Volatile Compounds Produced by Irradiation of Butterfat

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Abstract

Anhydrous butterfat was irradiated at 6 megarads in a special glass reaction flask, and the headspace and total condensate samples were examined by wide-range (−180C to 125C) gas chromatography combined with mass spectrometry. The nature and amounts of volatile compounds were not greatly influenced by whether the butterfat was irradiated under oxygen or under vacuum, nor (apart from *n*-alkanoic acids) by storage for 1 and 9 weeks.

Carbon dioxide was produced in greatest amount. Of the remaining compounds, aliphatic hydrocarbons predominated both in number and amount. Aliphatic oxygenated compounds including carbonyl compounds were isolated in relatively small amounts. The following compounds were identified positively: C_{1-13} *n*-alkanes; C_{4-9} 2-methylalkanes; C_{2-14} alk-l-enes; C_{2-9} alk-l-ynes; C_{2-5} *n*-alkanoic acids; C_{1-5} *n*-alkan-l-ols and propan-2-ol; C_{2-8} *n*-alkanals and 2-methylbut-2-enal; $C_{3-5,7}$ alkan-2-ones; C_{1-4} *n*-alkyl formates, vinyl and isoamyl formate, methyl acetate and methyl *n*-hexanoate; and carbon dioxide.

Introduction

INVESTIGATIONS of the volatile compounds produced during the irradiation of foods have been directed toward the determination of their roles in flavor and breakdown patterns. The complexity of the systems studied varies greatly and the results are dependent on many experimental factors. Meat has one of the more complex systems (6,8,9). Merritt and co-workers have identified carbonyls, sulfur compounds and hydrocarbons in irradiated beef, pork, mutton, and other meats. The hydrocarbons were attributed to free radicals formed from the lipids and the sulfur compounds to those formed from proteins (6,7). Monty et al. (11) suggested that long chain aliphatic aldehydes and ketones isolated from irradiated meats arose from hydrolysis of plasmalogens and other lipids rather than by oxidative breakdown, but this hypothesis is not supported by Merritt et al. (6) who have found both aldehydes and ketones in dry lipids irradiated under vacuum.

Milk fat has a simpler system than meat since the volatiles are derived entirely from lipid. Day and Papaioannou (3) isolated a large number of *n*-alkanals and alkan-2-ones from irradiated milk fat. More recently, Khatri, Libbey and Day (5) again irradiated milk fat and analyzed an ether extract of the steam volatiles by combined gas chromatography and mass spectrometry. Alkanes and alkenes up to C_{17}, *n*-alkanals, *n*-alkanones, and γ- and δ-lactones were identified.

Pure fatty acids or their esters provide data most amenable to interpretation. The formation of hydrocarbons and of aliphatic esters from methyl stearate, methyl oleate, tristearin and triolein under vacuum has been described by Merritt and his colleagues (6,7,9). Chipault and Mizuno (2), on the other hand, have studied the accumulation of peroxides, carbonyl compounds and reducing substances during irradiation of pure methyl myristate and methyl linoleate. Under vacuum, preformed hydroperoxides in methyl linoleate were destroyed whereas under oxygen, one-eighth of the peroxides formed arose from the direct reaction of irradiation-induced free radicals with oxygen while the rest was formed through a chain mechanism. Under oxygen, methyl myristate gave peroxides and carbonyl compounds.

In lipid systems carbonyl compounds have been regarded as the main contributors to radiation flavor. However, the knowledge of the presence and amounts of hydrocarbons and other compounds would contribute to our understanding both of irradiation flavor and mechanisms.

Experimental

The butterfat used in this study was taken from the lot used for the oxidation study (4). The same controls were used as in the oxidation study since these experiments were concurrent. Four 200 g samples were irradiated (6 megarads) in reaction flasks as previously described (1) and then placed in storage. Two samples, one with oxygen and one without oxygen, were analyzed for volatiles after a 1-day storage period. Analyses of volatiles from the remaining two samples, one with oxygen and one without oxygen, were carried out after a one- and a nine-week storage period, respectively. Aliquots of the headspace and total condensate samples were analyzed by wide-range programmed temperature gas chromatography and mass spectrometry (1,10).

