Characterization of the Pyrazines Formed during the Processing of Maple Syrup

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Pyrazine formation in maple syrup was investigated during the boiling of maple sap at 105 °C for 220 min. In general terms, there was an induction period, characteristic of the type of pyrazine, associated with the formation of all identified pyrazines. No pyrazine was detected before 60 min of heating at 105 °C; 2,5-dimethyl- and trimethylpyrazine were formed after 60 min of heating, whereas methyl-, 2,6-dimethyl-, ethyl-, 2,3-dimethyl-, and 2-ethyl-3-methylpyrazine were detected after 120 min of heating. The total level of pyrazines increased from 3.42 ng/g after 60 min of heating to 72.32 ng/g in the final syrup. The formation rate constants (0.04−0.13 ng of pyrazines/min) were determined from the slopes of plots of concentrations versus time of heating. These plots were consistent with *pseudo-zero*-order reactions. The formation of these pyrazines was influenced by the heating time and by the pH of the boiling sap. The pH values of the sap increased from 7.2 to 9.2 during the first 40 min of boiling, then decreased to 7.3; the decrease in pH values was associated with an increase in the total soluble solids, mainly sugars, from 3% in the sap to 65% in the syrup. Consequently, the levels of sucrose, glucose, and fructose increased from 23.21, 0.09, and 0.09 mg/g, respectively, in the sap to 416.97, 3.25, and 1.82 mg/g in the syrup.

Keywords: *Pyrazines; maple; maple syrup; maple sap*

INTRODUCTION

Flavor compounds of maple syrup include volatile phenolic compounds, carbonyl compounds, and alkylpyrazines (Kallio, 1988; Alli et al., 1990; Belford, 1991). The alkylpyrazines, typical products of the advanced stage of the Maillard reaction, have been the subject of numerous studies because of their impact on color and flavor of foods (Maga, 1982). Model systems consisting of a variety of carbohydrates and amino acids or nitrogen bases have been used to study the pathways and mechanisms associated with the formation of pyrazine compounds. Dawes and Edwards (1966) proposed that pyruvaldehyde, formed during sugar fragmentation, could react with amino acids to form aminopropanal, which can yield dimethyldihydropyrazine by condensation. Newell et al. (1967) postulated the formation of dimethylpyrazine from the Amadori 1,2-enaminol. Shibamoto and Bernhard (1977) proposed that the interactions between reducing sugars and amino compounds resulted in the formation of α-aminocarbonyl intermediates which could condense to form pyrazines. This hypothesis stipulates that free ammonia resulting from the decomposition of amino acids is the nitrogen source. The most widely accepted mechanism for the formation of pyrazines in food systems is via the Strecker degradation of amino acids which in the presence of α-diketones result in the formation of α-aminoketones and Strecker aldehydes. The condensation of α-aminoketones results in the formation of pyrazines (Koehler and Odell, 1970; Hwang et al., 1994).

The effects of several factors, including reactants, pH, the temperature/time relationship, water activity, and the presence of oxidizing and reducing agents, on pyrazine formation have been studied in both food and model systems (Koehler and Odell, 1970). The formation of pyrazine compounds is considered to require sugar fragments. Monte and Maga (1981) reported that alkaline conditions promote sugar fragmentation and hence resulted in an increased formation of pyrazines.

Pyrazines, mostly found in heat-treated foods and some raw vegetables, have organoleptic characteristics; the importance of their contribution to the overall flavor has been reviewed (Maga, 1982; Fors, 1983). Sucrose, glucose, and fructose (Jones and Alli, 1987; Leech and Kim, 1990) and amino acids (Morselli and Wholen, 1986; Kallio, 1988), present in maple sap, would be expected to be the principle precursors for the formation of pyrazines in maple syrup.

