Formation of the Intense Flavor Compound *trans*-4,5-Epoxy-(*E*)-2-Decenal in Thermally Treated Fats

Klaus Gassenmeier and **Peter Schieberle***
Deutsche Forschungsanstalt für Lebensmittelchemie, D-85748 Garching, Germany

Thermal degradation of several possible precursors of the intense flavor compound *trans*-4,5-epoxy-(*E*)-2-decenal in model experiments revealed that the odorant is formed in significant yields from 13-hydroperoxy-9,11-octadecadienoic acid (13-HPOD) and 9-hydroperoxy-10,12-octadecadienoic acid (9-HPOD). Of these hydroperoxides, arising in equal amounts during autoxidation of linoleic acid, the 9-HPOD was established as the more effective precursor. The key intermediates in the generation of the epoxyaldehyde were found to be 2,4-decadienal, arising from 9-HPOD, and 12,13-epoxy-9-hydroperoxy-10-octadecenoic acid, a degradation product of 13-HPOD. Isolation and characterization of the precursors from a baking margarine confirmed glycerine-bound 9- and 13-HPOD as the intermediates in the formation of the epoxyaldehyde during heating of fats that contain linoleic acid.

KEY WORDS: Autoxidation, baking margarine, 4,5-epoxy-(*E*)-2-decenal, flavor formation, flavor precursors.

The flavor compound *trans*-4,5-epoxy-(*E*)-2-decenal (ED) has been reported among the volatiles of thermally degraded trilinolein (1). This odorant, exhibiting the extremely low odor threshold of 0.5 pg/L air (2), was shown to contribute to the green, hay-like off-odor in soybean oil stored in the dark (3) and to the warmed-over flavor of stored beef (4). Furthermore, ED has been detected among the odorants of wheat bread crumb (2), popcorn (5) and roasted sesame seeds (6). Model studies have revealed that the epoxyaldehyde is formed among other volatiles during thermal degradation of methyl 12,13-epoxy-9-hydroperoxy-10-octadecenoate (7). Because this hydroperoxide has been characterized as a degradation product of methyl 13-hydroperoxy-9,11-octadecadienoate (13-HPOD) (8,9) a reaction scheme leading from linoleic acid to the epoxyaldehyde has been suggested (3).

Based on the results of an aroma extract dilution analysis and the calculation of odor activity values, we have recently (10) identified ED as one of the main contributors to the green, lard-like, fatty odor of puff-pastries prepared with a baking margarine (shortening). Previously, a homologue of ED, 4,5-epoxy-(*E*)-2-heptenal, had been reported to cause a fishy-tainted off-flavor in butterfat (11). The odor threshold of the epoxyheptenal is, however, a factor of 10^6 higher than the odor threshold of ED (2).

Because no exact quantitative data on the generation of ED from the postulated precursor compounds are available in the literature, the aim of this investigation was to measure the liberation of ED from different precursors in model experiments and to isolate and characterize its precursors from a commercial baking margarine by means of a stable-isotope dilution assay.

*To whom correspondence should be addressed at Bergische Universität/GH, Lebensmittelchemie (FB 9), Gauß-Straße 20, D-42097 Wuppertal, Germany.

EXPERIMENTAL PROCEDURES

Material. The fatty acid composition of the baking margarine (shortening-type), supplied by a manufacturer of commercial baking fats, is given in Table 1.

Chemicals. trans-4,5-Epoxy-(*E*)-2-decenal and (^{2}H)-*trans*-4,5-epoxy-(*E*)-2-decenal were synthesized according to the literature (2,12). Trilinolein and methyl linoleate (ML) (Sigma, Munich, Germany) were freed from peroxidic compounds by thin-layer chromatography (TLC) on silica gel with *n*-pentane/diethyl ether (9 + 1, by volume) as the solvent. (*E,E*)-2,4-Decadienal (Aldrich, Steinheim, Germany) was purified by TLC and by high-performance liquid chromatography (HPLC) as described recently (10).

13-HPOD was prepared as reported earlier (13). Methyl 9-hydroperoxy-(*E,Z*)-10,12-octadecadienoate (9-HPOD) was prepared from linoleic acid with a potato lipoxygenase as described by Grosch (14). The isomeric purity of both hydroperoxides was checked by HPLC of the reduced derivates (>92%) according to Chan and Prescott (15). Methyl *trans*-12,13-epoxy-9-hydroperoxy-(*E*)-10-octadecenoate (12,13-EP-9-HPOD) was prepared by photolysis of 13-HPOD in cyclohexane and isolated by TLC and HPLC (Fractions **IIIf** and **IIIh**) as previously reported (16).

