

Thermal Decomposition of Capsaicin. 1. Interactions with Oleic Acid at High Temperatures

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The thermal decomposition of capsaicin alone and in the presence of oleic acid was studied by GC-MS. Decomposition products of capsaicin included vanillin, methylnonenoic acid, and methylnonenamide. In mixture with oleic acid, two unique products, 9-octadecenamide and *N*-vanillyl-9-octadecenamide, are formed. The formation of expected oxidative decomposition products of oleic acid is suppressed by the presence of capsaicin.

INTRODUCTION

The goal of this study was to understand the chemical changes that occur during the process of cooking with capsaicin, the principle hot flavor in chili peppers. Specifically, the decomposition at high temperatures of capsaicin alone and in mixtures with oleic acid was investigated in the presence of air. The decomposition products were determined by coupled gas chromatography-mass spectrometry (GC-MS).

Capsaicin (CAP) is the active ingredient largely responsible for the irritating and pungent effects of hot pepper, sometimes referred to as red pepper or chili pepper. This spice is widely used in the preparation of curries, hot sauces, and Mexican and Asian cuisines. Recently, the popularity of these cuisines has increased. This is due in part to the desire to reduce fats in the diet by substituting foods that do not depend upon fat for their flavor and appeal.

The structure of CAP has been established as 8-methyl-*N*-vanillyl-6-nonenamide (Nelson, 1919; Nelson and Dawson, 1923) and is shown in Figure 1. In addition, it has been shown that natural chili extracts contain a series of closely related analogues which include dihydrocapsaicin (DC), nordihydrocapsaicin (NDC), homocapsaicin (HC), and homodihydrocapsaicin (HDC) (Benett and Kirby, 1968; Masada et al., 1971). The analogues vary in the length of the hydrocarbon chain and the degree of unsaturation.

Previous studies of capsaicin and its analogues have focused on its neurochemical and pharmacological effects (Virus and Gebhart, 1979; Buck and Burks, 1986). The separation and detection of CAP and its analogues by various chromatographic methods have been reported by a number of investigators (Iwai et al., 1979; Law, 1983; Weaver and Awde, 1986; Wood, 1987; Attuquayefio and Buckle, 1987; Krajewska and Powers, 1987; Cooper et al., 1991). However, there are no studies reported in the literature on the effects of heat on CAP. This study investigates the effects of cooking temperatures on CAP and discusses the products that result from that operation.

MATERIALS AND METHODS

Reagents. CAP, DC, oleic acid, linoleic acid, and linolenic were purchased from Sigma Chemical Co. (St. Louis, MO). HPLC grade dichloromethane (Fisher Scientific, Springfield, NJ) was used as a solvent for the heated samples. Methyl eicosanoate,

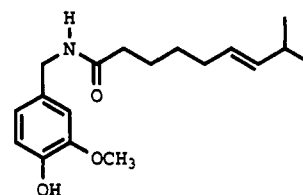


Figure 1. Structure of capsaicin.

used as the internal standard, was purchased from Analabs (North Haven, CT). Standard mixtures of hydrocarbons, acids, and aldehydes were purchased from Alltech Associates (Deerfield, IL). Vanillin, 2-methoxyphenol, and 2-methoxy-4-methylphenol were purchased from Aldrich Chemical Co. (Milwaukee, WI). Amides were synthesized from standard acids.

Heat Treatment. Samples were heated using three types of procedures described as follows: (a) A standard distillation apparatus was connected to a vacuum pump at pressures of 100 Torr. Dry air was admitted into the reaction flask to maintain the pressure and to sweep volatile products into the collection flask, which was cooled in liquid nitrogen. (b) A Standard distillation apparatus was run at atmospheric pressure. In each of the above samples, the materials were placed in a glass round-bottom flask and heated to about 200 °C for 2 h using a sand bath. (c) The third method consisted of heating the materials in Pyrex test tubes (9 mm o.d. × 160 mm) sealed with Teflon ferrules in a stainless steel compression plug (Parker-Hannifin, Huntsville, AL) in a 200 °C oven for 2 h.