Results

Gamma irradiation caused bleaching of the butterfat and destruction of the fresh butter odor. Many

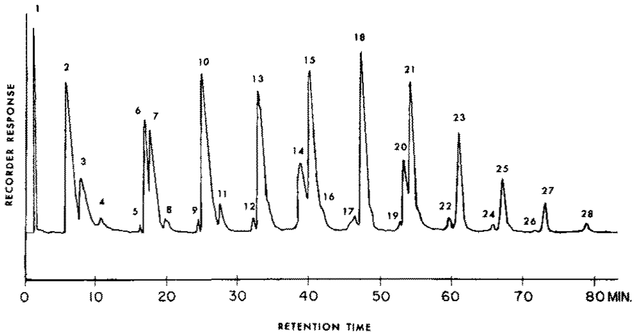

FIG. 1. Gas chromatogram of volatile compounds isolated from butterfat irradiated under vacuum. Flame ionization detector; Column 6 ft × ¼ in. O.D. 5% Carbowax 20M on 60–80 mesh C-22 firebrick, Temperature programmed from −80C to +80C at 2C/min. Carrier gas He at 40 ml/min.

Compounds identified: 1) Ethane, Ethene. 2) *n*-Propane. 3) Propene. 4) Ethyne. 5) 2-Methylpropane. 6) *n*-Butane. 7) But-l-ene. 8) Propyne. 9) Pent-l-ene. 10) 2 Methylbutane, *n*-Pentane. 11) But-l-yne. 12) Hex-l-ene. 13) Propanal, 2-Methylpentane, *n*-Hexane. 14) Hept-l-ene, 2-Methylhexane. 15) *n*-Heptane. 16) Butanal, Butanone, Ethanol. 17) Oct-l-ene, 2-Methylheptane. 18) *n*-Octane. 19) 2-Methyloctane. 20) Non-l-ene. 21) *n*-Nonane. 22) Dec-l-ene. 23) *n*-Decane. 24) Oct-l-yne, Undec-l-ene. 25) *n*-Undecane. 26) Dodec-l-ene. 27) *n*-Dodecane. 28) *n*-Tridecane.

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TABLE I

Volatile Compounds from Irradiated Butterfat

S — Small M — Moderate L — Large

	Stored under vacuum		Stored under oxygen	
	1 day	9 weeks	1 day	1 week
n-Alkanes				
Methane	M	M	S	S
Ethane	L	L	M	M
n-Propane	L	L	L	L
n-Butane	L	L	L	L
n-Pentane	L	L	L	L
n-Hexane	L	L[a]	L	L
n-Heptane	L	L	L	L
n-Octane	L	L	L	L
n-Nonane	L	L	L	L
n-Decane	L	L	L	L
n-Undecane	L	L	S	S
n-Dodecane	M	L	S	S
n-Tridecane	S	M		
2-Methylalkanes				
2-Methylpropane	M	M	S	M
2-Methylbutane	L	L	M	M
2-Methylpentane	L	L	M	M
2-Methylhexane	S	M	M	M
2-Methylheptane	S	M	M	M
2-Methyloctane	S	S	S	
n-Alk-l-enes				
Ethene	M	M	M	M
Propene	L	L	L	L
But-l-ene	L	L	L	L
Pent-l-ene	M	L	M	M
Hex-l-ene	M	L	M	L
Hept-l-ene	L	L	S	S
Oct-l-ene	M	M	M	M
Non-l-ene	L	L	L	L
Dec-l-ene	M	M	M	M
Undec-l-ene	S	S	M	S
Dodec-l-ene	S	S		
Tridec-l-ene	S	S		
Tetradec-l-ene		S		
n-Alk-l-ynes				
Ethyne	M	M	M	M
Propyne	M	M	M	M
But-l-yne	M	M	S	S
Pent-l-yne	S	S	M	M
Hex-l-yne	S	S	S	S
Hept-l-yne	S	S	S	S
Oct-l-yne	S	S	S	S
Non-l-yne	S	S		S
n-Alkanoic Acids				
Ethanoic	S	M	S	M
Propanoic	S	M	S	M
n-Butanoic	S	L	S	L
n-Pentanoic	S	M	S	M
Alkanols				
Methanol	S	S		S
Ethanol	S	S		S
Propan-l-ol		S	S	S
Propan-2-ol	S	S	S	S
Butan-l-ol	S	S		S
Pentan-l-ol		S	S	
Aliphatic Aldehydes				
Ethanal	S	S	S	S
Propanal	S	S	S	S
Butanal	S	S	S	S
2-Methylbut-l-enal		S		S
n-Pentanal		S		S
n-Hexanal		S		S
Hex-2-enal		S	S	
n-Heptanal	S	S	S	S
n-Octanal		S		
Alkan-2-ones				
Propanone	S	S	S	S
Butanone		S	S	S
Pentan-2-one	S	S	S	
Heptan-2-one		S		
Esters				
Methyl formate			S	S
Ethyl formate				S
Vinyl formate	S			
n-Propyl formate		S	S	S
n-Butyl-formate		S		S
Isoamyl formate				S
Methyl acetate	S	S	S	S
Methyl *n*-hexanoate		S		
Others				
Carbon dioxide	L	L	L	L
Oxygen			L	L

