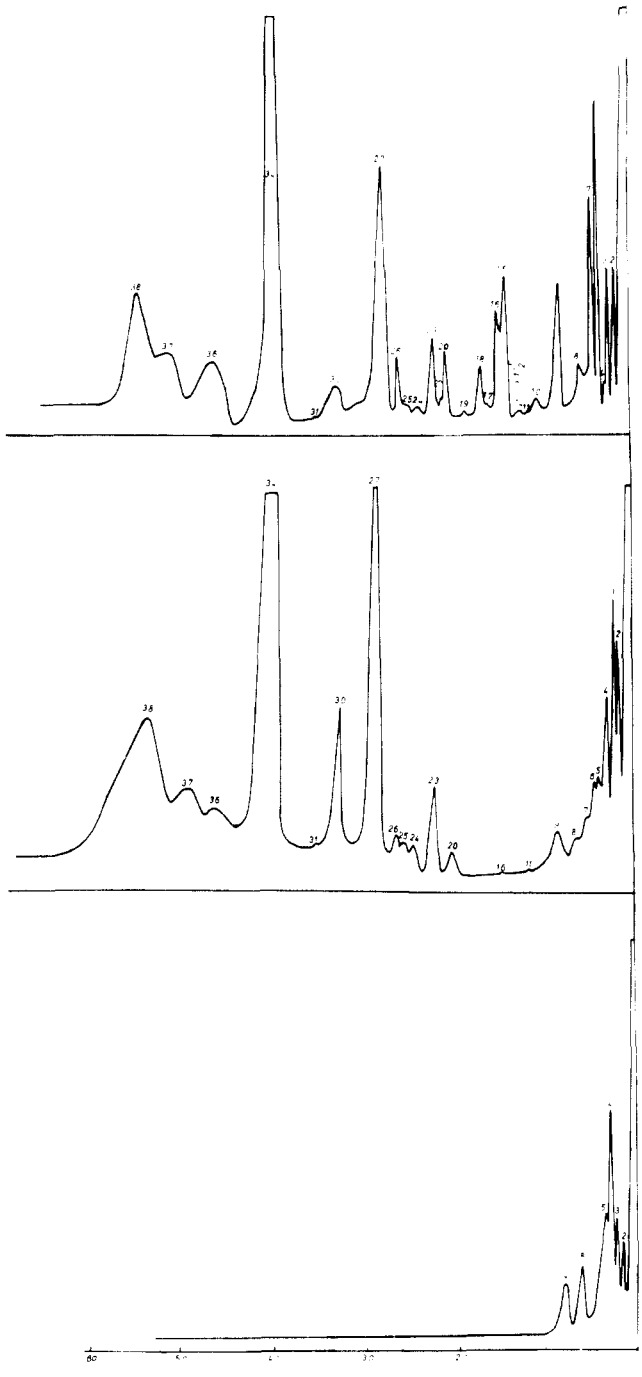

Figure 1. Gas–liquid chromatograms of the aroma concentrate of roasted white sesame seeds free from sesamine–sesamoline (top) and its neutral–acidic (middle) and basic (bottom) fractions, respectively.

Horticulture Ministry of Agriculture.

Standard reference compounds were products of International Flavors and Fragrance Inc., NJ, and Ochanomizue University, Food Chemistry Laboratory, Japan. All other reagents and solvents were extra pure or analytical grade.

Methods. (*1*) *Lipid Extraction.* Clean crushed white seeds (800 g) were subjected to *n*-hexane extraction with a Soxhlet extraction apparatus (Fieser, 1964) to isolate the lipid which amounted to 57% of the whole sesame.

(*2*) *Isolation of Sesamin and Sesamolin.* Sesame oil (400 mg) was dissolved in acetone (200 cm^3) and cooled overnight in acetone saturated with solid carbon dioxide (dry ice) at −50 °C. The glyceride crystals were separated by filtration while cool (−20 °C), and removal of acetone from the filtrate gave a yellow oil. The yellow oil gave crystals of sesamin when left to stand overnight.