Samples from the low-pressure decomposition were initially divided into two parts for analysis. The sample from the liquid nitrogen trap was dissolved in dichloromethane and dried over anhydrous sodium sulfate. The residue in the three-neck flask was also dissolved in dichloromethane. Since subsequent analysis indicated that both the volatile and residue fractions contained substantially the same compounds, the fractions were combined for quantitative studies. The atmospheric pressure samples were simply dissolved in dichloromethane. All samples were then subjected to GC-MS analysis. For purposes of quantitation, 10.0 mg of the internal standard, methyl eicosanoate, was added to the samples from the distillation apparatus, and they were brought to a volume of 10.0 mL. The samples from the sealed tube reactions were spiked with 1.0 mg of internal standard and were diluted to 1.00 mL final volume with the solvent. The amounts of oleic acid and CAP in each sample are shown in Table I.

Quantitative analysis of chromatograms was carried out by integrating the total ion chromatogram. The peak areas of the individual components were divided by the area of the internal standard peak and converted to percent. These were corrected for intersample comparisons to reflect the amounts of initial

Table I. Sample Composition for Oleic Acid-Capsaicin Studies

sample	oleic acid, mL	capsaicin, mg
low-pressure oleic	2.0	0
low-pressure 10% CAP	0.20	20
low-pressure 50% CAP	0.20	200
low-pressure CAP	0	100
atm pres oleic	0.20	0
atm pres 50% CAP	0.20	200
atm pres CAP	0	100
sealed tube 30% CAP	0.010	3.55
sealed tube 50% CAP	0.010	10.34

products shown in Table I (e.g., the peak areas for CAP alone were multiplied by two for comparison with the interaction samples). No corrections were attempted for the variation in response of the total ion trace for the various classes of products found.

Instrumentation. A Trio 2 MS system (VG Instruments, Danvers, MA) was directly interfaced to a Hewlett-Packard 5890 gas chromatograph. LABBASE software (VG Instruments) was used for all data manipulation and quantitation. The LABBASE software includes the NBS Library of Standard Spectra, which was used for identification. The MS was calibrated with perfluorotributylamine and operated in electron impact and chemical ionization modes. Methane and ammonia reagent gases were used in CI mode for the determination of molecular weights. Four fused silica columns (Restek, Bellefonte, PA) were used for separations. Three were 30 m \times 0.25 mm i.d. The stationary phases were RT_x-5, RT_x-200, and Stabliwax. The fourth column was 15 m \times 0.25 mm i.d. and contained Stabliwax-DA as the stationary phase. All columns had 0.25- μ m film thickness. Carrier gas was helium at 12 and 4 psi for the 30- and 15-m columns, respectively. The GC-MS transfer line was operated at 250 °C. While this was lower than the final column temperature, no peak tailing was observed. Operation at higher temperatures produced no obvious decomposition. The ion source was normally set to 180 °C to obtain reasonable molecular ions for most compounds.

RESULTS AND DISCUSSION

The results of this work indicate that the thermal decomposition of CAP alone and of CAP with oleic acid yields many interesting decomposition products. To carry out this study, it was also necessary to heat the oleic acid alone and compare the decomposition products found in the oil alone with those found in the oil-CAP interaction sample. The products of the decomposition of oleic acid, CAP, and the interaction of oleic acid with CAP are described in the following sections.

Thermal Decomposition of Oleic Acid. The thermal decomposition of oleic acid and other unsaturated fatty acids has been previously studied (Ramanathan et al., 1959;

Nawar, 1969; Henderson et al., 1980). The major products found in this study are similar to those found in earlier studies. Two homologous series of products are formed, acids and aldehydes. Four fatty acids were found: hexanoic, heptanoic, octanoic, and nonanoic. Octanoic acid was the predominant acid, with nonanoic acid present at only a slightly lower concentration. Three aldehydes were found: heptanal, octanal, and nonanal. Identification was based on comparison of mass spectra and retention times with authentic compounds.

