Effects of Cultivar and Tempering Procedures on Crude Soybean Oil Volatiles

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The effects of soybean cultivar and tempering procedures on crude soybean oil volatiles were investigated by gas chromatography/mass spectrometry (GC/MS). Varietal differences in volatiles were not obvious. Trends in volatile contents due to tempering were noted primarily when comparing mean peak areas of these compounds in oils stored 16 days at 60°C. Differences in volatiles in response to tempering were not apparent in oils that had not been stored. Equilibration of beans with water resulted in oils with higher volatile contents than those from untempered beans. Steaming lowered volatiles from equilibrated values, and pressure steaming had an even more dramatic lowering effect.

KEY WORDS: Crude soybean oil, headspace analysis, volatiles.

Oxidation of unsaturated lipids results in the formation of hydroperoxides, which are tasteless and odorless (1). Undesirable flavors and odors associated with rancidity of oxidized lipids arise from decomposition of these hydroperoxides. Some of the volatile products resulting from this decomposition include aldehydes, ketones, alcohols, hydrocarbons, esters, furans and lactones.

Because of the important role lipid oxidation plays in flavor deterioration of soybean oil, volatile analysis has been developed as a method to evaluate the degree of oxidation, especially in conjunction with sensory evaluation and peroxide value analysis.

Results from the identification and quantitation of volatile components may be related to flavor quality of the oil. Various techniques have evolved for gas chromatography (GC) volatile analysis, ranging from direct injection to various purge-trap systems (2).

In our previous paper (3), we showed that tempering procedures applied to cracked soybeans during oil extraction, and designed to result in various degrees of lipoxygenase (E.C.1.13.11.12) inactivation, had more impact on sensory characteristics and peroxide values than did changing cultivars of soybeans, including one lacking lipoxygenase-1 (L-1). In this paper the effects of soybean cultivar and tempering procedures on crude soybean oil volatiles are presented.

EXPERIMENTAL PROCEDURES

Materials. Two varieties of soybeans (*Glycine max* cv. Amsoy and Calland) were obtained from a local seed company. Lipoxygenase-1 null soybeans (Forest × PI 408251) were provided by Dr. E. Hartwig, USDA (Stoneville, MS). Linoleic acid and linolenic acid were purchased from Sigma Chemical Company (St. Louis, MO).

Oil extraction. Soybeans were coarsely ground. At this point, tempering, if required, was done by one of the three methods described below. Approximately 100 g beans (untempered or tempered) were blended in a Waring Blendor with 100–150 mL hexane for 2 min at high speed and cooled. Blending was repeated three times for a total of 8 min. The slurry was then transferred to a large flask with approximately 250 mL hexane and stirred in a water bath (approx. 60°C) for 1.5–2 h. Filtration of the slurry was followed by rinsing the meal with hexane and rotary vacuum evaporation of the hexane/oil mixture until there was no more detectable solvent.

Tempering. Three tempering procedures were designed to result in beans of 10–12% moisture. The first method was direct equilibration of 100 g coarsely ground soybeans with 7.5 g water in a closed container placed in a water bath at 67°C for 3 h. The second method was steaming (100°C) of 100 g coarsely ground beans for 2 min in a vegetable steamer. The third method was steaming 100 g coarsely ground beans under 0.67 atm. pressure (116°C) for 1 min. All procedures were replicated three times for statistical purposes. Each extraction represented an individual sample.

Lipoxygenase assays. Lipoxygenase-1, -2, and -3 activities were assayed as described in our previous paper (3).

Accelerated storage. Crude soybean oils were either frozen (−20°C) immediately after extraction in clear glass bottles with nitrogen in the headspace or held at 60°C for 16 days (accelerated storage) in clear glass bottles approximately 1/2–2/3 full with loosely fitting celophane-wrapped corks (4). After storage, the oils were also frozen (−20°C) under nitrogen for future analysis.