The yellow oil was saponified with 5% alcoholic potassium hydroxide (25 cm^3) for 1 h. Water (100 cm^3) was added and the soap solution was extracted with ether (60 cm^3) for three times. Removal of ether gave a yellow resin which was dissolved in ether and when left overnight yielded more crystals of sesamin (mp 118 °C). The filtrate, after removal of ether, was dissolved in chloroform and light petroleum (bp 90–120 °C was added until the onset of cloudness. Sesamolin separated as a white solid which crystallized from ethanol in white plates (mp 93–94 °C) (Haslam and Haworth, 1955).

(*3*) *Preparation of the Aroma Concentrate.* (*A*) *Roasted Sesamin and Sesamolin-Free Seeds.* Sample A was seeds that contained lipids that were free of sesamin and sesamolin. These seeds (228 g) were added to 172 g of defatted seeds. This mixture was roasted by using a silicon oil bath at 180 °C for about 2 h under reduced pressure (about 30 mmHg) with a continuous rotating device (Soliman et al., 1974), yielding 3.17 g of aroma concentrate.

(*B*) *Roasted Lipid-Free Seeds with Sesamin and Sesamolin.* Lipid-free seeds (302 g) were mixed with the

Table I. The Composition of Aroma Concentrate from Roasted White Sesame Seeds Free from Sesamin–Sesamolin and Its Neutral-Acidic and Basic Fraction (See Figure 1)

peak no.	concentration, % aroma concentrate	neutral–acidic	basic	compounds
1				
2	2.08	1.09	5.14	2-ethylpyrazine, 3-methylbutanal
3	2.21	4.33	5.67	hexanal, propylpyrazine
4	0.26	2.13	17.65	2,3-dimethylpyrazine, unknown
5		0.18	38.86	2,5-dimethylpyrazine, unknown
6	6.26	0.69		octanal
7	2.97	0.27		nonanal
8	1.46	0.49	17.26	2,5-diethylpyrazine, *n*-octanol
9	8.39	0.98	15.42	nonyl alcohol, unknown
10	0.99			
11	tr	tr		benzaldehyde
12				
13				
14	0.12			
15	2.65			
16	2.62	tr		2-furfural
17	tr			
18	1.09			
19	0.07	tr		5-methylfurfural, undecanol
20	u.15	0.99		2-acetylfuran, dodecanol
21				
22	0.06			heptyl ester
23	1.57	3.45		
24	0.28	0.93		
25	0.38	0.45		
26	0.83	0.70		heptanone
27	10.93	21.51		4-penten-2-one, furan
28				
29				
30	2.47	4.92		nonyl ester
31	tr	tr		benzyl alcohol
32				
33				
34	28.54	32.59		2,4-undecadienal
35				
36	6.71	2.76		
37	4.73	2.93		methyl undecanoate
38	11.18	18.61		2,4,6-dodecatrienal

Table II. Comparison between the Neutral-Acidic and Basic Fractions of the Aroma from Roasted White Sesame Seeds and the Neutral-Acidic and Basic Fractions of Aroma from Roasted White Sesame Seeds Free from Sesamin-Sesamolin

peak no.	concentration, % roasted white sesame seeds		concentration, % roasted white sesame seeds free from sesamin-sesamolin		compounds
	neutral-acidic	basic	neutral-acidic	basic	
1	9.00	14.64			2-methylpyrazine, ethanol
2	1.02	23.96	1.09	5.14	2-ethylpyrazine, 3-methylbutanol
3	2.79	3.00	4.33	5.67	propylpyrazine, hexanal
4		17.99	2.13	17.65	2,3-dimethylpyrazine, unknown
5		27.14	0.18	38.86	2,5-dimethylpyrazine, unknown
6	1.56		0.69		octanal
7	1.64	2.55	0.27		2,3-diethylpyrazine, nonanal
8	0.11	6.25	0.49	17.26	2,5-diethylpyrazine, *n*-octanol
9	0.86		0.98	15.42	nonyl alcohol
10		0.56			
11	0.40	1.99			benzaldehyde
12	0.27	1.71			
13		0.21			
14	0.05				
15					
16	1.00		tr		2-furfural
17	0.05				
18	0.17				
19	0.12		tr		5-methylfurfural, undecanol
20	0.18		0.99		2-acetylfuran, dodecanol
21	0.20				
22	0.05				heptyl ester
23	0.36		3.45		
24	0.06		0.93		
25	0.04		0.45		
26	0.06		0.70		heptanone
27	0.59		21.51		4-penten-2-one, furan
28	0.08				octyl ester
29	0.07				octanone
30			4.92		nonyl ester
31	0.90		tr		benzyl alcohol
32	1.08				nonanone
33	1.44				2-furfuryl alcohol
34	49.44		32.59		2,4-undecadienal
35	1.45				
36			2.76		
37	8.20		2.93		methyl undecanoate
38	6.90		18.61		2,4,6-dodecatrienal
39	9.86				

