Mechanism of Formation of Volatile Compounds by Thermal Degradation of Carotenoids in Aqueous Medium. 2. Lycopene Degradation

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During heat treatment of lycopene suspension in water, several volatile compounds are produced in an oxidative process. Beside 2-methyl-2-hepten-6-one and citral, previously characterized as a degradation products of lycopene, 5-hexen-2-one, hexane-2,5-dione, 6-methyl-3,5-heptadien-2-one, and geranyl acetate were identified and pseudoionone was tentatively identified by GC–MS. The temperature effect and the kinetic study of nonvolatile compound formation show that during the degradation process all-trans-lycopene is partially isomerized to the cis–trans isomer, which may considered an end reaction product. The same studies relative to volatiles show that the main compounds isolated, 2-methyl-2-hepten-6-one, geranial, and pseudoionone, arise from all-trans-lycopene. Subsequent degradation of this latter compound gives 6-methyl-3,5-heptadien-2-one, and geranial is isomerized to neral.

Thermal degradation of lycopene is less documented than that of β-carotene; however, Nakagawa et al. (1971) have shown that the initial decomposition of lycopene in organic solvents such as dioxane or n-heptane or in aqueous emulsion using Tween 20 followed a simple-order reaction and that lycopene was less stable than β-carotene in each solvent studied. Miki and Akatsu (1971) found that 5–15% of dispersed lycopene in broken tomato cells or of granular lycopene was degraded under heating at 95–130 °C for 10 min. Fe^{III}, Cu^{II}, and Sn^{II} have little stabilizing effect on lycopene.

Consequently the results concerning the formation of volatile compounds from lycopene are scarce; 2-methyl-2-hepten-6-one and to a less degree citral are described by several authors (Cole and Kapur (1957); Schormuller and Kochman (1969); Schreier et al. (1977); Sieso and Crouzet (1977); Coulibaly et al. (1979)) as degradation compounds of lycopene in tomato. According to these authors, thermal or enzymatic pathways are involved in the formation of these volatiles.

To our knowledge, only one work (Drawert et al., 1981) was devoted to the study of pure lycopene degradation in the solid state; 2-methyl-2-hepten-6-one was identified in the volatile fraction isolated.

In a previous work, Kanasawud and Crouzet (1990) reported the mechanism of formation of volatile compounds during heat treatment of β-carotene in a water system. The purpose of the present work was the study of the production of volatile compounds during heat treatment of a water suspension of lycopene, identification of volatiles, and their formation mechanism.

MATERIALS AND METHODS

Lycopene Preparation. Lycopene was extracted from tomato puree with carbon disulfide and purified by dissolution in carbon disulfide and precipitation with methanol as indicated in Karrer and Jucker (1950). The pureness of the product freshly

isolated and purified, checked by thin-layer chromatography and by spectrophotometry, was more than 90%.

Heat Treatment. A 15-mg portion of lycopene suspended by sonication in 50 mL of distilled water in a sealed Kjeldahl flask wrapped in aluminum foil was heated at 97 ± 2 °C in an oil bath for different reaction times or for 3 h from 30 to 97 °C. In some experiments, the suspensions were saturated with oxygen before the flask was sealed.

Isolation of Volatile Compounds. The volatile compounds were isolated by two techniques as indicated in Kanasawud and Crouzet (1990). The more volatile compounds were obtained by gas stripping with nitrogen and trapping on Tenax GC. The less volatile compounds were isolated by direct extraction with dichloromethane of the filtrate obtained by elimination of undissolved compounds of the reactive medium.

Gas Chromatography. A Varian 3700 gas chromatograph fitted with a FID detector, an on-column injector, and a WCOT glass capillary column (40 × 0.4 mm (i.d.)) coated with Carbowax 20 M was used under the conditions described by Kanasawud and Crouzet (1990). In this case, the internal standard was 1-heptanol.

Gas Chromatography–Mass Spectrometry. A Hewlett-Packard 5996 apparatus fitted with a fused silica capillary column DB 5 (J&W) was used under the conditions described in Kanasawud and Crouzet (1990). Reference compounds used for identification were obtained from commercial suppliers.

Study of Nonvolatile Compounds. These compounds were isolated and separated by TLC on aluminum oxide F_{254} Type T (Merck) under the conditions used for nonvolatile compounds resulting from heat treatment of β-carotene (Kanasawud and Crouzet, 1990). The absorption spectra of fractions recovered from preparative TLC performed under the same conditions were recorded in hexane solution.