Isolation of the precursors in baking margarine. The fat (10 g), dissolved in *n*-pentane/diethyl ether (20 mL, 85 + 15 by volume), was placed on the top of a water-cooled column (30 cm × 3.2 cm), filled with a slurry of silica gel in *n*-pentane, and separated into two fractions: Fraction **I** (400 mL *n*-pentane/diethyl ether, 85 + 15 by volume) and Fraction **II** (600 mL diethyl ether/ethanol, 100 + 2 by volume). Fraction **II** was further separated into three subfractions by TLC on silica gel in the solvent system *n*-pentane/diethyl ether (7 + 3 by volume): Fraction **IIa** (R_f: 0.2–0.6), Fraction **IIb** (R_f: 0.63–73) and Fraction **IIc** (R_f: 0.8–1.0). HPLC-separation of Fraction **IIb** was performed isocratically on a stainless-steel column (50 cm × 0.46 cm) filled with Shandon Hypersil (5 μ; Shandon Products, Eastmore, Great Britain) by using a pump of type 110B (Beckman, Munich, Germany). The effluent (*n*-hexane/di-

TABLE 1

Fatty Acid Composition of the Baking Margarine[a]

Fatty acid	Relative peak area (%)
C16:0	36.1
C18:1 (*cis*)	33.6
C18:2	9.6
C18:1 (*trans*)	8.6
C18:0	7.1
C18:3	2.3

[a]Fatty acids occurring in amounts >2% are displayed.

TABLE 2

Amounts of *trans*-4,5-Epoxy-(*E*)-2-Decenal (ED) Formed from Several Precursors

Experiment	Precursor[a]	ED (μg/100 μmol precursor)
1	Methyl linoleate	1.9
2	Methyl 13-hydroperoxy-(*Z,E*)-9,11-octadecadienoate (13-HPOD)	18.6
3	13-HPOD[b]	43.9
4	13-HPOD[c]	5.2
5	Methyl 12,13-epoxy-9-hydroperoxy-(*E*)-10-octadecenoate	49.0
6	Methyl 9-hydroperoxy-(*E,Z*)-10,12-octadecadienoate (9-HPOD)	27.0
7	(*E,E*)-2,4-Decadienal	128.4[d]
8	(*E,E*)-2,4-Decadienal	35.5

[a]The precursor (100 μmol) dissolved in trioctanoate (16 g) was heated for 7 min at 150°C in a closed vessel.
[b]$FeSO_4$ (2 mg) and water (300 μL) were added.
[c]*Tert.* butyl hydroxyanisol (25 mg) was added.
[d]9-HPOD (80 μmol) was added prior to heating. The amounts liberated from the added 9-HPOD were subtracted.

ethyl ether, 100 + 0.3 by volume; 2 mL/min) was monitored at 230 nm with an SP-4 UV-detector (Gynkotek, Munich, Germany).

Model experiments; quantification of trans-*4,5-epoxy-(E)-2-decenal.* Each of the precursors given in Table 2 and each of the fractions obtained by separation of the margarine as described above was dissolved in trioctanoate and then heated for 7 min at 150°C in a closed vessel. After cooling, diethyl ether (80 mL), containing (^{2}H)-*trans*-4,5-epoxy-(*E*)-2-decenal (10 μg), was added, and the flavor compound and the internal standard were isolated by high-vacuum sublimation (17). ED was then quantitated by a stable-isotope dilution assay with a 8230 mass spectrometer (MS) (Finnigan, Bremen, Germany) running in the chemical ionization (CI) mode with isobutane as the reactant gas (MS/CI) (12).

Characterization of the precursors; capillary gas chromatography (HRGC)/MS. Fraction **IIb**, which gave a positive reaction with potassium iodine/starch, was reduced with triphenylphosphine (9), the hydroxy-triacylglycerides formed were then converted into methyl esters by treatment with sodium methylate (18) and silylated (9). The derivatives were analyzed by HRGC on a silicone SE-54 column (25 m × 0.32 mm fused-silica capillary column; Fa. Machery and Nagel, Düren, Germany) by the on-column injection technique at 35°C. An initial hold at 35°C for 2 min was followed by temperature programming to 150°C and then to 260°C at 8°C/min, with a final hold at 260°C. The peak areas, calculated by using an integrator, were not corrected for response factors. For the identification experiments, the capillary column was coupled with the MS 8230 (Finnigan) running in the electron impact (EI) mode at 70 eV (MS/EI).