The oxidation of unsaturated fatty acids occurs by hydrogen abstraction from α -methylene sites and formation of the hydroperoxides at these positions, as described by Farmer et al. (1942). Thermal breakdown of the hydroperoxides results in hydroxy radicals and alkoxy radicals. The latter then undergo scission of the carbon-carbon bond adjacent to the carbonyl group. The eight-carbon aldehyde, which was found in this study, is an expected product for oleic acid. The formation of the seven- and nine-carbon aldehydes is explained by the formation of hydroperoxides on sites other than the α -methylene position as previously reported in the literature (Krishnamurthy et al., 1967).

Thermal Decomposition of Capsaicin. While there is much information on the thermal decomposition of fatty acids in the literature, there is no evidence of any studies performed on the thermal decomposition of CAP. In this study, a number of major and minor decomposition products were identified. The major decomposition product is thought to be 8-methyl-6-nonenamide (MW 169), a primary amide whose mass spectrum is shown in Figure 2. Two additional products are observed at significant levels: vanillin (MW 152) and a component thought to be 8-methyl-6-nonenic acid (MW 170). The spectrum and retention index of vanillin matched that of the authentic compound. The spectrum of the peak thought to be 8-methyl-6-nonenic acid is shown in Figure 3.

A number of other decomposition products were identified. These include substituted phenols due to the vanillyl moiety and a series of amides all derived from the nonenamide side chain. Nonanoic acid was also observed along with 2-heptene. Authentic compounds were run for a number of compounds as indicated in Table II.

Finally, an additional compound, which has been tentatively identified as *N*-vanillyl-di(8-methyl-6-nonen)-imide (MW 457), was found. This compound could logically be formed by the condensation of CAP with 8-methyl-6-nonenic acid, which is present due to de-

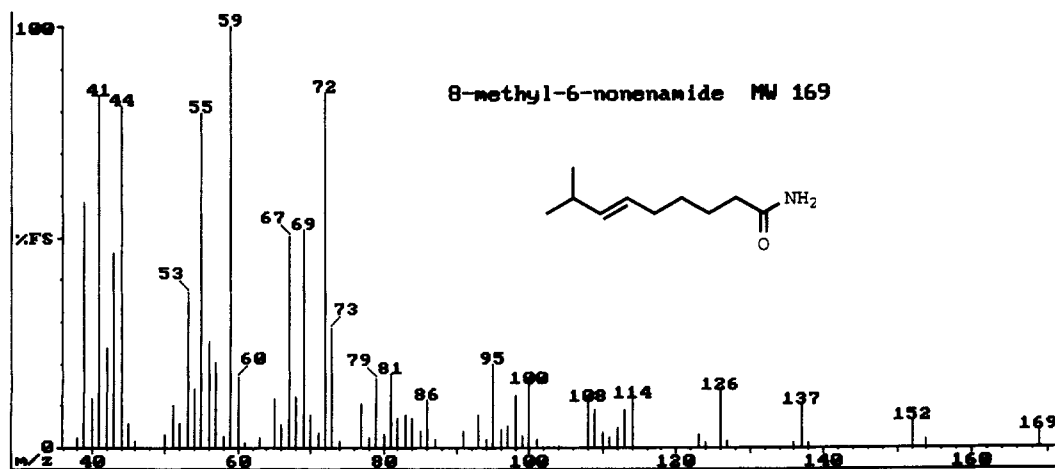


Figure 2. Mass spectrum and tentative structure of 8-methyl-6-nonenamide.