RESULTS

Identification of Volatile Compounds. The volatile compounds isolated, as indicated in Materials and Methods, were identified by GC–MS (Figures 1 and 2; Table I). These two extraction techniques were used in as far as complementary results may be obtained, the more volatile compounds being partially lost during solvent elimination when direct extraction is carried out whereas the less volatile compounds are little or not swept

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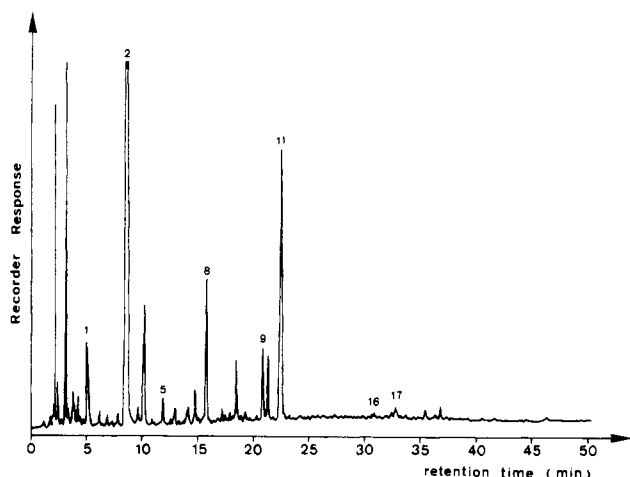
Figure 1. Chromatogram of volatile compounds isolated by adsorption on Tenax GC and thermal desorption after treatment of lycopene at 97 °C for 3 h in the presence of oxygen (glass capillary column, 40 × 0.4 mm (i.d.), Carbowax 20 M, operated at 50 °C for 10 min and then programmed to 170 °C at 4 °C/min).

by the nitrogen stream during gas stripping. As seen in Figures 1 and 2, compounds 14–17 are present in detectable amounts only in the solvent extract. Under these conditions quantitative determinations were performed with use of only solvent extracts.

Among the compounds identified, only three of them, 2-methyl-2-hepten-6-one, geranial, and neral, have been reported as degradation products of lycopene (Stevens, 1970; Drawert et al., 1981). The other compounds, 5-hexen-2-one, hexane-2,5-dione, 6-methyl-3,5-heptadien-2-one, geranyl acetate, and pseudoionone, were identified or tentatively identified for the first time among the volatile compounds produced by thermal degradation of lycopene. In the case of pseudoionone the mass spectrum obtained matches with those published by Thomas et al. (1969).

Influence of Temperature on Volatile Compound Formation. In a preliminary study, formation of the main volatile compounds identified was studied for different treatment temperatures, from 30 to 97 °C, in the presence of air for 3 h (Figure 3). For 2-methyl-2-hepten-6-one, 6-methyl-3,5-heptadien-2-one, and neral there is an increase of the quantities of product formed with the increase of temperature with a lag phase until 25 °C for the two latter compounds. On the contrary, for geranial and pseudoionone an increase of production to 50 °C was followed by a decrease beyond this temperature.

Kinetics of Volatile Compound Formation. In the presence of air (Figures 4 and 5) the rate of volatile compound formation generally increases with the length of treatment; however, a light decrease in the rate of formation of pseudoionone was observed at the end of the holding time; 2-methyl-2-hepten-6-one is the more important compound produced under these conditions.

In the presence of oxygen (Figure 6) the quantities of volatiles produced by lycopene degradation increase significantly; 2-methyl-2-hepten-6-one is always the most important compound present in the extracts. The decrease in the rate of pseudoionone formation observed after 2 h of treatment in the presence of air is confirmed when the reaction medium is saturated with oxygen. Under these conditions the same phenomenon was noticed for geranial.

Study of Nonvolatile Compounds. The thin-layer chromatograms of lycopene and of nonvolatile com-

pounds resulting from heat treatment at 97 °C during 1 h in the presence of air and 1–3 h in the presence of oxygen are given Figure 7. These results show that the lycopene extracted and purified from tomato puree was more than 90% pure (Figure 7-I); the purity of the product was checked by spectrophotometry. The chromatogram of nonvolatile compounds isolated after heat treatment at 30 and 50 °C for 3 h in the presence of air (results not reported) and at 97 °C for 1 h under the same aeration conditions (Figure 7-II) shows only a slight increase of nonvolatile compounds produced by lycopene degradation.