Although the presence of pyrazines has been reported in maple syrup (Alli et al., 1990; Akochi-K et al., 1993, 1994), as far as the authors are aware, the presence of these compounds has not been investigated. In the present work, the levels of sucrose, glucose, and fructose were found in maple sap and heated maple sap to be 9, 16, and 65.5% total soluble solids. The formation of pyrazine compounds was monitored during the conversion of maple sap to syrup.

MATERIAL AND METHODS

Materials. Maple sap (3% total dissolved solids) from the 1993 harvest season was obtained from the Morgan Arboretum (Macdonald Campus of McGill University, Ste Anne de Bellevue, Québec). The sample was kept frozen at −15 °C and thawed just before analyses or processing. Commercial pure maple syrups were purchased from Les Producteurs de Sirop d'Erable du Québec (Plessiville, Québec).

pH. The pH values for maple sap and syrup samples were measured using a multichannel Accumet pH meter (Fisher Scientific Ltd., Ottawa, ON).

Total Dissolved Solids. Total soluble solids (TS) of sap and syrup samples were determined by measurement of refractive index (R_i) using an Abbé refractometer (Belford et al., 1991).

Determination of Individual Sugars. Lyophilized maple sap and maple syrup samples were analyzed for individual sugars. A quantity (5.0 mg) of sample was used to convert

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Table 1. Content of Fructose, Glucose, and Sucrose in Maple Sap, Heated Maple Sap, and Maple Syrup

samples	sugar (mg/g)[a] fructose	glucose	sucrose	total
unheated maple sap	0.09 (0.01)[b]	0.09 (0.00)[b]	23.21 (0.02)[b]	23.39
percentage[c]	0.38	0.38	99.27	
heated maple sap				
9% TS[c]	0.19 (0.02)[b]	0.38 (0.01)[b]	58.98 (1.15)[b]	59.55
percentage	0.32	0.64	99.04	
16% TS[d]	0.24 (0.00)[b]	0.50 (0.01)[b]	109.34 (1.02)[b]	110.08
percentage	0.22	0.45	99.33	
maple syrup				
65.5% TS[b]	1.64 (0.22)[b]	2.77 (0.13)[b]	417.63 (0.17)[b]	422.04
percentage	0.39	0.66	98.96	

[a] Concentration in mg of sugar/g of sap or syrup. [b] Results are means (standard deviations) of triplicate determination. [c] Relative percentage of the total identified sugars. [d] Total soluble solids.

the sugars to their trimethylsilyl derivatives and subjected to gas−liquid chromatographic (GC) analyses (Jones and Alli, 1987). Glucose, fructose, and sucrose, obtained from Aldrich Chemical Co. Inc. (Milwaukee, WI), were used as reference sugars for identification; sorbitol was used as the internal standard. GC analyses were performed using a Varian gas chromatograph, model 3700, equipped with a flame ionization detector and a DB 1701 (7% cyanopropylphenyl silicone liquid phase) fused silica capillary column (30 m length × 0.25 mm i.d. with a 0.25 μm film thickness; J&W Scientific, Montréal, PQ). The analysis was carried out using the following conditions: the injector and detector temperatures were 230 and 280 °C, respectively; the nitrogen carrier gas flow rate was 10 mL/min. The oven temperature was programmed from 120 to 250 °C at a rate of 4 °C/min, with a 6 min initial temperature hold. All chromatograms were recorded and integrated using an HP-3390A integrator (Hewlett-Packard, Montréal, Québec) programmed to calculate the response factors for each individual sugar used in the standard mixture.

Determination of Individual Free Amino Acids. Individual free amino acids in maple sap were determined by high-performance liquid chromatography (HPLC) according to the procedure previously described by Spackman et al. (1958).