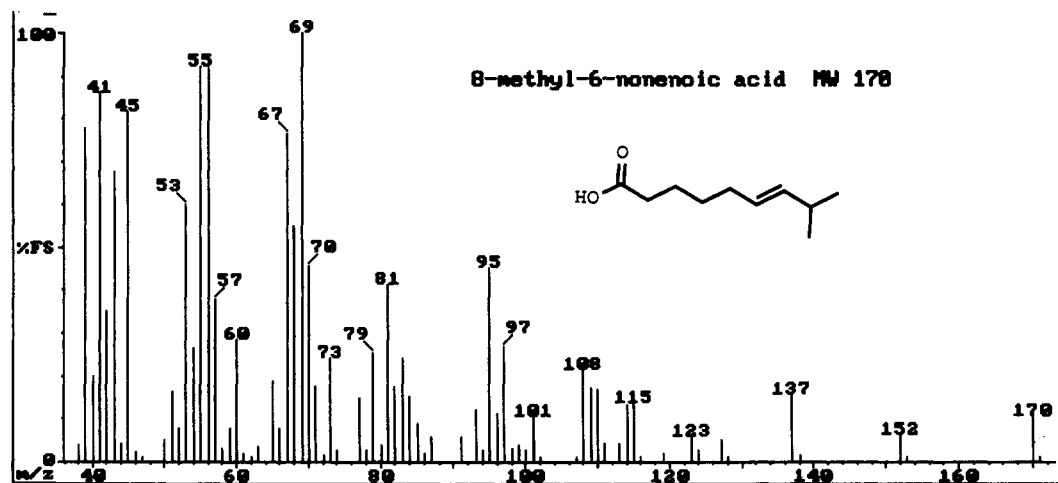


Figure 3. Mass spectrum and tentative structure of 8-methyl-6-nonenic acid.

Table II. Products of Thermal Decomposition of Capsaicin

compd	MW	method of identification	rel % ^a
heptene	98	NBS 1213	<1
2-methoxyphenol	124	authentic compd	<1
2-methoxy-4-methylphenol	138	authentic compd	1-10
pentanamide	101	authentic compd	<1
nonanoic acid	158	authentic compd	1-10
8-methyl-6-nonenic acid	170	interpretation	1-10
isononanamide	157	interpretation	1-10
nonanamide	157	authentic compd	1-10
methylvanillin	166	interpretation	1-10
8-methyl-6-nonenamide	169	interpretation	>10
vanillin	152	authentic compd	>10
N-vanillyl-di(8-methyl-6-nonen)imide	457	interpretation	<1

^a Quantitation of peak areas relative to internal standard.

composition of the CAP. Molecular ions at m/z 457 were confirmed by methane CI-MS. The spectra and structure are shown in Figure 4.

The compounds found and the methods of identification used are summarized in Table II. It is evident from the results presented in Table II that the primary route for the formation of products is by cleavage of the bond between the amide group and the vanillin moiety. This leads to the production of vanillin and 8-methyl-6-nonenamide. The other amides found in this study, including pentanamide, nonanamide, and isononanamide, arise from subsequent cleavage, hydrogenation, and rearrangement of methylnonenamide. In addition, the free

acid, methylnonenoic acid, was also identified in the CAP sample. This acid is formed by deamination of 8-methyl-6-nonenamide.

Other products arising from the vanillin (4-hydroxy-3-methoxybenzaldehyde) portion of the CAP molecule include 2-methoxyphenol, 2-methoxy-4-methylphenol, and methylvanillin.

Capsaicin-Oleic Acid Interaction Products. The chromatograms of the major interaction products of CAP with oleic acid are shown in Figure 5. The probable identity, method of identification, and relative amounts of all of the products identified are given in Table III.

The unique products formed as a result of the CAP interaction with oleic acid were amides. The principal product is *cis*-9-octadecenamide (oleamide). The mass spectrum was identified by comparison with the NBS Library of Mass Spectra (spectrum 31474). This compound appears to result from the reaction of ammonia with oleic acid.

A second amide was also identified having a molecular weight of 417. The spectrum and structure of this compound are shown in Figure 6. The identity of this product, *N*-vanillyl-9-octadecenamide, was confirmed by repeating the reaction using stearic and linoleic acid. These two reactions yielded analogous products with molecular weights of 419 and 415, respectively. The reaction of oleic acid with DC produced the same product as the reaction with CAP. This confirms that the unsaturated moiety from the CAP molecule does not contribute to this product. The formation of this product is most probably due to the