Five nonvolatile compounds, produced during heat treatment of lycopene at 97 °C over 3 h in the presence of oxygen, were separated by TLC on alumina. Three of them were isolated in sufficient quantity to allow determination of their absorption spectra. Compound 5, quantitatively the greatest, presents absorbance maxima at 444, 472, and 504 nm indicative of the all-trans structure of lycopene. For the compound corresponding to band 4, less important than the former, the maxima in absorbance at 442, 469, and 501 nm agree with the spectral characteristics of the cis–trans-lycopene, denoted 1A and 1B (Magoon and Zechmeister, 1957). The compound isolated from band 2, λ_{max} 517 and 484 nm, was not identified.

Comparison of the chromatograms obtained after 3 h of reaction at different temperatures (30, 50, 97 °C) shows at 97 °C an increase of the band corresponding to the compound identified as the cis–trans isomer and a parallel decrease of band corresponding to all-trans isomer. This phenomenon is more apparent when lycopene is heated at 97 °C for 1–3 h in the presence of oxygen (Figure 7-III–V).

DISCUSSION

Three compounds produced during heat degradation of lycopene suspensions, 2-methyl-2-hepten-6-one, neral, and geranial, have been previously identified among the volatile fraction of tomato (Cole and Kapur, 1957; Schormuller and Kochmann, 1969) or tomato products (Schreier et al., 1977; Sieso and Crouzet, 1977; Coulibaly et al., 1979). According to these authors, the isolated compounds could be produced by degradation of lycopene. On the other hand, Stevens (1970) has shown that there is a correlation between the presence of 2-methyl-2-hepten-6-one and citral and the neurosporene, lycopene, prolycopene, and γ- and δ-carotene content in tomato products. Moreover this ketone and citral were present after cooxidation of lycopene by linoleic acid catalyzed by soybean lipoxygenase. More recently, Drawert et al. (1981) reported that the thermal degradation of lycopene at 190 and 220 °C in air leads to 60% 2-methyl-2-hepten-6-one. As for β-carotene, some volatile compounds produced by thermal degradation of lycopene under the conditions studied are the same as those identified after heat treatment of this compound in the solid phase or detected in tomato products submitted to heat treatment.

As indicated in Figure 8 there are evident structural connections between lycopene and pseudoionone on the one hand and pseudoionone and 6-methyl-3,5-heptadien-2-one on the other. Concerning 5-hexen-2-one and hexane-2,5-dione, which are minor products of the reaction, their formation from lycopene is plausible but mechanisms remain highly speculative.

Among the components identified for the first time in the volatile fraction obtained after thermal degradation of lycopene, only the geranyl acetate is questionable. This compound may arise from the tomato concentrate (Seck
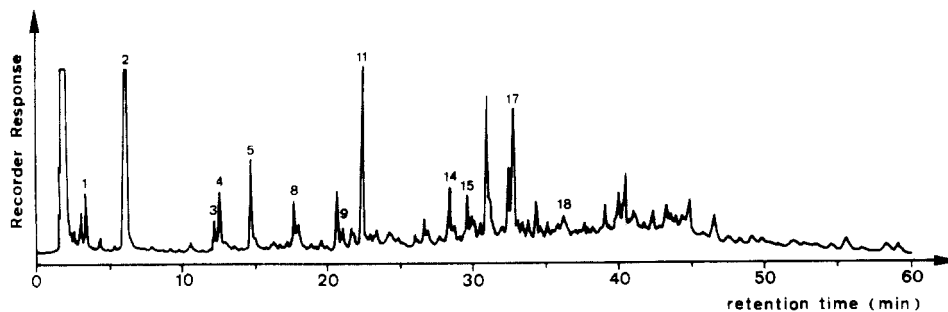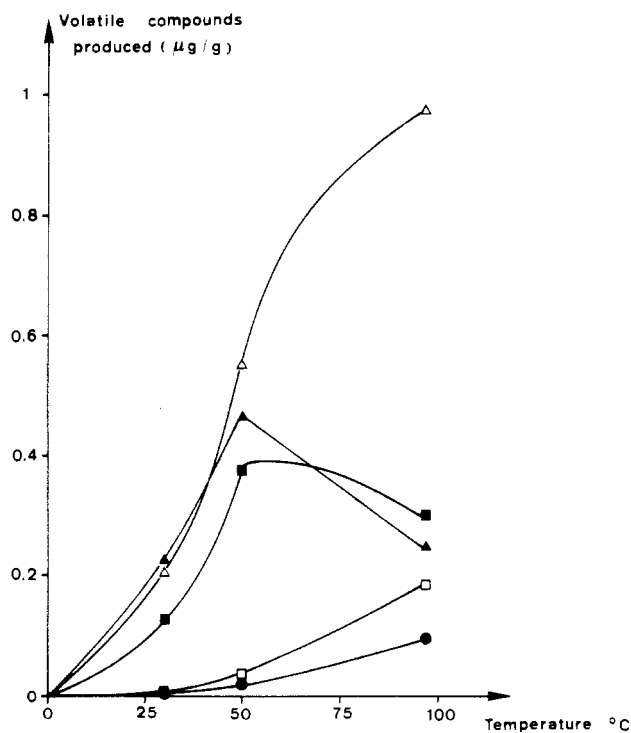
Figure 2. Chromatogram of volatile compounds isolated by solvent extraction with dichloromethane after treatment of lycopene at 97 °C for 3 h in the presence of oxygen (glass capillary column, 40 × 0.4 mm (i.d.), Carbowax 20 M, operated at 50 °C for 10 min and then programmed to 170 °C at 4 °C/min).