Volatile analysis. A Chemical Data Systems (CDS) 330 concentrator unit with Tenax trap (CDS Data Systems, Oxford, PA) was used to purge and trap volatiles prior to GC analysis in a Hewlett-Packard GC with capillary column and flame-ionization detector (Palo Alto, CA). Approximately 7 mg crude soybean oil was accurately weighed into a previously dried sample tube with stainless steel top, joined by an inert Teflon seal. A 15-min holding period in the concentrator unit (impinger) at 180°C preceded progression through the rest of the sequence (Table 1). Sample volatiles were purged with helium at 1.34 atm. with a flow rate of 20–24 mL/min and collected on Tenax trap (A) over a 10-min period. The trap temperature was 25°C. As the trap temperature rose to 250°C, volatiles were desorbed and transferred to trap B (200°C) over a 5-min period. The volatiles were then swept onto the GC column, which was temperature programmed from −60 to 250°C at 3°C/min. A "bake out" program was run to purge the system and remove any carryover material. Peak areas and retention times were generated with a Hewlett-Packard electronic integrator.

Mass spectra of eluted compounds were obtained with a Hewlett-Packard Model 5890 GC-MS instrument equipped with a data system for recording and manipulation of mass spectra. The capillary column, a 50 m × 0.25 mm fused silica column bonded with 5% methyl silicone (Hewlett-Packard) was interfaced with the mass spectrometer. The column was temperature programmed from −60 to 250°C at 3°C/min. Mass spectra of eluting components were collected at an ionizing voltage of 70 volts. Chem-

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TABLE 1

Relative Yields of Alkanals and Hydrocarbons from Crude Oils[a]

Oil	Pentane	Propanal	Pentanal	Hexanal	Heptanal	Total
Amsoy, 0-day						
not tempered	7.03	1.67	3.49	14.66	1.53	28.38
equilibrated	3.89	1.38	4.33	11.66	1.10	22.36
steam, 100°C	6.36	1.80	5.79	11.42	1.40	26.77
steam, 116°C	6.13	1.63	6.48	12.88	1.35	28.47
Amsoy, 16-day						
not tempered	6.74	1.25	3.77	10.83	1.10	23.69
equilibrated	6.15	1.52	5.27	11.88	1.18	26.00
steam, 100°C	4.10	1.05	2.98	8.21	0.92	17.26
steam, 116°C	3.29	0.59	2.28	4.96	0.50	11.62
Calland, 0-day						
not tempered	7.35	1.62	7.96	19.15	1.61	37.69
equilibrated	5.28	1.38	4.69	10.93	0.93	23.21
steam, 100°C	6.67	2.13	7.73	17.80	1.55	35.88
steam, 116°C	5.05	1.27	4.31	10.40	0.95	21.98
Calland, 16-day						
not tempered	5.76	1.63	3.19	14.13	1.25	25.96
equilibrated	6.73	2.12	10.98	18.51	1.66	40.00
steam, 100°C	5.36	1.15	4.60	11.33	1.01	23.45
steam, 116°C	3.57	0.79	3.21	6.76	0.60	14.93
L-1 Null, 0-day						
not tempered	6.86	1.28	5.86	14.86	1.29	30.15
equilibrated	3.26	0.93	11.64	19.32	1.87	37.02
steam, 100°C	4.44	0.74	2.97	6.72	0.64	15.51
steam, 116°C	4.07	0.95	3.58	7.65	0.78	17.03
L-1 Null, 16-day						
not tempered	4.61	0.96	3.78	9.03	0.93	19.31
equilibrated	7.01	1.85	8.67	19.32	1.90	38.75
steam, 100°C	4.98	1.28	7.84	13.22	1.22	28.54
steam, 116°C	4.81	0.94	2.82	9.28	0.87	18.72

[a]Values represent areas of eluted compounds from a capillary column $\times 10^6$.

ical ionization spectra were determined with methane as the reactant gas. All spectra were stored on disc for subsequent background subtraction and manipulation. Peak identification was carried out by library search and also by standard matching when appropriate standards were available.

RESULTS AND DISCUSSION

Gas chromatography was used to analyze volatiles in an attempt to draw a relationship between volatile profile, soybean cultivar and tempering procedure. Figures 1 and 2 represent typical gas chromatograms for two different oils. Figure 1 is a chromatogram of an oil extracted from untempered Amsoy beans, which was stored for 16 days. Figure 2 is a chromatogram of an oil from L-1 null beans, which were steamed under pressure and not stored. A number of these peaks were identified by gas chromatography-mass spectrometry (GC-MS). The chromatograms show many more predominant peaks in the stored oil from untempered Amsoy beans. The oil from L-1 null beans contained two components at retention times near 24 min that were identified as hexane and possibly methylcyclopentane. Both compounds resulted from residues remaining in the oil after hexane extraction.