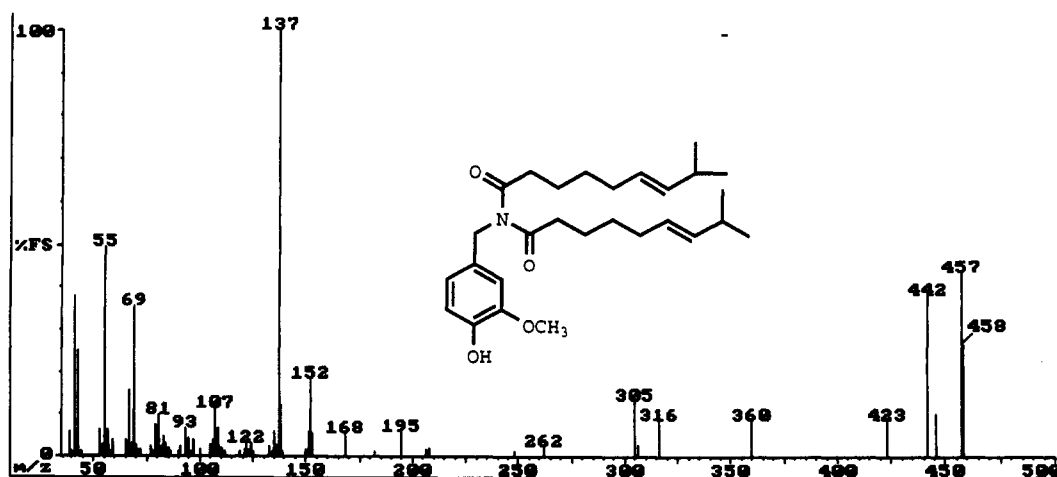


Figure 4. Mass spectrum and tentative structure of imide MW 457.

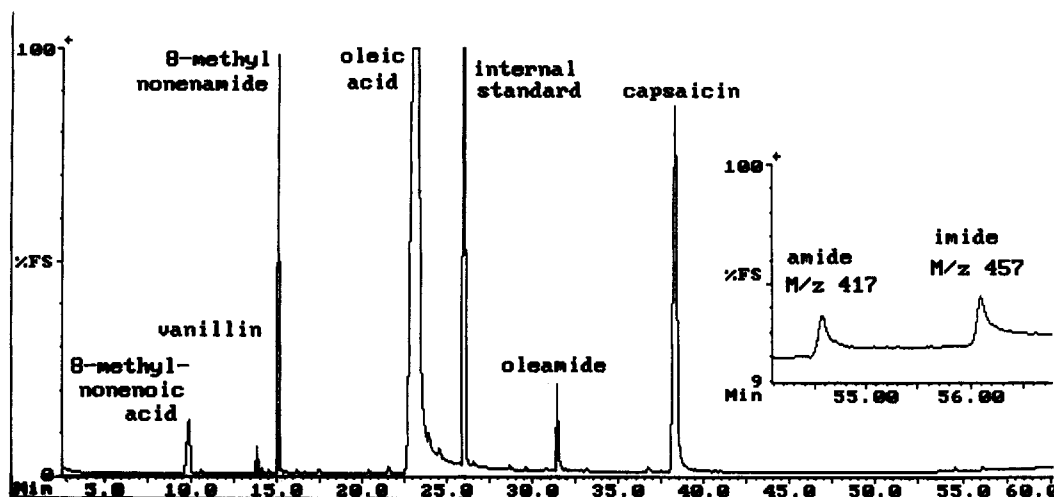


Figure 5. Total-ion chromatogram of atmospheric pressure interaction mixture of 50–50 CAP–oleic acid on RT₁-200 column. Injector (splitless), 275 °C; temperature program, 70 °C for 2 min, 8 °C/min to 175 °C, 3 °C/min to 310 °C; carrier gas helium at 12 psi.