Peroxide values. Hydroperoxides were quantitated with the Fe(II)-test (19) by using a standard curve obtained with known amounts of 13-HPOD (13).

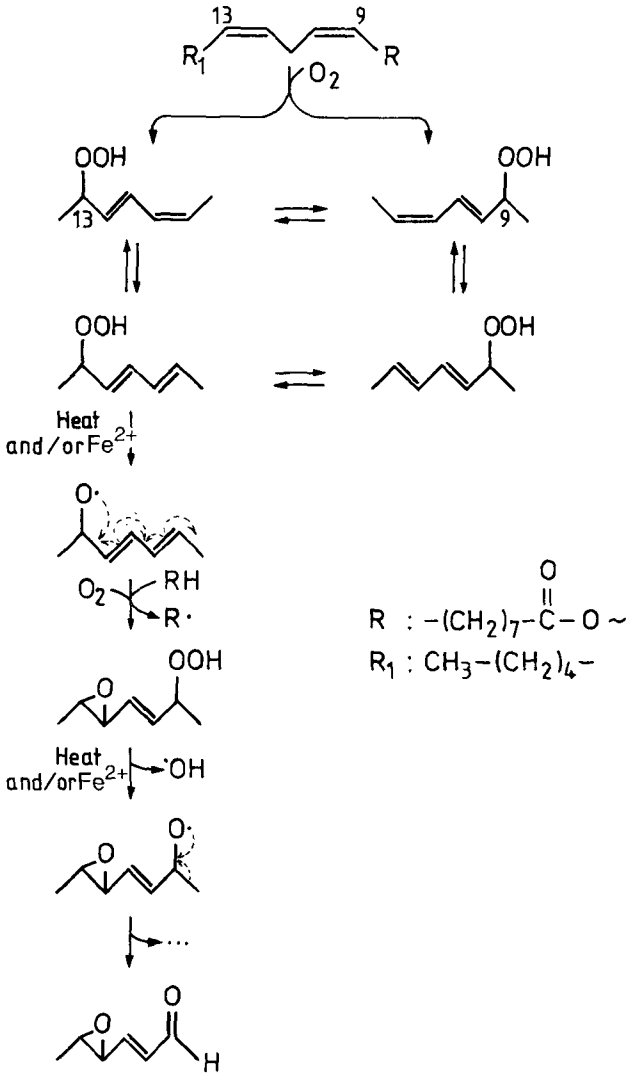
RESULTS AND DISCUSSION

Model experiments. ML, 13-HPOD and 12,13-EP-9-HPOD were thermally degraded in a model system, and the amounts of ED formed were measured by a stable-isotope dilution assay. The results summarized in Table 2 revealed that only low amounts of the odorant were formed from ML (Experiment 1) and trilinolein (data not shown). Substitution of ML by 13-HPOD or 12,13-EP-9-HPOD increased the yields of the epoxydecenal by factors of ten or twenty-five, respectively (Experiments 1, 2 and 5, Table 2). The data confirmed especially the 12,13-EP-9-HPOD as an effective precursor of the epoxydecenal (7) and were in good agreement with the reaction pathway leading from linoleic acid to ED *via* 13-HPOD as the intermediate (Scheme 1). Because 12,13-EP-9-HPOD was closest to the epoxydecenal, lower amounts of this precursor were consumed by side reactions compared to 13-HPOD or ML, respectively.

Additions of Fe(II)-ions increased the formation of epoxydecenal from 13-HPOD by a factor of more than two (*cf.* Experiments 2 and 3, Table 2). Formation of 12,13-EP-9-HPOD from 13-HPOD as well as the formation of ED from 12,13-EP-9-HPOD requires the cleavage of the hydroperoxy group into an alkoxyradical (*cf.* Scheme 1). Because Fe(II)-ions initiate this cleavage of the hydroperoxy group (8), the results are a further confirmation of the reaction pathway shown in Scheme 1. Additionally, in the presence of an antioxidant, much lower amounts of the ED were formed from 13-HPOD (*cf.* Experiments 2 and 4, Table 2).

In a further model experiment, the amounts of ED formed by thermal degradation of 9-HPOD were determined. Comparatively higher amounts of the epoxydecenal were formed from 9-HPOD than from the 13-HPOD (*cf.* Experiments 2 and 6, Table 2). It is known that, during heat treatment, 9-HPOD isomerizes into 13-HPOD (15), which in turn liberates the epoxydecenal (*cf.* Scheme 1). However, this pathway does not explain the comparatively higher yields obtained from 9-HPOD.