Determination of Pyrazines. Laboratory prepared maple syrups and commercial maple syrups were analyzed for their pyrazine content by gas chromatography according to the method described by Akochi-K et al. (1994). Quantities of 100 g of commercial maple syrup or 10−25 g of laboratory prepared syrup were diluted with deionized water, adjusted to pH 2.0 with 11% HCl solution, and extracted with diethyl ether. The aqueous phase was adjusted to pH 12 with 3 M NaOH solution and extracted five times with 20 mL of dichloromethane. The organic phase was concentrated to approximately 5 mL using a rotary evaporator, then to a final volume of 0.5 mL with a stream of nitrogen. The pyrazine peaks were identified by comparison of their retention times to those of reference pyrazines obtained from Aldrich Chemical Co. Inc. (Milwaukee, WI).

Processing of Maple Sap. An evaporation process, in which glass beakers of 1 L capacity were placed on a sand bed within a stainless steel pan, was used for the conversion of maple sap to maple syrup in the laboratory. The sap was heated with burners, adjusted in a way that the temperature of heating (105 °C) and the rate of evaporation (average of 6 mL per min) were maintained constant. The temperature of boiling was measured using a multichannel tele-thermometer (YSI model 42SC; Yellow Spring Instrument, Yellow Springs, OH).

Maple sap was boiled to total soluble solid values of 9, 16, and 65.5%; these samples were analyzed for their sugar and total solid (TS) contents as well as for their pH values. In addition, the syrup of 65.5% TS was subjected to pyrazine analyses.

Formation of Pyrazines during Processing. The formation of pyrazines in maple sap was monitored during a single volume boiling cycle and a continuous boiling process.

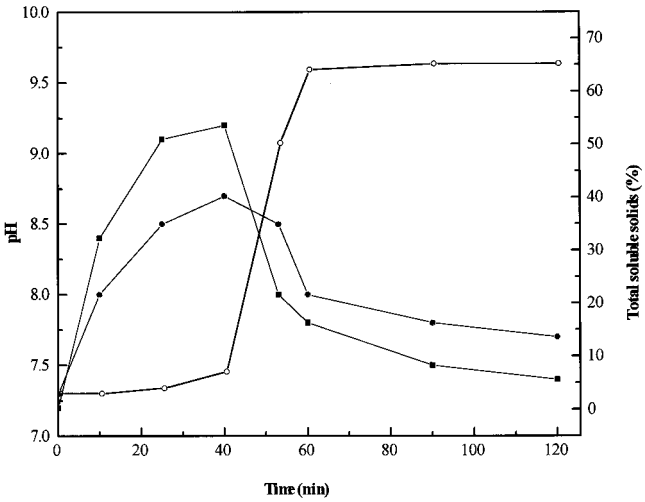

Figure 1. Monitoring of pH values and total soluble solid contents during the heating of maple sap: changes in pH (■) open boiling and (●) under reflux boiling, and (○) changes in total soluble solid contents.

For the single boiling cycle, the sap was boiled in batches of 600 mL for 30, 60, 90, and 120 min at a constant temperature of 105 °C. For the continuous boiling process, a starting sap volume of 600 mL was heated with periodic additions of fresh sap (95 mL at 15 min intervals). To simulate the processing of sap to syrup on a commercial scale, large volumes of sap (4−5 L) were heated. In addition, commercial maple syrup (500 g) was subjected to extended heating at 105 °C for 30 and 50 min.

RESULTS AND DISCUSSION

The determination of sugars and amino acids served to establish that the precursors required for the formation of pyrazines are present. The results of the sugar analyses (Table 1) show the presence of sucrose, glucose, and fructose in maple sap and heat-treated maple sap. These findings agree with those reported by Jones and Alli (1987), who identified these sugars in maple sap. The GC analyses indicated that the relative percentages of sucrose, glucose, and fructose in maple sap were 99.27, 0.38, and 0.38, respectively. The total concentration of these three sugars was 23.39 mg/g of sap; however, individual concentrations of these sugars increased to reach 417.63 mg for sucrose, 2.77 for glucose, and 1.64 for fructose when the sap turned into syrup with a total concentration of 422.04 mg/g. The increase in glucose and fructose concentrations may have been the result of heat degradation of sucrose. The overall increase of these three sugars may also be attributed to water evaporation. Figure 1 shows a gradual increase in total solids (TS) values from 3 to 5% during the first 40 min of boiling of maple sap. This was followed by a rapid increase in TS values to 65% during the subsequent 20 min. The dramatic increase in TS values of the boiling sap, associated to decreases in pH values, could be attributed to the increase in the rate of evaporation of water.