Table I. Volatile Degradation Products of Lycopene by Thermal Degradation for 3 h at 97 °C under Oxygen

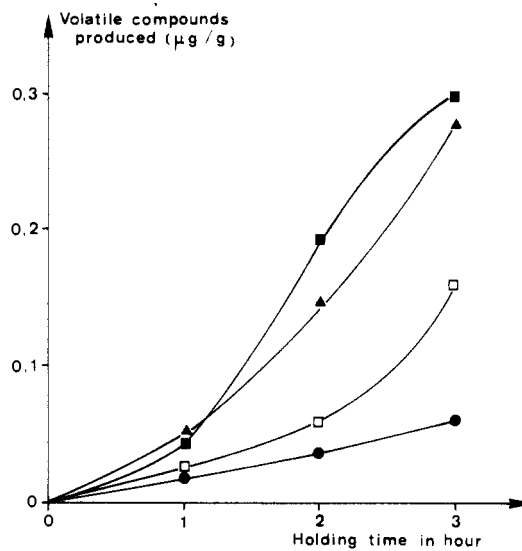| peak in Figures 2 and 3	compound	$R_I{}^a$	identification MS	identification RT^b
1	5-hexen-2-one		+	+
2	2-methyl-2-hepten-6-one	981	+	+
3	hexane-2,5-dione	1041	+	+
4	unknown (143, 99, 71, 114)	1069	+	−
8	6-methyl-3,5-heptadien-2-one	1102	+	+
9	neral	1247	+	+
11	geranial	1277	+	+
14	geranyl acetate	1358	+	+
17	pseudoionone	1585	+	−

^{a} Retention index on Carbowax 20 M. ^{b} Retention time.

Figure 4. Kinetics of formation of volatile compounds during heat treatment of lycopene at 97 °C in the presence of air: (●) neral; (▲) geranial; (□) 6-methyl-3,5-heptadien-2-one; (■) pseudoionone.
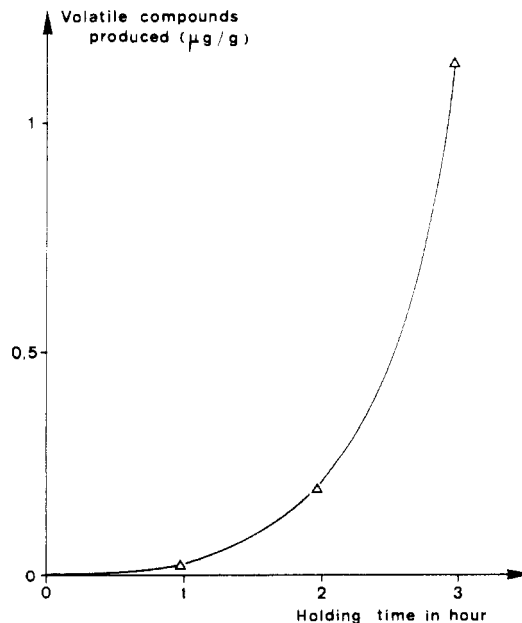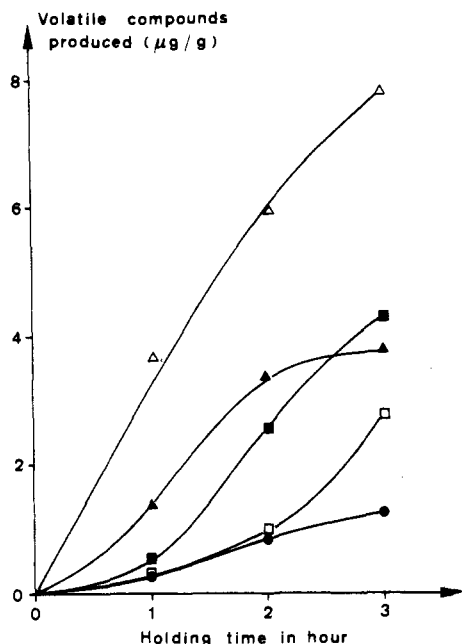