[a] Estimated from gas chromatographic data, 100 μM/kg.

TABLE II

Compounds Tentatively Identified from Irradiated Butterfat

Pent-2-ene
2-Methylbut-l-ene
2-Methylpent-l-ene
3-Methylpent-l-ene
Hex-2-ene
2-Methylhex-l-ene
3-Methylhex-l-ene
Oct-2-ene
Oct-4-ene
Non-2-ene
n-Hexanoic acid
2-Methylbutan-l-ol
2-Methylpropanal
2-Methyl-*n*-butanal
3-Methyl-*n*-hexanal
Non-2-enal
Heptan-3-one

volatile compounds were isolated. The chromatogram in Fig. 1 shows the compounds produced in largest amounts by gamma irradiation of butterfat under vacuum. Hydrocarbons were produced far in excess of any other class of compounds. Table I summarizes the qualitative and semiquantitative analyses of the volatiles produced by irradiation of butterfat, under vacuum and oxygen at different storage periods. Table II lists compounds which could not be positively identified for one or more of the following reasons: 1) interfering mass spectra of more abundant compounds; 2) incomplete chromatographic separation; 3) unavailability of reference mass spectra; or 4) insufficient amounts.

Carbon dioxide was produced in relatively large quantities both under vacuum and under oxygen and constituted the most abundant single component isolated. Aliphatic hydrocarbons were produced in the greatest abundance. Relatively large amounts (100 μM[2] *n*-hexane/kg) of C_{3-10} *n*-alkanes were produced in all irradiated samples. Ethane, and C_{4-7} 2-methylalkanes were found in moderate amounts. $C_{1,11-13}$ *n*-alkanes and 2-methyloctane were found in small amounts. Irradiation produced appreciable amounts of C_{2-10} alk-l-enes, but in general not as much as the corresponding alkanes. The C_{2-9} alk-l-ynes were produced in moderate and small amounts, but each one correspondingly less than the alk-l-ene with the same carbon number.

C_{2-5} fatty acids were found in all irradiated samples and the amounts increased on storage. C_{1-5} *n*-alkan-l-ols and propan-2-ol, C_{2-8} *n*-alkanals and 2-methylbut-2-enal, $C_{3,4,5,7}$ alkan-2-ones and eight esters were also isolated. The number of compounds in most classes increased with storage, and the presence of oxygen did not appear to affect their production.

Small amounts of chlorinated hydrocarbons and substituted aromatics were found in both control and irradiated samples. These are probably contaminants in the fat resulting from exposure to insecticides, refrigerants and/or insecticide metabolites.

Discussion

The predominating amounts of hydrocarbons produced by irradiation of butterfat suggests formation of hydrocarbon free radicals from alkyl chains as the primary mechanism involved. This mechanism is supported by the results of studies of the irradiation of pure fatty acid esters and triglycerides (6,7). The wide range in chain length of hydrocarbons produced indicates a number of cleavage points. The following scheme can account for the hydrocarbons produced by gamma irradiation. Cleavage may occur at any bond resulting in the formation of free radicals:

$R_1R_2 \rightarrow R_1\cdot + R_2\cdot$ where $R_1\cdot$ is a hydrocarbon radical with 1–20 carbon atoms and $R_2\cdot$ is the corresponding residual triglyceride radical. R_1 and R_2 may be unsaturated if derived from an unsaturated fatty acid such a linoleic. $R_1\cdot$ may abstract hydrogen from the fatty acid chain of a triglyceride, or from $R_2\cdot$ producing a hydrocarbon and a doubly charged

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[2] Estimated from gas chromatographic data.

radical which will change to a vinyl compound. Subsequent cleavage of the hydrocarbon chain of the latter would then lead to the formation of an alk-1-ene. $R_1\cdot$ and $R_2\cdot$ may combine, and the presence of branched hydrocarbons and of nonterminally monounsaturated compounds (Tables I and II) suggests this does occur.