The analyses of maple sap for its individual free amino acids content showed the presence of trace quantities of aspartic acid, serine, glycine, alanine, valine, and lysine. Morselli and Whalen (1986) reported the presence of aspartic acid, asparagine, glutamine, proline, ammonia, and urea as nitrogenous compounds in maple sap. Heating of amino acids in the presence of reducing sugars generates aminocarbonyl fragments, which combine with other aminocarbonyl fragments to

Table 2. Pyrazines Formed When Maple Sap Is Subjected to a Single Heating Cycle

time[b] (min)	pH	TS[c] (%)	pyrazine (ng/g)[a] 2,5-dimethylpyrazine	trimethylpyrazine
0	7.2	3.0	*d*	*d*
10	8.3	3.2	*d*	*d*
25	9.1	3.3	*d*	*d*
40	9.2	5.0	*d*	*d*
60	7.8	63.0	1.68 (0.05)[e]	2.54 (0.01)[e]
90	7.5	65.0	4.76 (0.02)[e]	0.96 (0.00)[e]
120	7.3	65.0	2.86 (0.11)[e]	0.67 (0.00)[e]

[a] Concentration in ng of pyrazine/g of syrup. [b] Time of heating of maple sap. [c] Total soluble solids. [d] Not detected. [e] Results are means (standard deviations) of triplicate analysis.

form pyrazines; with glysine and lysine being highly reactive and serine and alanine moderately reactive in the formation of N-heterocyclic compounds (Baltes, 1990).

Figure 1 shows the changes in pH values during the processing of maple sap. The pH values of sap increased from 7.2 to 9.2 after 30 min boiling at 105 °C, before it decreased to 7.3. The initial increase in pH values could be due to the formation of Amadori rearrangement products, secondary and tertiary amines, which are more basic than amino acids. These changes could also be attributed to the loss of organic acids present in maple sap (Mollica and Morselli, 1984; Kallio, 1988) as a result of the decarboxylation of these acids. In addition, Strecker degradation of amino acids is accompanied by the loss of CO_2 from the acid moiety and this would contribute to the increase in pH values. However, the decrease in pH values may be due to the decomposition of Amadori products which are implicated in further reactions (Namiki, 1988) as well as to the concentration of organic acids. The changes in pH values during the heating of maple sap under refluxing conditions were similar to the changes observed for the open heating (Figure 1). These results suggest that chemical reactions occurring during the heating process rather than the losses of acids through evaporation are responsible for changes in pH values.

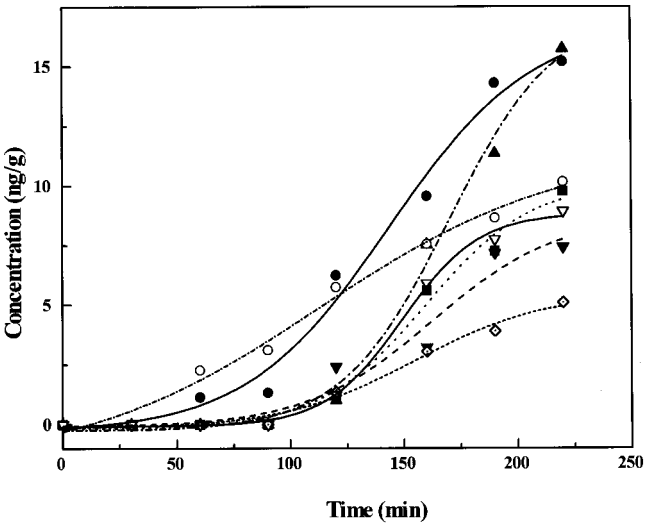

Figure 2. Accumulation of pyrazines during the boiling of maple sap: methyl- (■), 2,5-dimethyl- (●), 2,6-dimethyl (▲), ethyl- (▼), 2,3-dimethyl- (◇), trimethyl- (○) , and 2-ethyl-3-methyl pyrazine (▽).