Table III. Products of Thermal Decomposition of Capsaicin with Oleic Acid

compd	identification method	Kovats index		oleic ^a	50–50 ^a	capsaicin ^a
		RT ₁ -200	Stab-DA			
2-heptene	NBS 1213		1149	0.0	0.1	0.5
heptanal	authentic compd	1087	1195	7.6	0.3	0.3
octanal	authentic compd	1188	1299	14.9	0.1	0.0
nonanal	authentic compd	1285	1401	28.8	0.3	0.1
pentanoic acid	authentic compd	<i>b</i>	1747	1.4	0.0	0.0
2-methoxyphenol	authentic compd	1275	1860	0.0	0.5	0.7
2-methoxy-4-methylphenol	authentic compd	1387	1956	0.0	0.4	1.0
heptanoic acid	authentic compd	<i>b</i>	1963	10.0	0.1	0.0
pentanamide	authentic compd	1356	2006	0.0	0.1	0.4
octanoic acid	authentic compd	<i>b</i>	2076	17.5	0.2	0.1
nonanoic acid	authentic compd	<i>b</i>	2188	17.8	1.1	2.9
8-methyl-6-nonenoic acid	interpretation	<i>b</i>	2259	0.5	44.5	4.0
isononanamide	interpretation	1721	2397	0.0	0.7	2.0
methylvanillin	interpretation		2409	0.0	0.3	0.9
nonanamide	authentic compd	1742	2451	0.0	0.4	1.2
8-methyl-6-nonenamide	interpretation	1857	2529	3.0	41.7	84.5
vanillin	authentic compd	1753	2556	0.3	7.2	10.6
9-octadecenamide	NBS 31474	2776	3240	0.0	47.5	963.3
<i>N</i> -vanillyl-9-octadecenamide	interpretation	4062		0.0	tr	0.0

^a Percent normalized peak areas relative to internal standard. ^b Retention varies with amount of material.

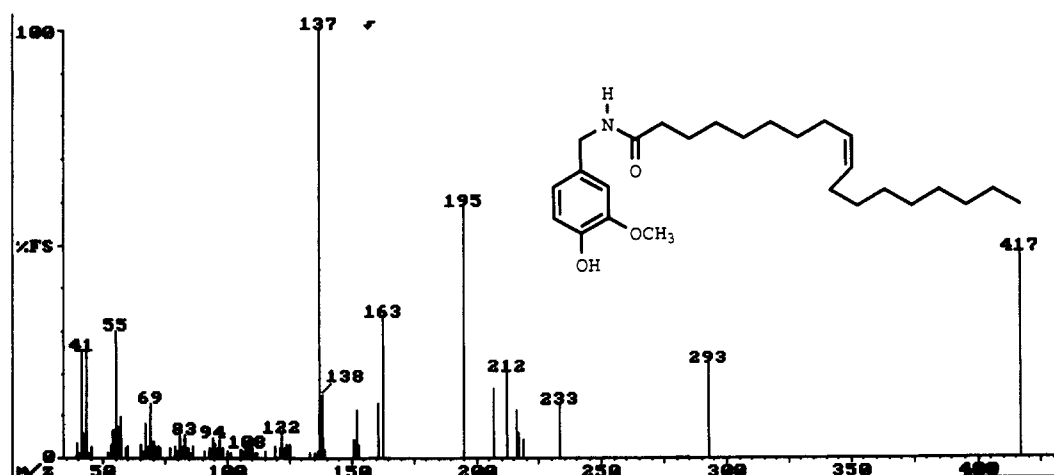


Figure 6. Mass spectrum and tentative structure of amide MW 417.

formation of an amine by cleavage of the bond between the nitrogen and the carbonyl carbon in CAP. The resulting amine can then form a salt with the carboxylic acid present. This is then dehydrated to form the amide.

If the mechanism proposed for the formation of *N*-vanillyl-9-octadecenamide is correct, one would expect to see amides formed by other free acids present during

heating. The other major acid present in these reactions has been tentatively identified as 8-methyl-6-nonenoic acid. The analogous reaction of the amine with this acid would produce CAP. This would regenerate the starting material, and therefore this reaction would be undetectable.