isolated sesamin and sesamolin. This mixture was used for the preparation of aroma concentrate by the same method mentioned above, yielding 1.41 g of aroma concentrate.

(*4*) *Fractionation of Aroma Concentrate.* The aroma concentrate was fractionated into neutral-acidic and basic fractions according to Takei et al. (1974), as shown in Scheme I.

Gas-Liquid Chromatography-Mass Spectroscopy (GC-MS) Coupling. The identification of the components of aroma concentrate was done by using gas-liquid chromatography-mass spectrometery, Varian 1400-Mat 112, under the following conditions: column package, 3% SE 30 on chromosorb w 100-120 mesh, length 6 ft with internal diameter $^1/_8$ in; column temperature, 70-190 °C with programming rate 4 °C/min; chart speed, 1 cm/min; flow rate (He), 20 mL/min; temperature of ion source of mass spectrometry, 200 °C, under reduced pressure, 10^{-6} torr, and electron volt, 70 ev.

The area of the peak was measured by the triangle method. The concentration of each component was measured by the normalization method. Concentration of each component = (area of peak/($\sum$ area)100.

RESULTS AND DISCUSSION

Aroma of Roasted White Sesame Seeds Free from Sesamin and Sesamolin. Figure 1 and Table I are a record of the compounds constituting the aroma of roasted white sesame seeds free from sesamin-sesamolin together with its neutral-acidic and basic fractions.

It is noticed that the aroma concentrate has both nutty and roasted sesame seed-like aroma, the first being the predominant. Thus, it contains both the compounds characteristic for the nutty aroma (pyrazine derivatives) and those characteristic for the roasted sesame seed aroma (furan derivatives, aldehydes and ketones, etc.).

The neutral-acidic fraction has roasted oily aroma. It's components together with their concentrations are represented in Table I. It is observed that the two unsaturated aldehydes (2,4-undecadienal and 2,4,6-dodecatrienal) have the highest concentrations (32.59% and 18.61%).

These two aldehydes are liberated due to lipid oxidation and in particular linoleic acid which is the major acid of sesame lipid (Meyer, 1964). So, removing sesamin and sesamolin may cause oil reversion.

The saturated short-chain aldehydes, hexanal, octanal, and nonanal, have the lower concentrations 4.33%, 0.69%, and 0.29%, respectively. It is observed from Table II that the concentration of most of the compounds in the neutral-acidic fraction of the sesamin-sesamolin-free seeds differed from those of the same fraction of whole roasted seeds. The great variation was observed in the concentration of 2,4-undecadienal (49.44%) in whole roasted seeds (Soliman et al., 1984) compared to that (32.59%) in the