Table 2 shows the presence of pyrazine compounds in maple syrup obtained by the single boiling cycle process. The boiling of maple sap for 30 and 40 min at 105 °C did not result in the formation of pyrazine compounds. However, boiling the sap for 60 min resulted in the formation of 1.68 and 2.54 ng/g of dimethyl- and trimethylpyrazine, respectively. The level of 2,5-dimethylpyrazine increased thereafter while that of trimethylpyrazine decreased; these variations may be attributed to the volatilization and/or decomposition of these pyrazines. In their study of a rhamnose−ammonia model system, Shibamoto and Bernhard (1977) proposed several fragmentation pathways of sugar and amino acids, leading to α-aminocarbonyl fragments that resulted in the early formation of pyrazines such as 2,5-dimethyl- and trimethylpyrazine. The gradual formation of 2,5-dimethyl- and trimethylpyrazine, during the early stages of roasting of cocoa

Table 3. Pyrazines Formed When Maple Sap Is Subjected to Continuous Boiling

time[b] (min)	pyrazine (ng/g)[a] methyl-	2,5-dimethyl-	2,6-dimethyl-	ethyl-	2,3-dimethyl-	trimethyl-	2-ethyl-3-methyl-	total
30	*c*	*c*	*c*	*c*	*c*	*c*	*c*	*c*
60	*c*	1.15 (0.03)[e]	*c*	*c*	*c*	2.27 (0.15)[e]	*c*	3.42
90	*c*	1.33 (0.01)[e]	*c*	*c*	*c*	3.11 (0.22)[e]	*c*	4.44
120	1.18 (0.05)[e]	6.25 (0.32)[e]	1.01 (0.00)[e]	2.40 (0.11)[e]	1.35 (0.01)[e]	5.75 (0.73)[e]	1.22 (0.08)[e]	19.16
160	5.60 (0.12)[e]	9.57 (1.01)[e]	7.53 (0.09)[e]	3.21 (0.15)[e]	3.05 (0.00)[e]	7.55 (1.05)[e]	5.90 (0.00)[e]	42.41
190	7.25 (0.19)[e]	14.30 (1.21)[e]	11.35 (1.00)[e]	7.17 (1.02)[e]	3.90 (0.05)[e]	8.65 (0.16)[e]	7.75 (0.93)[e]	60.37
220	9.77 (0.65)[e]	15.20 (0.00)[e]	15.73 (0.55)[e]	7.42 (0.00)[e]	5.10 (0.37)[e]	10.17 (1.23)[e]	8.93 (0.82)[e]	72.32
%[d]	*13.51*	*21.02*	*21.75*	*10.26*	*7.05*	*14.06*	*12.35*	

[a] Concentration in ng of pyrazine/g of syrup. [b] Time of heating of sap. [c] Not detected. [d] Relative percentage of individual pyrazine after 220 min of boiling. [e] Results are means (standard deviations) of triplicate analyses.