Small amounts of octanoic and nonanoic acid which are

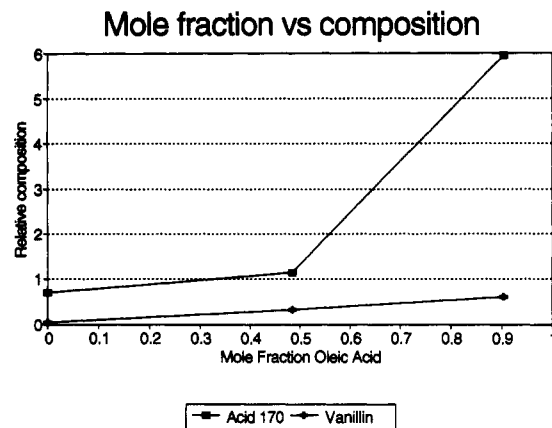


Figure 7. Relative amounts of 8-methyl-6-nonenic acid and vanillin as a function of oleic acid concentration.

detected in the oleic acid decomposition would also be expected to form amides with the aromatic amine. The products of these reactions would be shorter chain CAP analogues, and these could have sensory significance. The products of the reactions of these acids were undetectable by the present method. This may be due in part to the suppression of oleic acid decomposition seen in the presence of CAP discussed below.

All of the products of the decomposition of CAP alone also appear in the interaction samples. However, the decomposition products of oleic acid alone are present in substantially lower concentrations in the interaction samples than in the oil alone. A comparison of the acid and aldehyde concentrations in the samples from the atmospheric pressure decomposition of oleic acid alone and the 1:1 (w/v) CAP-oleic interaction mixture was made. The oleic acid decomposition products in the interaction sample appeared at less than 10% of the concentrations present in the absence of CAP. Most products were present at only 1–2% of the concentration in the pure oleic acid sample.

The low-pressure samples produced fewer decomposition products as expected from the lower partial pressure of oxygen and the fact that products were swept from the system as soon as they formed. The ratio of aldehydes to acids in the products also increased as the pressure was reduced, as would be expected under milder oxidative conditions.

The sealed tube reactions yielded much higher concentrations of decomposition products than either of the other heating methods. The fact that the entire tube was at a constant high temperature makes this a much more reactive environment. The heating procedure using the distillation apparatus allows volatile products to rise above the heated portion of the pot and therefore to be protected from additional reaction. While the distillation apparatus is a better simulation of normal cooking processes, the sealed tubes provided better speed and reproducibility. They also allow studies to be carried out with smaller amounts of reagents.

The concentration of one major decomposition product of CAP, 8-methyl-6-nonenic acid, varied directly as a function of the concentration of oleic acid. As the concentration of oleic acid increased, the concentration of 8-methyl-6-nonenic acid also increased, while the concentration of the corresponding amide decreased. A graph of this relationship is shown in Figure 7. It appears that the nucleophilic substitution in which the amino group is replaced by a hydroxyl group is enhanced by the presence of an acid such as oleic acid. The presence of the oleic

acid may shift the equilibrium of the system toward the deaminated product.

CONCLUSIONS

The thermal decomposition of CAP yields products that arise mainly from cleavage of the alkyl group attached to the amide. This cleavage and subsequent oxidation leads to the formation of vanillin. Further breakdown of the vanillin into other substituted phenols is observed. In addition, several products resulting from the alkylamide are also observed.

The heating of oleic acid and CAP yields some products that are unique to the interaction. The products formed result from the reaction of oleic acid with ammonia to give 9-octadecenamide and from the reaction of oleic acid with the vanillyl moiety to form a secondary amide.

Finally, the oxidation of the oleic acid appears to be inhibited by the presence of CAP. The oxidation of CAP appears to be preferred over the oxidation of oleic acid, and thus CAP may serve as an antioxidant in nonaqueous systems. Ongoing investigations in this laboratory are examining the extent of this behavior on oleic and other fatty acids.

The structure of CAP is quite similar to that of several other important spices, most significantly ginger. The extension of the present studies to gingerols is also in progress.

The release of vanillin during heating is particularly interesting in light of the fact that folklore related to cooking with CAP indicates that heating in oil releases the flavor of the peppers. This observation may be based on the release of vanillin. Since this release is accompanied by the formation of several amides, it is reasonable to speculate that these amides may have significant flavor characteristics.

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