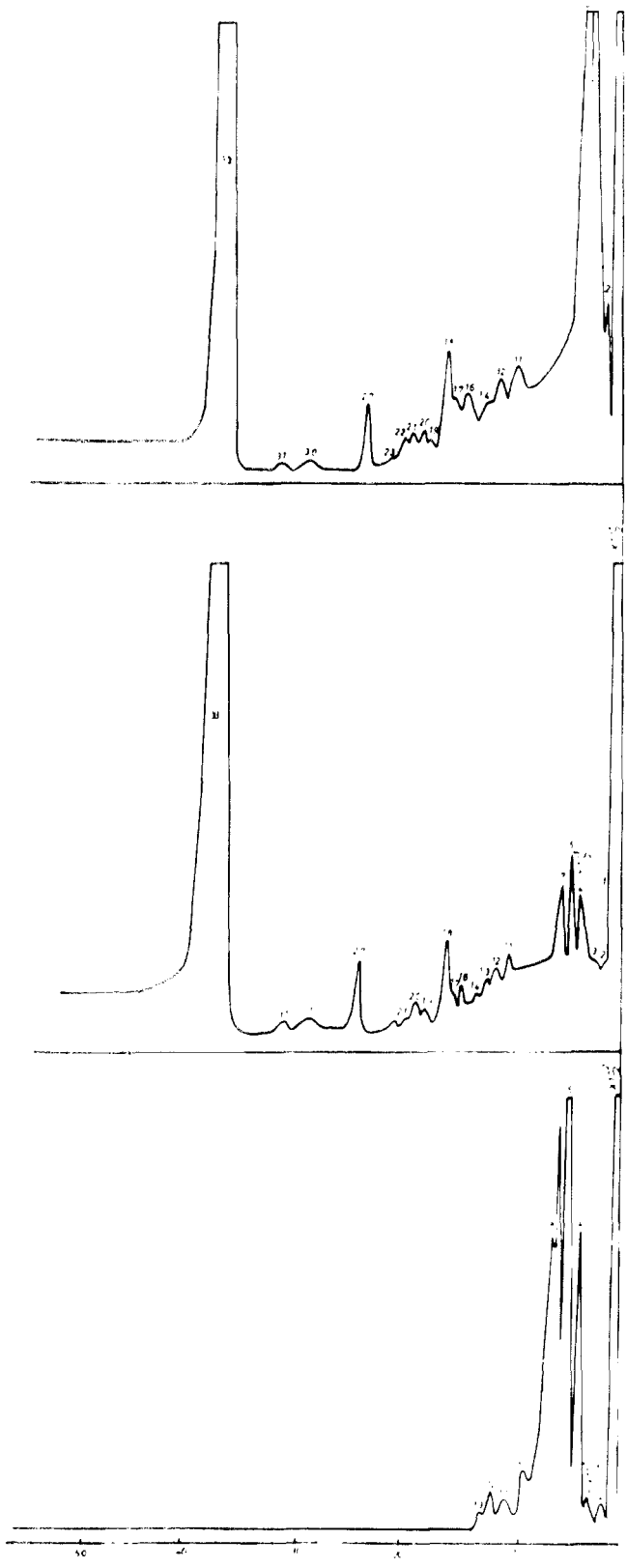

Figure 2. Gas–liquid chromatograms of the aroma concentrate of roasted defatted white sesame seeds mixed with sesamine–sesamoline (top) and its neutral–acidic (middle) and basic (bottom) fractions, respectively.

sesamin–sesamolin-free sample. On the contrary 2,4,6-dodecatrienal increased from 6.90% in whole roasted seeds to 18.61% in the sesamin–sesamolin-free sample. While the other aliphatic alcohol and aldehydes of C_6, C_8, and C_9 show approximately the same concentration.

Table III. Composition of Aroma Concentrate from Roasted Defatted White Sesame Seeds Mixed with Sesamin–Sesamolin and Its Neutral–Acidic and Basic Fractions (See Figure 2)

peak no.	concentration, % aroma concentrate	neutral–acidic	basic	compounds
1				
2	0.54	0.47	2.10	2-ethylpyrazine, 3-methylbutanal
3	19.86	1.42	1.57	hexanal, propylpyrazine
4	30.80	3.33	18.37	2,3-dimethylpyrazine, unknown
5		3.22	32.18	2,5-dimethylpyrazine, unknown
6				
7		3.35	12.07	2,3-diethylpyrazine, nonanal
8			26.05	2,5-diethylpyrazine
9				
10			3.31	
11	0.80	0.60	1.57	benzaldehyde
12	0.62	0.54	1.42	
13		0.42	1.36	
14	0.70	0.27		
15				
16	0.81	0.50		2-furfural
17	0.16	0.42		
18	1.70	3.16		
19	0.07	0.84		5-methylfurfural
20	0.08	0.61		2-acetylfuran
21	0.13	0.24		
22	0.29	0.19		
23	tr			
24				
25				
26				
27	1.31	2.84		4-penten-2-one, furan
28				
29				
30	0.47	1.00		benzyl alcohol
31	0.31	0.58		
32				
33	41.35	76.00		2-furfuryl alcohol

Benzaldehyde, benzyl alcohol, and all furan derivatives decreased remarkably in the neutral–acidic fraction of sesamin–sesamolin-free roasted seeds except 4-(5-methyl-2-furyl)-3-buten-2-one recorded a much higher concentration (21.51%) compared to that in whole roasted seeds (0.50%).