Figure 3. Influence of temperature on volatile compound formation during heat treatment of lycopene from 30 to 97 °C in the presence of air for 3 h: (△) 2-methyl-2-hepten-6-one; (●) neral; (▲) geranial; (□) 6-methyl-3,5-heptadien-2-one; (■) pseudoionone.

Figure 5. Kinetics of formation of 2-methyl-2-hepten-6-one during heat treatment of lycopene at 97 °C in the presence of air.

and Crouzet, 1973) from which lycopene was obtained. However, its formation in tomato extract by lycopene degradation cannot be rejected.

As for β-carotene (Kanasawud and Crouzet, 1990), the oxidative nature of the heat-induced degradation process of lycopene is clearly established. Effectively, in the presence of a saturating level of oxygen an important

increase in the quantities of 2-methyl-2-hepten-6-one, geranial, neral, pseudoionone, and 6-methyl-3,5-heptadien-2-one was observed. The quantities of volatile compounds

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Figure 6. Kinetics of formation of volatile compounds during heat treatment of lycopene at 97 °C in the presence of oxygen: (△) 2-methyl-2-hepten-6-one; (●) neral; (▲) geranial; (□) 6-methyl-3,5-heptadien-2-one; (■) pseudoionone.
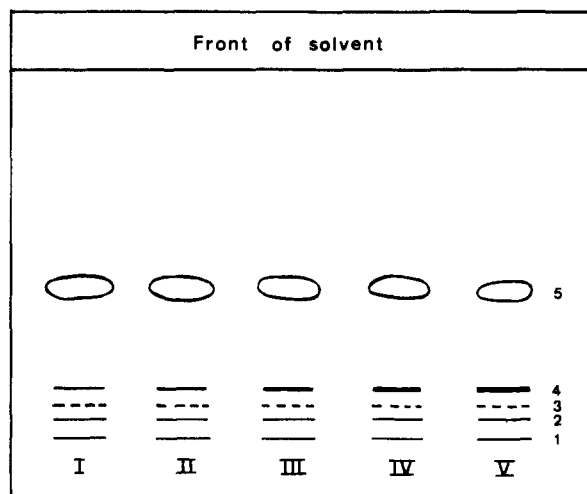
Figure 7. Thin-layer chromatography on aluminum oxide F_{254} of lycopene (I) and of nonvolatile compounds produced after heat treatment of this compound at 97 °C for 1 h in the presence of air (II) and for 1–3 h in the presence of oxygen (III–V): 1–3, nonidentified compounds; 4, cis-trans-lycopene; 5, all-trans-lycopene.

produced under these conditions are 10 times more important than those detected in the presence of air.

Three of these volatile compounds, 2-methyl-2-hepten-6-one, geranial, and pseudoionone, are generated under relatively mild conditions (temperatures below 50 °C in the presence of air and may be considered as the primary products of the degradation process. The fact that the amounts of compounds produced under these conditions are approximately the same indicates that the sensitiveness of C_6–C_7, C_8–C_9, and C_{10}–C_{11} bonds (Figure 8) to oxidative cleavage at low oxygen pressure is equivalent. However, the decrease of geranial and pseudoionone concentrations associated with the increase in geranial and 6-methyl-3,5-heptadien-2-one are indicative of the transient nature of the former compounds detected. Trans–cis isomerization of the terpenic aldehyde and a further cleavage of pseudoionone, even if 2-methylbutanal was not detected, are probably involved.
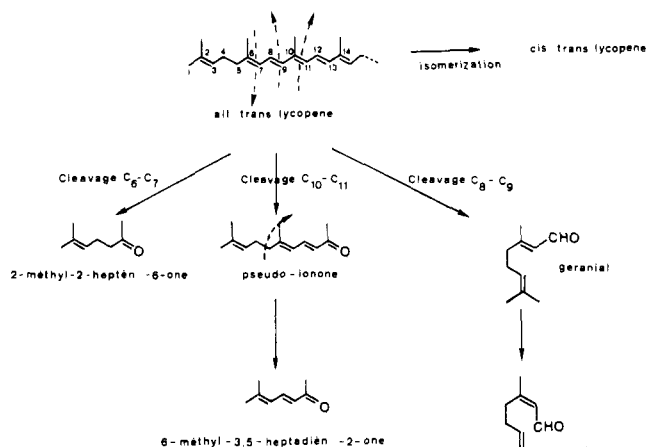
Figure 8. Reaction sequence for the formation of volatile compounds during heat treatment of lycopene.