Table 4. Pyrazines in Commercial Maple Syrup and Heated Commercial Maple Syrup

samples	pyrazine (ng/g)[a] methyl-	2,5-dimethyl-	2,6-dimethyl-	ethyl-	2,3-dimethyl-	trimethyl-	2-ethyl-3-methyl-	total
maple syrup	7.90 (0.25)[d]	16.17 (0.11)[d]	21.30 (1.21)[d]	*b*	1.32 (0.02)[d]	6.75 (1.00)[d]	3.20 (0.00)[d]	56.64[a]
%[c]	13.95	28.55	37.61		2.33	11.92	5.65	
heated maple syrup								
30 min	21.53 (1.55)[d]	6.86 (0.91)[d]	6.24 (0.02)[d]	*b*	2.01 (0.01)[d]	3.76 (0.07)[d]	3.15 (0.10)[d]	43.55[b]
%	49.44	15.75	14.33		4.62	8.63	7.23	
50 min	25.82 (0.88)[d]	5.03 (0.03)[d]	7.18 (0.31)[d]	*b*	2.55 (0.00)[d]	3.47 (0.12)[d]	1.26 (0.01)[d]	45.31[b]
%	56.99	11.10	15.85		5.63	7.66	2.78	

[a] Concentration in ng of pyrazine/g of syrup. [b] Not detected. [c] Relative percentage of individual pyrazine. [d] Results are means (standard deviations) of triplicate analysis. Mean scores with the same letter within the same column are not significantly different at 0.05 level.