A specific degradation product of 9-HPOD is 2,4-decadienal (20). To gain an insight into the extent of isomerization of the hydroperoxides, the amounts of 2,4-decadienal formed during thermal treatment of 13-HPOD and 9-HPOD were compared (Table 3). The results showed that more than seven times higher amounts of the decadienal were formed from 9-HPOD, indicating that a rearrangement of the hydroperoxy group was negligible under our reaction conditions. To explain the favored formation of the epoxydecenal from 9-HPOD, it might, therefore, be assumed that oxidation of the γ-double bond in 2,4-de-



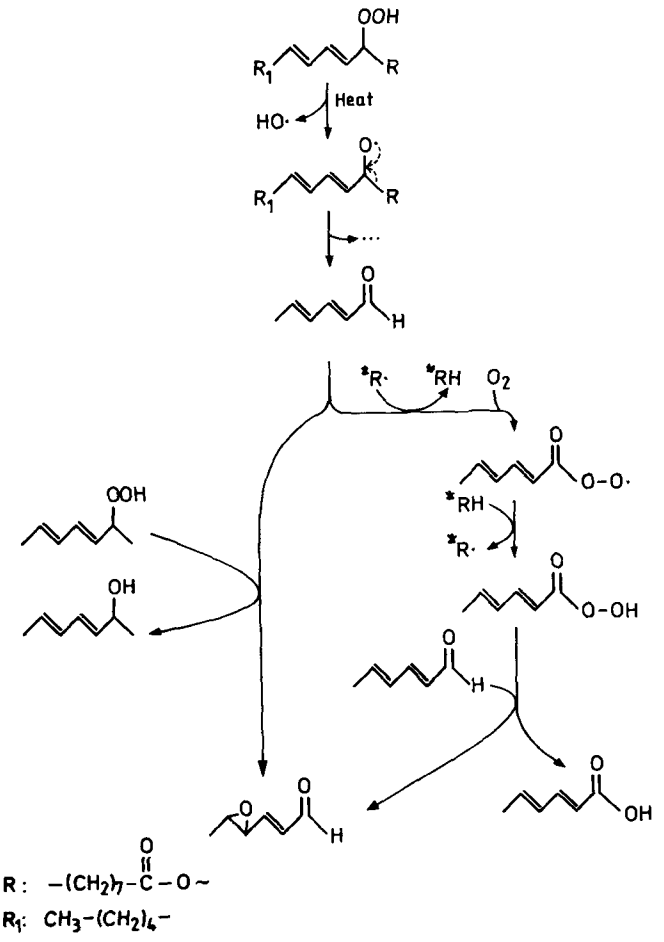
SCHEME 1

TABLE 3

Amounts of (*E,E*)-2,4-Decadienal Formed from 9-HPOD and 13-HPOD[a]

Precursor	(*E,E*)-2,4-Decadienal[b] (μg/mmol)
9-HPOD	1824
13-HPOD	243

[a]The precursor (100 μmol) was dissolved in trioctanoate (16 g) and heated for 7 min at 150°C in a closed vessel. See Table 2 for abbreviations.
[b]The decadienal was determined by a stable-isotope dilution assay (Ref. 12).



SCHEME 2

cadienal by, e.g., hydroperoxides, in analogy to the well-known *Prilezhaev* reaction (21) would result in the formation of epoxydecenal. To clarify the role of the dienal in the formation of the epoxydecenal, (*E,E*)-2,4-decadienal was thermally treated in the presence and absence of hydroperoxides (9-HPOD was added), and the amounts of the ED formed were measured. The results indicated that heating of 2,4-decadienal in the presence of 9-HPOD (Experiment 7, Table 2) yielded nearly five times higher amounts of the epoxydecenal than from 9-HPOD itself. These results established that the epoxydecenal was indeed formed from 9-HPOD *via* 2,4-decadienal as the intermediate, as suggested on the left side of Scheme 2; reaction pathway leading from 9-HPOD to ED. Because ED was already generated in significant amounts singly by heating 2,4-decadienal alone (Experiment 8, Table 2), it might be speculated that traces of unknown peroxides, possibly occurring in the 2,4-decadienal used in the experiments, initiated the formation of further peroxides, such as the peroxy decadienoic acid, which then oxidized another molecule of the unsaturated aldehyde (Scheme 2, right side).