The basic fraction has nutty-like aroma. Its components are shown in Table I. For this fraction, 2-methylpyrazine disappeared, while 2-ethylpyrazine and propylpyrazine have low concentrations (5.14% and 5.67%, respectively). On the contrary 2,5-dimethylpyrazine and 2,5-diethylpyrazine, which are markedly considered as responsible for the nutty flavor, have the highest concentrations (38.86% and 17.65%, respectively).

Comparing the components of the fraction to those of the whole roasted seeds (Soliman et al., 1984), it is obvious that 2-ethylpyrazine decreased while 2,5-dimethylpyrazine and 2,5-diethylpyrazine increased.

The shift of aroma toward the nutty flavor is clearly a result of a change in the balance of the percentage of the individual compounds causing both flavors. Therefore, an increase in some pyrazine derivatives which cause the nutty flavor at the expense of a decrease in some furan derivatives responsible for the sweet roasted seed-like aroma was observed. This gave rise to a nutty flavor for the whole concentrate rather than the sweet roasted sesame-like aroma.

Aroma of Roasted Defatted White Sesame Seeds Mixed with Sesamin–Sesamolin. The gas chromato-

Table IV. Comparison between the Neutral-Acidic and Basic Fractions of the Aroma from Roasted Defatted White Sesame Seeds and the Neutral-Acidic and Basic Fractions of the Aroma from Roasted Defatted White Sesame Seeds Mixed with Sesamin-Sesamolin

peak no.	concentration, % roasted defatted white sesame seeds neutral–acidic	roasted defatted white sesame seeds basic	roasted defatted white sesame seeds mixed with sesamin–sesamolin neutral–acidic	roasted defatted white sesame seeds mixed with sesamin–sesamolin basic	compounds
1	4.00	23.14			2-methylpyrazine, ethanol
2	1.45	10.03	0.47	2.10	2-ethylpyrazine, 3-methylbutanal
3	0.20		1.42	1.57	propylpyrazine, hexanal
4	0.25	20.66	3.33	18.37	2,3-dimethylpyrazine, unknown
5		33.50	3.22	32.18	2,5-dimethylpyrazine, unknown
6					
7		5.63	3.35	12.07	2,3-diethylpyrazine, nonanal
8		7.04		26.05	2,5-diethylpyrazine
9					
10	0.93			3.31	
11	tr		0.60	1.57	benzaldehyde
12	0.25		0.54	1.42	
13			0.42	1.36	
14			0.27		
15					
16	0.60		0.50		2-furfural
17			0.42		
18			3.16		
19	0.42		0.84		5-methylfurfural
20	1.17		0.61		2-acetylfuran
21			0.24		
22			0.19		
23	4.10				
24	0.50				
25	0.40				
26	0.35				
27	2.55		2.84		4-penten-2-one, furan
28					
29	0.46				
30			1.00		benzyl alcohol
31	4.10		0.58		
32	1.53				
33	72.66		76.00		2-furfuryl alcohol
34					
35	4.08				
36					

gram and percent compositions of aroma concentrate as well as its neutral–acidic and basic fractions are shown in Figure 2. The compounds eluted from GLC and their concentrations are shown in Table III.

The major components of the neutral–acidic fraction are the furan derivatives. 2-Furfuryl alcohol has the highest concentration, 76%; this compound is known to have popcorn-like flavor. It is developed from the caramelization of sugar.

Other components such as 2-furfural, 5-methylfurfural, 2-acetylfuran, and 4-(5-methyl-2-furyl)-3-buten-2-one have low concentrations (0.5%, 0.84%, 0.61%, and 2.84%, respectively).

Concerning the basic fraction, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, 2,3-diethylpyrazine, and 2,5-diethylpyrazine are present in high concentrations.