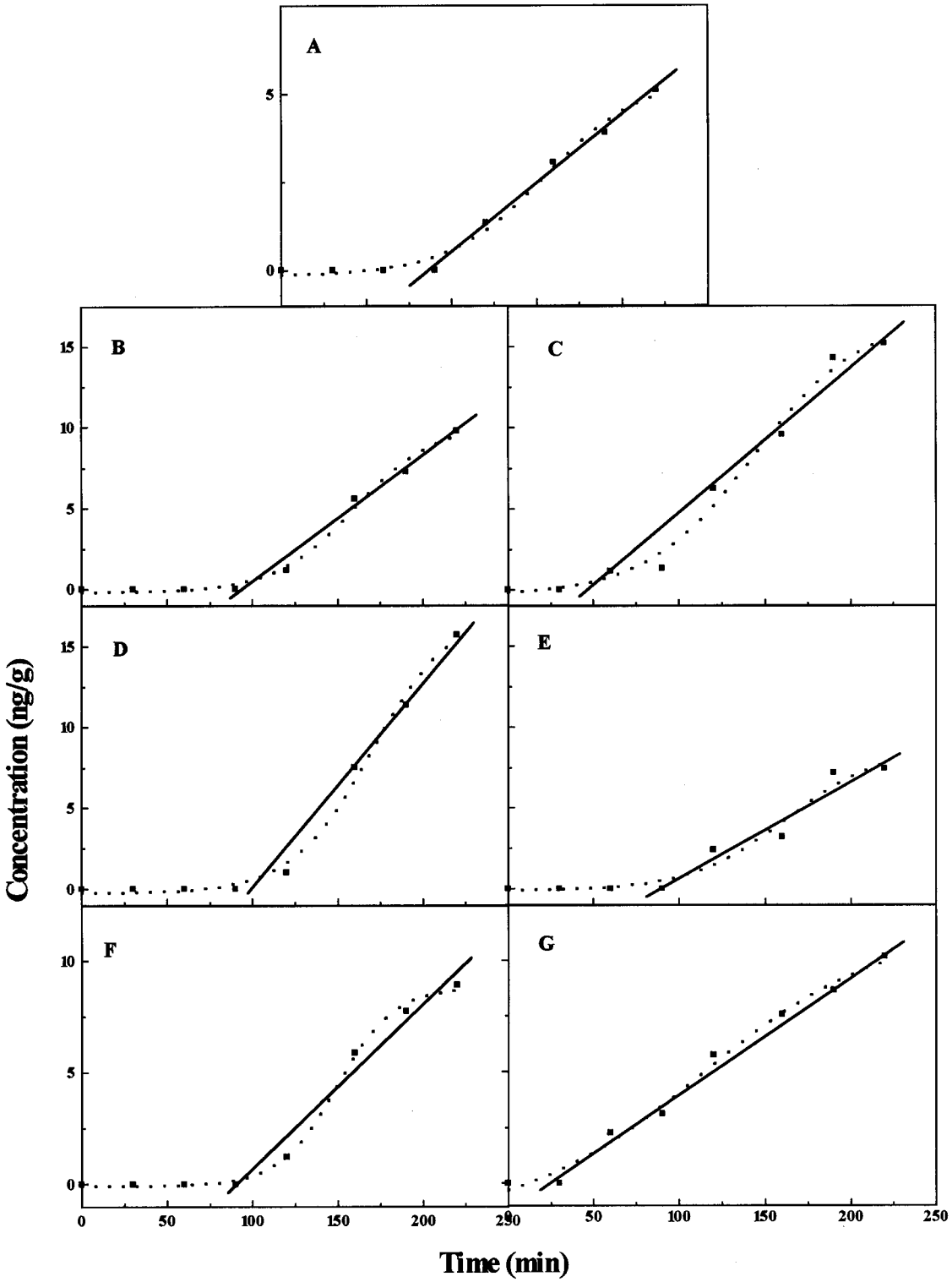

Figure 3. Accumulation of pyrazines during the boiling of maple sap: 2-ethyl-3-methyl- (A), methyl- (B), 2,5-dimethyl- (C), 2,6-dimethyl- (D), ethyl- (E), 2,3-dimethyl-(F), and trimethylpyrazine (G).

beans was also reported (Chaveron et al., 1989). It has been suggested that the formation of heterocyclic compounds, such as pyrazines, in Maillard reaction is followed by their decomposition that lead eventually to the formation of melanoidine (Reinccius et al., 1972). These authors showed a similar pattern for the formation of methyl-, 2,5-dimethyl-, trimethyl-, and tetramethylpyrazine during the first 30 min of roasting of cocoa beans at 150 °C. The results (Table 2) suggest that the formation of pyrazines in maple syrup was affected by the period of heating, pH, and water content of the sap. As a result of these effects, pyrazines were not detected before 60 min of heating, the time when TS increased from 3 to 63% and pH decreased from 9.2 to 7.8 (Table 2; Figure 1).

In the continuous boiling process, the heating of maple sap at 150 °C for a period of 220 min resulted in the formation of seven alkylpyrazines (Table 3). The boiling of maple sap with continuous addition of fresh sap for 30 and 40 min at 105 °C did not result in the formation of pyrazine compounds. After 60 min of heating, 2,5-dimethyl- and trimethylpyrazine were detected. The boiling of maple sap for 120 min at 105 °C resulted in the formation of methyl-, 2,6-dimethyl-,

Table 5. Rate Constants for Pyrazine Formation in Maple Syrup

pyrazine compounds	k (ng/min)[a]	R^2 [b]
2-methylpyrazine	0.08	0.98
2,5-dimethylpyrazine	0.09	0.95
2,6-dimethylpyrazine	0.13	0.98
2-ethylpyrazine	0.06	0.93
2,3-dimethylpyrazine	0.04	0.99
trimethylpyrazine	0.04	0.99
2-ethyl-3-methylpyrazine	0.07	0.97

[a] Rate constant of formation. [b] Linear correlation coeffecient.

ethyl-, 2,3-dimethyl-, and 2-ethyl-3-methylpyrazine. The accumulation of pyrazines increased as the time of heating increased (Figure 2). There was a steady increase in the formation of 2,5-dimethylpyrazine and a decrease in trimethylpyrazine after 150 min of heating. In addition, 2,6-dimethylpyrazine, which was detected after 120 min of heating, showed a rapid increase from 1.15 to 15.73 ng/g in the final syrup, a level comparable to that of 2,5-dimethylpyrazine (15.20 ng/g) formed after 60 min of heating. Methyl-, ethyl-, 2,3-dimethyl-, and 2-ethyl-3-methylpyrazine showed a slow increase to 9.77, 7.42, 5.10, and 8.93 ng/g, respectively, in the final syrup. Gwen et al. (1993) reported larger yields and distributions of pyrazines at basic pH values (9.00 and 9.64) than those at acidic conditions, in glucose−glycine model systems; 2,5-dimethyl- and trimethylpyrazine were predominant.