The predominant peaks of interest resulting from hydroperoxide decomposition were: 2-propenal, pentane, propanal, pentanal, hexanal, 2-hexenal, heptanal, 2-heptenal, 2,4-heptadienal, and 2,4-decadienal because of their influence on oil quality (5). Tables 1, 2 and 3 show relative yields of alkanals and hydrocarbons, alkenals and alkadienals, respectively. Table 4 is a summary of the total yields of the chosen volatiles. These values are presented as the means of at least two replications. These compounds have been used either singly or in combination as indices of soybean oil deterioration. For 2,4-heptadienal and 2,4-decadienal, two isomers (*trans, cis* and *trans, trans*) of each were found, and areas of the two peaks were combined for one peak total.

Varietal differences in volatiles of crude oils were not obvious. Lipoxygenase-1 null beans produced oils that were generally lower than Amsoy and Calland in the chosen volatile components for oils from untempered beans (0 and 16 days' storage) as well as for oils from steamed beans that were not stored. Stored oils from L-1 null beans that had been pressure-steamed were generally higher in these volatiles than those from Amsoy and Calland. Oils from equilibrated L-1 null beans generally were higher in these volatiles as well. If lipoxygenase is responsible for flavor and oxidative deterioration of soybean oil, because of the initiation of autoxidation during the grinding and tempering of the beans prior to the extraction procedure, L-2 and L-3 may be more important than L-1. It appeared that L-1 beans were not superior to Amsoy and Calland varieties. Lipoxygenase-2 and -3 activities of L-1 null beans were almost as great or greater than those in untempered, steamed and equilibrated Amsoy and Calland beans (3).

Trends in volatile contents due to tempering were noted primarily when comparing mean areas of these components in oils that had been stored 16 days at 60°C. Differences in volatiles in response to tempering were not apparent in oils that had not been stored. Storage appeared necessary to observe differences in oil quality. Storage