Both the defatted white sesame seeds and the defatted white sesame seeds mixed with sesamin–sesamolin have the same pyrazine derivatives with the same concentration for 2,3-dimethylpyrazine and 2,5-dimethylpyrazine, while 2-methylpyrazine and 2-ethylpyrazine decreased. On the contrary, 2,3-diethylpyrazine and 2,5-diethylpyrazine showed remarkable increase (Table IV).

Regarding the furan derviatives, again all the components are represented giving rise to an almost identical flavor. However, the defatted sample showed stronger roasted sesame-like flavor when admixed with sesamin–sesamolin, which might be partly due to the increase of furfuryl alcohol concentration (Table IV).

In general, development of the roasted sesame seed aroma increased by removing the lipid due to the increase of 2-furfuryl alcohol, while removing the sesamin–sesamolin resulted in stronger roasted oil aroma in the neutral–acidic fraction. On the other hand, the addition of sesamin–sesamolin to the defatted sample created a much stronger roasted sesame seed-like aroma in the neutral–acidic fraction. However, the basic fractions developed, in all cases, more or less the same nutty aroma.

Registry No. 2-Ethylpyrazine, 13925-00-3; 3-methylbutanol, 590-86-3; hexanal, 66-25-1; propylpyrazine, 18138-03-9; 2,3-dimethylpyrazine, 5910-89-4; 2,5-dimethylpyrazine, 123-32-0; octanal, 124-13-0; nonanal, 124-19-6; 2,5-diethylpyrazine, 13238-84-1; *n*-octanol, 111-87-5; nonyl alcohol, 143-08-8; benzaldehyde, 100-52-7; 2-furfural, 98-01-1; 5-methylfurfural, 620-02-0; undecanol, 30207-98-8; 2-acetylfuran, 1192-62-7; dodecanol, 112-53-8; heptanone, 29299-43-2; 4-penten-2-one, 13891-87-7; benzyl alcohol, 100-51-6; 2,4-undecadienal, 13162-46-4; methyl undecanoate, 1731-86-8; 2,4,6-dodecatrienal, 85057-30-3; furan, 110-00-9; ethanol, 64-17-5; 2,3-diethylpyrazine, 15707-24-1; octanone, 27457-18-7; nonanone, 30642-09-2; 2-furfuryl alcohol, 98-00-0; sesamin, 607-80-7; sesamolin, 526-07-8; 2-methylpyrazine, 109-08-0.

LITERATURE CITED

Fieser, L. F. "Organic Experiments"; D.C. Heath and Company: Boston, MA, 1964.

Haslam, E.; Haworth, R. *J. Chem. Soc.* **1955**, 827.
Meyer, L. H. "Food Chemistry"; Reinhold Publishing Corporation: New York, Tokyo, 1964.
Segal, B.; Stover, V.; Covaropol, G. *Ind. Aliment. (Bucharest)* **1971**, *22* (10), 582.
Soliman, M. M.; El-Sawy, A. A.; Fadel, H. M.; Osman, F. *Acta Aliment. Acad. Sci. Hung.*, in press.
Soliman, M. M.; Kinoshita, S.; Yamanishi, T. *Agric. Biol. Chem.* **1974**, *39* (5), 973.
Takei, Y.; Shimada, K.; Watanabe, S.; Yamanishi, T. *Agric. Biol. Chem.* **1974**, *38*(1), 645.

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An Enzyme-Linked Immunosorbent Assay for Metalaxyl in Foods

William H. Newsome

An enzyme-linked immunosorbent assay was developed for residues of the fungicide metalaxyl in foods. The procedure is applicable to the analysis of methanol extracts without prior cleanup and will quantitate from 0.1 to 2.0 ppm of the parent compound in various commodities. Accuracy and precision compare favorably to a gas chromatographic method involving a solvent partitioning and adsorption column cleanup, although specificity is less with cross reactivity being observed with the herbicides metolachlor and diethatyl ethyl and to a lesser extent the fungicide furalaxyl. The simplicity of the method permits 4.5 times more samples to be analyzed per day than a conventional gas chromatographic procedure.