Precursors in margarine. In a previous investigation (10), we found that pastry prepared with margarine formed significant amounts of ED during baking. To investigate the margarine as the source of the odorant, we compared the changes in the concentrations of ED and, additionally, 2,4-decadienal, which are induced by heating of the margarine itself. Data showed that heat treatment increased ED in the margarine by 38 times, indicating a significant amount of its precursors in the fat (Table 4).

TABLE 4

Amounts of ED and 2,4-Decadienal Isomers Present in the Margarine Before and After Heating

Odorant	Amount (μg/kg)	
	Before	After heating[a]
ED	30	1130
2,4-Decadienal	219	21300[b]

[a]The margarine (10 g) was heated for 7 min at 150°C. The quantitation of the odorants was performed by a stable-isotope dilution assay as recently described (Ref. 7). See Table 2 for abbreviation.
[b]The sum of the *E,E*- and *E,Z*-isomers is given.

In addition, 2,4-decadienal showed a more than ninetyfold increase during the thermal treatment (Table 4). Similar data were found for two different batches of margarines (data not shown). To localize the precursors of ED, the margarine was fractionated by column chromatography, followed by TLC. Each fraction was made up to the same weight (10 g) by adding the respective amounts of trioctanoate. After heating, the amount of ED formed was determined. The results, summarized in Table 5, indicate that Fraction **IIb** showed by far the highest precursor activity. Compared with the control, a 115-fold enrichment of the precursors could be achieved (*cf.* Experiments 1 and 5, Table 5).

The fraction was further separated by HPLC. Figure 1 shows that no complete separation could be achieved. Because the FE-test (16) indicated the presence of peroxides in all four subfractions (1 to 4, Fig. 1), the compounds eluting between 11 and 22 min were combined, treated with triphenylphosphine to reduce the peroxides, and then the hydroxy-triacylglycerides formed were converted into the corresponding methyl esters. After silylation, the derivatives were analyzed by HRGC and HRGC/MS. The results revealed (data not shown) that Fraction **IIb** consisted of methyl palmitate, methyl stearate, methyl oleate and ML (74%) and a mixture of methyl 9-trimethylsiloxy- and 13-trimethylsiloxyoctadecadienoates (26%). The latter were identified by comparing their mass spectra with literature data (22). From these results, it can be calculated that about 80% of the triacylglycerides present in Fraction **IIb**, contained one molecule of either 9-HPOD or 13-HPOD. Assuming that these acids are the precursors of ED in Fraction **IIb**, it can be roughly calculated that

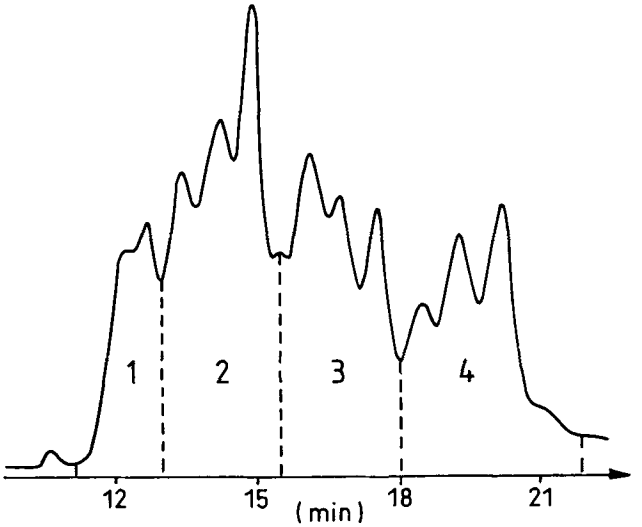


FIG. 1. High-performance liquid chromatography-separation of Fraction IIb (*cf.* Table 5), which contain the precursors of *trans*-4,5-epoxy-(*E*)-2-decenal.

from about 23 mg of 9- and 13-HPOD present in this fraction (26% of 90 mg; *cf.* Table 5), 10.3 μg of ED were generated (no. 5, Table 5). Because, in the model experiments, pure methyl 13- or 9-HPOD liberated the ED in the same concentration level (13.7 μg or 19.7 μg per 23 mg, respectively, *cf.* Experiments 2 and 6, Table 2), both hydroperoxides can be regarded as the main precursors of ED in the margarine.

Further experiments revealed that the amounts of ED formed during the heating of different margarines were not correlated with the amounts of linoleic acid present in the triacylglycerides (data not shown). Recently we demonstrated that during the baking of fat-rich products, the epoxydecenal is only formed if the hydroperoxides are already present in the baking fat (23). These data imply that the hydroperoxidation of linoleic acid during the baking process is only of minor importance in the generation of the epoxydecenal.