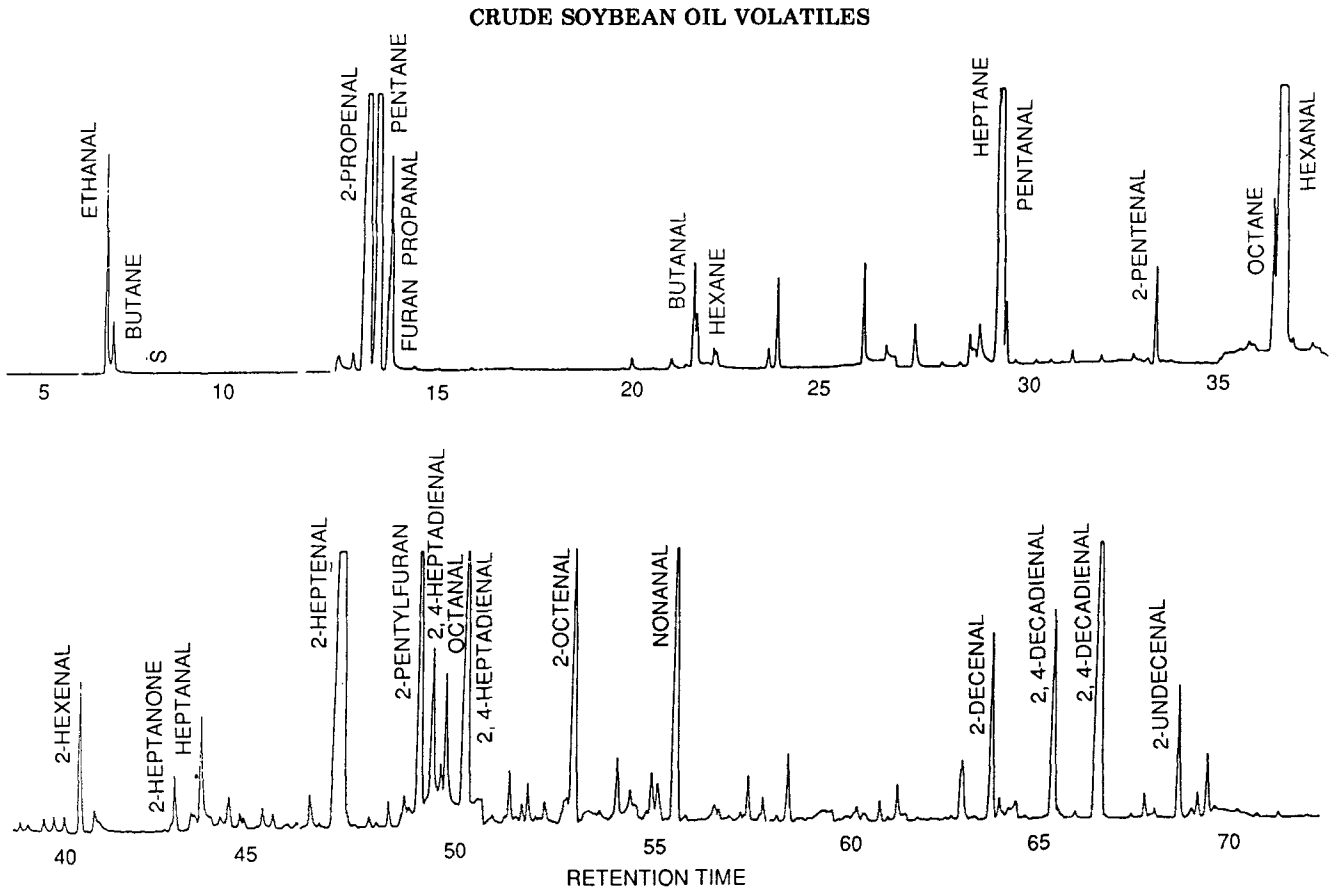

FIG. 1. Gas chromatography chromatogram of crude soybean oil from untempered Amsoy beans—stored 16 days at 60°C.