Laboratory prepared syrup (Table 3) contained lower proportions of 2,5-dimethyl- and 2,6-dimethylpyrazine (21.02 and 21.75%, respectively) and higher proportions of ethyl-, 2,3-dimethyl-, and 2-ethyl-3-methylpyrazine (10.26, 7.05, and 12.35%, respectively) than those previously reported in commercial syrups (Akochi-K et al., 1994); these differences could be attributed to the extended heating time (6−8 h) used in the preparation of commercial maple syrup compared to that of 220 min used for the preparation of syrup in laboratory.

The heating of commercial maple syrup for 30 min led to a decrease in the total level of pyrazines (Table 4), with a concurrent increase in 2-methylpyrazine and dramatic decreases in 2,5-dimethyl-, 2,6-dimethyl-, and trimethylpyrazine; however, 2,3-dimethyl- and 2-ethyl-3-methylpyrazine remained unchanged. After 50 min of heating, there was an increase in 2-methylpyrazine and a decrease in 2-ethyl-3-methylpyrazine. The changes in pyrazines content may have been the result of their volatilization or their involvement in the final stage of Maillard reaction (Reineccius et al., 1972).

Figure 3A−G shows the plots of pyrazine concentrations versus time of boiling of maple sap. An induction period, characteristic of the type of pyrazine, was associated with the formation of all identified pyrazines. This period could be attributed to the formation of precursors which interact to form pyrazines. After the induction period, the concentration of pyrazines increased linearly with time of boiling. This permitted the determination of the rate constants (k) for the formation of the pyrazines in maple syrup (Table 5). The k values for the pyrazines, generated by heating maple sap for 220 min at 105 °C, ranged from 0.04 to 0.13 ng/min. The slope of the linear section of the plots were characterized by a rate of *pseudo-zero*-order reaction. These findings indicate that the accumulation of pyrazines may not be dependent on the concentrations of the initial reactants (Stamp and Labuza, 1983). Leahy and Reineccius (1989) indicated that k values were dependent on the model and the temperature of heating. Huang et al. (1989) reported a *pseudo-zero*-order rate for the formation of pyrazine, methyl-, and 2,6-dimethylpyrazine in model systems, with activation energies of 19.5, 24.8, and 20.8 kcal/mol, respectively. The reported k values for methyl- and 2,5-dimethylpyrazine at 120 °C were 0.38 and 0.08 ng/min, respectively.

These results suggest that pyrazine formation in maple syrup could be separated into two main stages, the induction period which corresponds to the formation of the necessary precursors and the second stage which corresponds to the actual formation of pyrazines. This two-step process could explain the observed lack of a mathematical relationship to describe the formation of pyrazines. This is similar to observations reported for the formation of pigments in Maillard reactions (Labuza and Satlmarch, 1981). This lack of quantitative relationship could be explained by a high reactant/product ratio (Leahy and Reineccius, 1989) as well as by the occurrence of intermediate steps and competitive simultaneous reactions.

CONCLUSION

The analyses of maple sap showed the presence of sucrose, glucose, fructose, and trace amounts of amino acids. These are known precursors that participate in the formation of alkylpyrazines in foods. Pyrazines were formed in boiling sap after 60 min of heating. However, extended heating of maple syrup resulted in changes of individual and total pyrazine concentrations. The formation and accumulation of methyl-, 2,5-dimethyl-, 2,6-dimethyl-, ethyl-, 2,3-dimethyl-, trimethyl-, and 2-ethyl-3-methylpyrazine in maple syrup were influenced by temperature, time of heating, and pH of boiling maple sap. Rate of accumulation for each identified pyrazines in maple syrup was preceded by a period of induction. The period of induction and rate of accumulation were characteristic for each pyrazine.

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