Metalaxyl (methyl *N*-(2-methoxyacetyl)-*N*-(2,6-xylyl)-alaninate) is a fungicide registered for use in the United States on a variety of fruit and vegetable crops. Several methods have been published for the determination of crop residues by gas chromatography with nitrogen-selective detectors (e.g., Caverly and Unwin, 1981; Tafuri et al., 1981; Speck and Dirr, 1980). Cleanup techniques have ranged from simple water partitioning for tomato extracts (Waliszewski and Szymczynski, 1983) to sweep codistillation followed by silica gel chromatography for soil and sunflower foliage extracts (Tafuri et al., 1981). It has been our experience that a cleanup step involving adsorption chromatography was necessary when analyzing certain commodities on which metalaxyl is registered to prevent anomalous quantitative results arising from accumulation of coextractives on the gas chromatographic column.

An alternative approach to residue analysis with the potential of more efficient processing of samples is immunochemical determination based on competitive binding to an antibody (Hammock and Mumma, 1980). Radioimmunoassay procedures have been applied successfully to the determination of such pesticides as paraquat (Fatori and Hunter, 1980), 2,4-dichlorophenoxyacetic acid and (Rinder and Fleeker, 1981) parathion (Ercegovich et al., 1981), and benomyl (Newsome and Shields, 1981). More recently, enzyme-linked immunosorbent assay (ELISA) has been employed for the analysis of diflubenzuron (Wie and Hammock, 1982) and paraquat (Niewda et al., 1983) and has the advantage of not requiring a radioligand and associated counting equipment. The following report describes development of an ELISA procedure for metalaxyl residues in foods and compares it to a gas chromatographic method.

EXPERIMENTAL SECTION

Materials. Analytical standards of metalaxyl, its free acid, CGA 109097, and furalaxyl were gifts from Ciba-Geigy Ltd., Mississauga, Ont., while those of metolachlor, alachlor, propachlor, and benzoylprop-ethyl were obtained from Agriculture Canada, Ottawa, Ont. Diethatyl ethyl was obtained from Hercules Inc., Wilmington, DE. Stock solutions consisting of 0.5 mg mL^{-1} of each compound were prepared by dissolving in HPLC grade methanol (Caledon Laboratories, Georgetown, Ont.). Working standards of metalaxyl were prepared by serial dilution in methanol to give concentrations of 2.5, 5.0, 10, 20, and 40 ng mL^{-1}. Freund's complete adjuvant was purchased from Difco Laboratories, Detroit, MI. Bovine serum albumin (RIA grade), human serum albumin, ovalbumin, antirabbit IgG peroxidase conjugate, *o*-phenylenediamine dihydrochloride, and Tween 20 (polyoxyethylene sorbitan monolaurate) were obtained from Sigma Chemical Co., St. Louis, MO. 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride was supplied by Aldrich Chemical Co., Inc., Milwaukee, WI, while tri-*n*-butylamine and isobutyl chloroformate were obtained from Eastman Kodak Co., Rochester, NY.

Instruments. The optical density of microtiter plate well content was read on a Dynatech MR 600 dual beam plate reader. Gas chromatography was performed on a Varian 1400 fitted with a N–P detector, a 0.25 μm DB-5 capillary column (0.25 mm × 10 m, J:W Scientific, Inc., Rancho Cordova, CA), and a J:W oncolumn injector. Helium carrier gas was supplied at a linear velocity of 33 cm s^{-1}. Flows of nitrogen makeup gas, air, and hydrogen to the detector were 30, 175, and 4.5 mL^{-1}, respectively. The detector operating temperature was 250 °C. Injections of 1.0 μL were cold trapped and after a 1-min delay temperature programmed at 50 °C min^{-1} to 210 °C. Under these conditions, approximately 50% full scale deflection was obtained from the injection of 1.1 ng of metalaxyl.

Buffers. Phosphate buffered saline (PBS) contained 2.42 g of NaH_2PO_4 and 8.26 g of NaCl per L of distilled water. The pH was adjusted to 7.2 with NaOH before making to volume. PBS-Tween washing solution was prepared by adding 0.5 mL of Tween 20 per L of PBS. Antiserum diluent contained 0.1% bovine serum albumin in PBS. Coating buffer, pH 9.6, contained 1.59 g of

Food Research Division, Food Directorate, Health Protection Branch, Department of National Health and Welfare, Ottawa, Canada K1A 0L2.

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