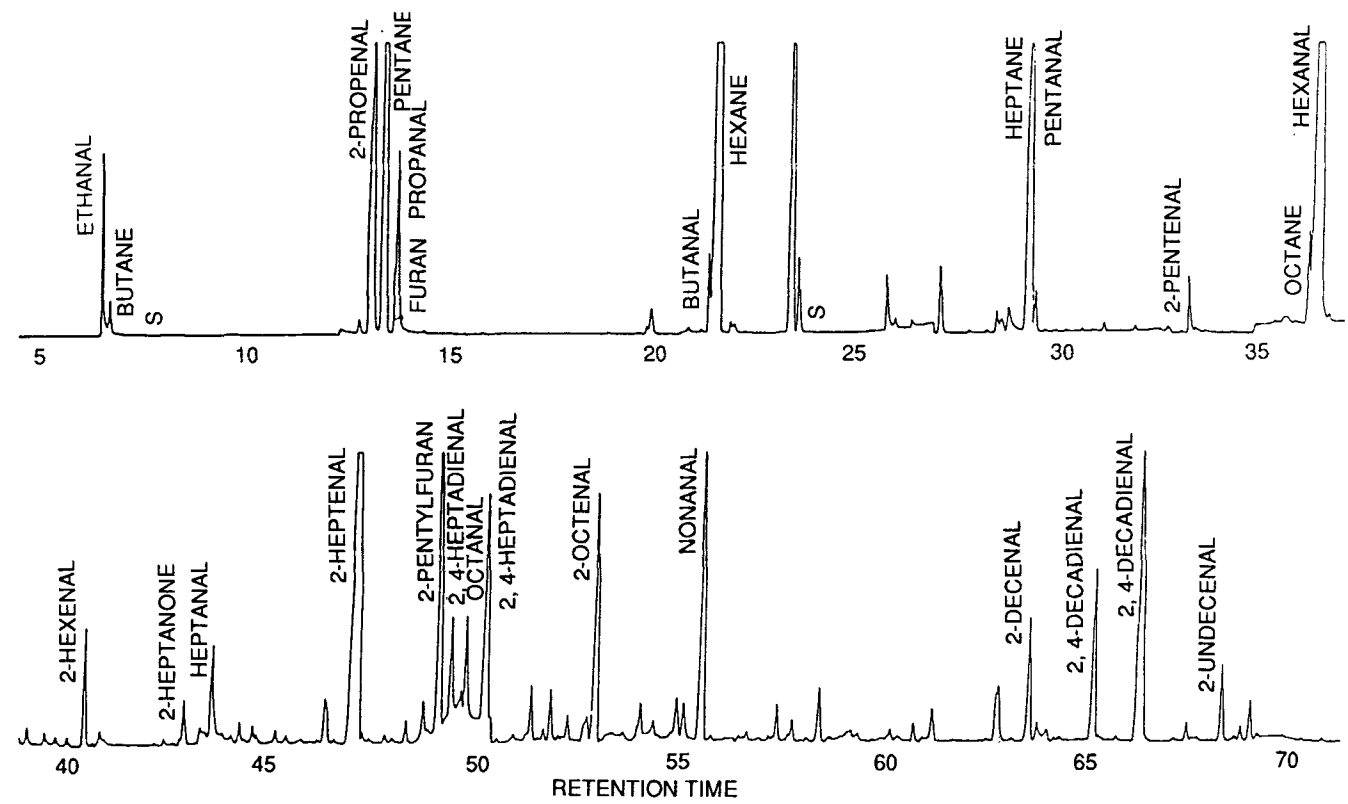

FIG. 2. Gas chromatography chromatogram of crude soybean oil from pressure-steamed L-1 Null beans—not stored.

TABLE 2

Relative Yields of Alkenals from Crude Oils[a]

Oil	Propenal	Hexenal	Heptenal	Total
Amsoy, 0-day				
not tempered	3.28	1.14	10.58	15.00
equilibrated	0.64	0.85	8.04	9.53
steam, 100°C	1.12	1.07	10.47	12.66
steam, 116°C	4.45	1.01	9.80	15.26
Amsoy, 16-day				
not tempered	1.87	0.93	8.56	11.36
equilibrated	4.36	1.01	9.58	14.95
steam, 100°C	2.24	0.68	6.86	9.78
steam, 116°C	1.63	0.36	3.64	5.63
Calland, 0-day				
not tempered	1.25	1.43	13.28	15.96
equilibrated	0.95	0.98	9.32	11.25
steam, 100°C	1.24	1.33	13.14	15.71
steam, 116°C	2.39	0.75	7.79	10.93
Calland, 16-day				
not tempered	3.67	0.96	11.05	15.68
equilibrated	5.34	1.44	14.12	20.90
steam, 100°C	1.81	0.09	9.10	11.81
steam, 116°C	2.14	0.52	5.10	7.76
L-1 Null, 0-day				
not tempered	1.48	0.71	6.82	9.44
equilibrated	0.92	1.33	12.70	14.95
steam, 100°C	1.96	0.51	4.92	7.39
steam, 116°C	1.62	0.55	5.51	7.68
L-1 Null, 16-day				
not tempered	1.91	0.71	6.82	9.44
equilibrated	4.14	1.46	14.02	19.62
steam, 100°C	2.14	0.89	8.67	11.70
steam, 116°C	2.41	0.66	6.70	9.77

[a]Values represent areas of eluted compounds from a capillary column $\times 10^6$.

TABLE 3

Relative Yields of Alkadienals from Crude Oils[a]

Oil	2,4-Heptadienal	2,4-Decadienal	Total
Amsoy, 0-day			
not tempered	5.23	7.26	12.49
equilibrated	4.09	4.67	8.76
steam, 100°C	5.16	5.66	10.82
steam, 116°C	4.94	7.20	12.14
Amsoy, 16-day			
not tempered	4.24	7.90	12.23
equilibrated	4.82	6.85	11.67
steam, 100°C	3.72	4.93	8.65
steam, 116°C	2.15	2.54	4.69
Calland, 0-day			
not tempered	5.12	9.29	14.41
equilibrated	4.74	8.13	12.87
steam, 100°C	5.49	8.18	13.67
steam, 116°C	4.14	6.12	10.26
Calland, 16-day			
not tempered	5.23	7.92	13.15
equilibrated	5.43	8.82	14.25
steam, 100°C	5.15	5.43	10.58
steam, 116°C	2.66	3.14	5.80
L-1 Null, 0-day			
not tempered	3.72	4.75	8.47
equilibrated	4.30	9.17	13.47
steam, 100°C	2.29	4.65	6.94
steam, 116°C	2.30	4.39	6.69
L-1 Null, 16-day			
not tempered	2.68	6.72	9.40
equilibrated	4.37	7.82	12.19
steam, 100°C	3.24	5.40	8.64
steam, 116°C	2.93	4.03	6.96

[a]Values represent areas of eluted componds from a capillary column $\times 10^6$.