The results of kinetic studies, the decrease in the quantities of pseudoionone produced in the two cases and of geranial in the presence of oxygen after 2 h of heat treatment, are consistent with the proposed mechanism. On the other hand, acceleration of 2-methyl-2-hepten-6-one production with time and with oxygen level in the reactive medium shows that the double bond in position C_6–C_8 is more sensitive to oxidation by molecular oxygen than the other two bonds. In the case of β-carotene degradation, Enzell (1981) indicates that several bonds are preferentially broken during photooxidation or other nonenzymatic oxidation.

According to the observed decrease in the quantity of all-trans-lycopene as a function of time and temperature, this compound may be considered as the most important precursor for volatiles produced during the degradation process. Only a weak increase in the quantity of the cis-trans-lycopene initially present as contaminent in the product used in this study occurs under the same conditions. This increase with time, temperature, and partial pressure indicates that the trans–cis isomerization observed is, as for geranial and neral, the result of treatment and is not induced by aluminum oxide during TLC separation (Meunier et al., 1951). Moreover, according to the nature of volatile compounds isolated, it appears that the weak increase noticed for cis-trans-lycopene is not the consequence of a very fast degradation of this compound into volatiles. Consequently, cis-trans-lycopene must be considered as end reaction product rather than as an intermediary compound.

According to the results obtained in the present work, the sequence presented in Figure 8 for volatile compound formation during oxidative degradation of lycopene may be proposed.

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Identification of Pyrazines in Maple Syrup

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Commercial samples of Quebec maple syrup (1, amber; 3, dark) were extracted successively with diethyl ether, an acid solution, a basic solution, and then finally dichloromethane. Capillary gas chromatographic analysis of the dichloromethane extract indicated the presence of methylpyrazine, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, ethylpyrazine, and trimethylpyrazine. Gas chromatography–mass spectrometry confirmed the presence of the six pyrazine compounds and also suggested the presence of the 2-ethyl-6-methylpyrazine.

Pyrazines are heterocyclic, nitrogen-containing compounds found both in many processed foods as well as naturally in many plant foods (Maga, 1982; Maga and Sizer, 1973). These compounds contribute characteristic flavor and aroma to foods. For example, alkylmethoxypyrazines have been reported to be responsible for the "earthy" or "potato-like" odors of many vegetables and plants (Gallois and Grimont, 1985) while the contribution of alkylpyrazines to aroma, flavor, and color of a large number of thermally processed or cooked foods has been reported by several workers (Shibamoto 1986; Fors and Erickson, 1986; Masuda and Mihara, 1986). Previous reports have been reviewed by Maga (1982).

The formation of pyrazine compounds in many thermally processed foods results from Maillard-type reactions between reducing sugars and free amino acids or amides (Koehler and Odell, 1970; Koehler et al., 1969). The mechanisms by which pyrazines are formed have been proposed (Shibamoto et al., 1979; Newel et al., 1967; Koehler et al., 1969). These reports have been reviewed by Maga (1982).

Traditionally, the conversion of maple sap to maple syrup involves concentration by boiling of the sap for several hours. The presence of small quantities of reducing sugars (Jones and Alli, 1987) and free amino acids and amides, particularly aspartic acid, glutamic acid, glutamine, asparagine, and citrulline, has been reported (Morselli and Whelan, 1986; Kallio, 1988). Although the conditions are favorable for formation of pyrazine compounds, a systematic attempt to identify pyrazine compounds in maple syrup has not been reported in the literature. Nevertheless, there have been sporadic reports indicating some relationship between maple flavor and pyrazine compounds. Masuda and Mihara (1986) indi-

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