The potent flavor compound *trans*-4,5-epoxy-(*E*)-2-decenal was formed from both 13-HPOD and 9-HPOD, which arose in equal amounts from peroxidation

TABLE 5

Amounts of ED Liberated by Heating Fractions of a Margarine[a]

Experiment	Fraction	ED (μg/10 g of margarine)	Fraction[b] weight (g)	ED (μg/10 g of fat fraction)
1	Unfractionated (control)	9.9	10	9.9
2	**I**	2.9	9.20	3.1
3	**II**	9.8	0.80	122.5
4	**IIa**	2.6	0.65	39.4
5	**IIb**	10.3	0.09	1144.4
6	**IIc**	2.5	0.06	416.7

[a]The margarine contained 9.6% of linoleic acid (*cf.* Table 2). See Table 2 for abbreviation.
[b]The fractions were made up to 10 g by adding trioctanoate and then heated for 7 min at 150°C in a closed vessel.

of triacylglycerides that contained linoleic acid (20), but the latter was found to be the more effective precursor. In model studies, 2,4-decadienal and 12,13-epoxy-9-hydroperoxy-10-octadecenoic acid were established as the key intermediates in the thermally induced formation of the odorant. Because glycerine-bound 9- and 13-HPOD have been identified as the precursors of the epoxyaldehyde in margarine, it can be assumed that the flavor compound will undoubtedly be formed during heat treatment of fat-rich products, such as pastries, if these precursors are already present in the raw material.

REFERENCES

1. Selke, E., W.K. Rohwedder and J.H. Dutton, *J. Am. Oil Chem. Soc. 57*:25 (1980).
2. Schieberle, P., and W. Grosch, *Z. Lebensm. Unters. Forsch. 192*:130 (1991).
3. Guth, H., and W. Grosch, *Lebensm. Wiss. Technol. 23*:59 (1990).
4. Konopka, U.C., and W. Grosch, *Z. Lebensm. Unters. Forsch. 193*:123 (1991).
5. Schieberle, P., *J. Agric. Food Chem. 39*:1141 (1991).
6. Schieberle, P., *Progress in Flavour Precursor Studies*, edited by P. Schreier, and P. Winterhalter, Allured Publishing Corporation, Carol Stream, 1993, p. 343.
7. Gardner, H.W., and E. Selke, *Lipids 19*:375 (1984).
8. Gardner, H.W., D. Weisleder and R. Kleiman, *Ibid. 13*:246 (1978).
9. Schieberle, P., Y. Trebert, J. Firl and W. Grosch, *Chem. Phys. Lipids 37*:99 (1985).
10. Gassenmeier, K., and P. Schieberle, *Lebensm. Wiss. Technol. 27*:282 (1994).
11. Swoboda, P.A.T., and K.E. Peers, *J. Sci. Food Agric. 29*:803 (1978).
12. Guth, H., and W. Grosch, *Lebensm. Wiss. Technol. 23*:513 (1990).
13. Schieberle, P., B. Tsoukalas and W. Grosch, *Z. Lebensm. Unters. Forsch. 168*:448 (1979).
14. Grosch, W., *Ibid. 163*:4 (1977).
15. Chan, H.W.-S., and F.A.A. Prescott, *Biochim. Biophys. Acta 380*:141 (1975).
16. Schieberle, P., Trebert, Y., J. Firl and W. Grosch, *Chem. Phys. Lipids 48*:281 (1988).
17. Guth, H., and W. Grosch, *Fat Sci. Technol. 90*:225 (1989).
18. Christie, W.W., *Lipid Analysis*, 2nd edn., Pergamon Press, Oxford, 1982, p. 53.
19. Stine, C.M., H.A. Harland, S.T. Coulter and R. Jeness, *J. Dairy Sci. 37*:202 (1954).
20. Grosch, W., *Autoxidation of Unsaturated Lipids*, edited by H.W.-S. Chan, Academic Press, London, 1986, pp. 95–139.
21. Dryuk, V.G., *Tetrahedron 32*:2855 (1976).
22. Kleiman, R. and G.F. Spencer, *J. Am. Oil Chem. Soc. 50*:31 (1973).
23. Schieberle, P., and K. Gassenmeier, *Proceedings of the 4th Wartburg Aroma Symposium*, Eisenach, Germany, 1995, in press.

[Received February 11, 1994; accepted September 8, 1994]