TABLE 4

Total Relative Yields of Alkanals, Alkenkals and Alkadienals from Crude Oils[a]

Oil	Alkanals and hydrocarbons	Alkenals	Alkadienals	Total
Amsoy, 0-day				
not tempered	28.38	15.00	12.49	55.87
equilibrated	22.36	9.53	8.76	40.65
steam, 100°C	26.77	12.66	10.82	50.25
steam, 116°C	28.47	15.26	12.14	55.87
Amsoy, 16-day				
not tempered	23.69	11.36	12.23	47.28
equilibrated	26.00	14.95	11.67	52.62
steam, 100°C	17.26	9.78	8.65	35.69
steam, 116°C	11.62	5.63	4.69	21.94
Calland, 0-day				
not tempered	37.69	15.96	14.41	68.06
equilibrated	23.21	11.25	12.87	47.33
steam, 100°C	35.88	15.71	13.67	65.26
steam, 116°C	21.98	10.93	10.26	43.17
Calland, 16-day				
not tempered	25.96	15.68	13.15	54.79
equilibrated	40.00	20.90	14.25	75.15
steam, 100°C	23.45	11.81	10.58	45.84
steam, 116°C	14.93	7.76	5.80	28.49
L-1 Null, 0-day				
not tempered	30.15	12.92	8.47	51.54
equilibrated	37.02	14.95	13.47	65.44
steam, 100°C	15.51	7.39	6.94	29.84
steam, 116°C	17.03	7.68	6.69	31.40
L-1 Null, 16-day				
not tempered	19.31	9.44	9.40	38.15
equilibrated	38.75	19.62	12.19	70.56
steam, 100°C	28.54	11.70	8.64	48.88
steam, 116°C	18.72	9.77	6.96	35.45

[a]Values represent areas of eluted compounds from a capillary column $\times 10^6$.

TABLE 5

Mean Peroxide Values (meq/kg) of Crude Soybean Oils from Three Varieties of Soybeans (stored at 60°C for 16 days or not stored)

	Amsoy		Calland		L-1 Null	
	0-day	16-day	0-day	16-day	0-day	16-day
Not tempered	0.00	6.98	0.00	16.45	0.00	9.83
Equilibrated	0.00	3.46	2.49	4.75	1.28	4.19
Steamed	0.00	3.12	0.00	0.68	0.00	2.78
Pressure-steamed	0.00	1.04	0.00	0.74	0.00	2.70

usually results in increases in peroxide value, hydroperoxide concentration and volatile content (6). The general trend in stored oils was an increase in volatiles of oils from equilibrated beans when compared to those from untempered beans. Peroxide values of the oil samples are listed in Table 5 (3). Steaming lowered the volatiles from equilibrated values, and pressure steaming had an even more dramatic lowering effect. In some cases, however, the steaming procedures lead to increased volatiles over equilibration. The oils from Amsoy beans did not follow this general trend prior to storage. Overall volatile contents were greater in oils from pressure steaming than from steaming and equilibration (Table 4). This was particularly apparent for measurement of 2-propenal (Table 2), 2,4-decadienal and 2,4-heptadienal (Table 3). Also, steam treatment (100°C) of Calland beans resulted in oils (day 0) with volatiles higher than those from oils of equilibrated beans and almost the same as those from nontempered beans (Table 4).

Soybean oil acceptability has been limited due to characteristic off-flavor (and odor) development, which is believed to result primarily from volatile components formed in lipid peroxidation. Most reports of volatile analysis in the literature are on processed oils. Crude soybean oil analysis has not been intensively studied. This, together with the fact that crude oils are more stable oxidatively than refined oils (7), may account for compositional differences seen when comparing the present results to those from other studies. Also, a variety of techniques has been used for volatile analysis. Snyder *et al.* (8) compared techniques for volatile analyses of soybean oil (direct injection, static headspace and dynamic headspace). Volatile profiles differed between methods. The characteristics of each was discussed. Dynamic headspace, as done in this study, was reported to be the slower method. However, because of the lower temperatures this method may be better to measure actual oxidation products without thermal decomposition of flavor precursors. Because a stan-

dard method has not yet been developed, some caution must be exerted when making comparisons.

ACKNOWLEDGMENTS

We thank David Hendren for his assistance with the GC-MS. This study was part of Project No. 60-323 of the Agricultural Experiment Station, College of Agriculture, University of Illinois at Urbana. It was supported in part by a grant from the Illinois Soybean Program Operating Board.

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[Received October 13, 1991; accepted May 20, 1992]