Butterfat contains 35% unsaturated fatty acids including 30% oleic acid. Alka-dienes and alka-trienes might also be expected, but are not found. Alk-1-ynes were found in small amounts. It would appear that scission α to the double bond occurs, followed by disproportionation to the acetylenic compound (13). This is probably due to rearrangement of the dienyl radical since less energy is required to form the triple bond.

Cleavage by gamma radiation seems to be random for all bonds within the molecule and is not selective.

Acids, alcohols, aldehydes, ketones, and esters were produced in much smaller amounts than hydrocarbons. Since no great difference was detectable in compounds produced by irradiation of butterfat in vacuo or under oxygen, it appears that formation of peroxy free radicals by reaction of alkyl radicals with oxygen is a minor reaction. Acids, alcohols, aldehydes, and ketones could have been produced by cleavage of the ester linkages followed by reactions with other free radicals or molecules. Alternatively these compounds could also arise from decomposition of any hydroperoxides present in the butterfat before radiation. Cleavage of enol-ether linkages (12) which bind some aldehydes to glycerol in butterfat could also produce aldehydes. Esters could be formed by reactions of acyl and alkoxy free radicals. However, the absence of formic acid and the fact that the majority of esters found are formates suggests some other mechanism for formation of most of the esters.

The carbon dioxide formed in such large amounts could have been derived from cleavage of the alkoxyl linkage followed by decarboxylation.

Compounds belonging to all the above classes but with boiling points above 250C would be expected to be present in the irradiated butterfat, but they could not be separated and identified by the method employed.

A comparison of the volatiles isolated from gamma irradiated butterfat with those found in oxidized butterfat (4) shows a much higher degree of specificity in the point of chain breakdown by oxidation. This is consistent with the mechanisms discussed in this paper and those postulated for copper-catalyzed oxidation (4).

It can be concluded that although both gamma-irradiation and autoxidation cause degradation of fats by a free radical mechanism the types of free radical differ. Gamma-irradiation produces free radicals of random chain length followed by hydrogen abstraction, by recombination and possible exchange giving major amounts of hydrocarbons. Since this reaction proceeds so fast, the presence of oxygen, limited by its solubility in the fat, has little effect on the degradation products of gamma-irradiated fats. On the other hand, copper-catalyzed oxidation of butterfat is much slower, allowing oxygen consumed in solution reactions to be replenished from headspace oxygen. This gives rise to mainly carbonyl compounds whose specific nature depends on the position of the double bond in the alkyl chain. In the absence of oxygen, scission of preformed hydroperoxides may produce hydrocarbons. A limited amount of hydrocarbons may also be formed by random reactions of alkyl free radicals with themselves or with hydrogen. Fat degradations by autoxidation and irradiation result in completely different ranges of products formed. The main difference, the relative amounts of *n*-alkanes and *n*-alkanals, is shown in Fig. 2.

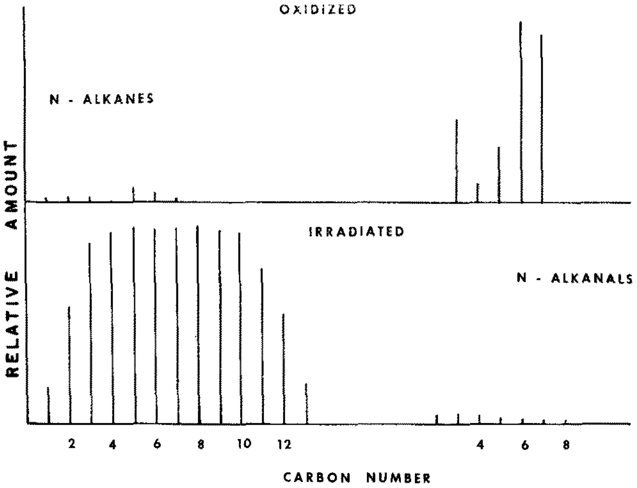

FIG. 2. Distribution of alkanes and alkanals among volatile compounds from treated butterfat.

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