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CONTRIBUTION OF ALKYLPYRAZINES TO THE FLAVOR OF MAPLE SYRUP

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Abstract

Alkylpyrazines contribute to the flavor and aroma of numerous thermally processed foods. Several of these compounds have been identified in maple syrup, the thermally processed product from maple sap. It is postulated that the pyrazine compounds in maple syrup result from thermally induced reactions between amino acids and reducing sugars present in maple sap. The quantities of various alkylpyrazine compounds in graded samples of maple syrup (Canada amber, medium and light), were determined by gas liquid chromatography. The level of individual pyrazines in the various syrups ranged from 2 ng to 33 ng/g of sample. The same samples of maple syrups were evaluated for flavor and taste preferences by an untrained sensory panel. The results indicated that the content of total alkylpyrazines in medium maple syrup was significantly higher ($p < 0.05$) than those of the other maple syrup and that the taste preference of amber was significantly lower ($p < 0.05$) than that of light syrup. Amber and medium syrups which were preferred over the light syrup contained higher levels of 2,3-dimethyl and 2-ethyl-3-methyl pyrazines but lower lels of 2,5-dimethylpyrazine when compared with the light syrup. The amber syrup which was jugded to have the highest maple flavor contained the lowest quantity of trimethylpyrazine.

Introduction

Maple syrup, in North America in general and in Québec in particular, is a well established speciality food product. The consumption of this product is directly related to its unique, characteristic "maple flavor"; marketability therefore depends on this flavor. The flavor of maple syrup, like that of many heat processed foods, is derived during processing. Maple syrup is derived from sap of maple tree (*Acer Saccharum MARSH*) through thermal processing. The freshly drawn sap does not reflect the characteristic and unmistakable maple syrup flavor, which gradually develops during the thermal processing of sap (Nelson, 1928; Findlay *et al.* 1935). Over the years, scientists have identified in maple syrup, several flavor compounds which are believed to produce the distinctive maple flavor; These include phenolic compounds (Filipic *et al.* 1965), carbonyl compounds (Kallio, 1988; Alli et al. 1990), alcohols and acids (Kallio, 1988). Recently, Alli *et al.* (1990) introduced pyrazine compounds into the list of maple flavor components; these included 2-methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2-ethylpyrazine, 2,3-dimethylpyrazine, 2,3,5-trimethylpyrazine and 2-ethyl-3-methylpyrazine. However, the extent of the contribution of these volatiles to maple flavor has not beeen established.

Alkylpyrazines contribute both desirable and undesirable flavors to processed foods , mainly nutty, roasted and burnt notes (Maga, 1982). Pyrazine compounds are formed in heat treated foods by Maillard reaction, which involve reducing sugar and amino compounds. Sucrose along with traces of reducing sugars (glucose and fructose) were found in maple sap (Jones and Alli ,1987; Leech and Kim, 1990); in addition, relatively minor quantities of amino acids (glutamine, glutamic acid, asparagine and proline) have been reported in maple sap (Morselli *et al.* 1986; Ahtonen and Kallio, 1987; Kallio, 1988; Rizzi, 1972; Shibamoto and Bernhard, 1977, 1978).

Maple syrups are categorized according to their color with light syrups having higher market value when compared to darker (amber) syrups (Sendak, 1982); However, panelists in sensory tests have shown a preference for darker syrup (Belford *et al.* 1991), indicating an inconsistency between comsumer preference and maple syrup pricing. It is evident therefore that a more thorough understanding of the role of flavor compounds to the characteristic maple flavor is required in order to assign grades for flavor quality and preference. The present work was aimed at quantitating pyrazine compounds in three graded maple syrup and investigating the relationship between pyrazines and sensory characteristics (taste) of the syrup.

Material and methods

Material

Commercial samples of pure maple syrups graded as Canada #1 light, Canada #1 medium and Canada #1 amber were purchased from Les Producteurs de sirop d'érable du Québec (Plessiville, Québec). Pyrazine standards were purchased from Aldrich Chemical Co. (Milwaukee, WI.). Diethylether and dichloromethane chromatographic grade were obtained from BDH (Montréal, Canada). Analytical grade sodium salt (NaCl) was purchased from Fisher Co. (Montréal, Canada).

Methods

Preparation of pyrazine standards

Solutions of pyrazine standards were prepared by dissolving known weights of each of the following pyrazines in 100 mL of dichloromethane: 2-methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2-ethylpyrazine, 2,3-dimethylpyrazine, 2,3,5-trimethylpyrazine and 2-ethyl-3-methylpyrazine. Pyrazine was used as internal standard.

Extraction of pyrazines

Pyrazines were extracted using a modification of the method described by Alli et al. (1990). A quantity 30 g of sodium chloride was solubilized in 100 mL distilled water. To this solution, 100 g of maple syrup sample was added with mixing. The mixture was adjusted to pH 3 by addition of HCl solution (11%), and the mixture was stirred at medium speed with a magnetic stirrer for 10 min. Diethylether (300 mL) was added and the mixture stirred for a further 30 min and the organic and aqueous phases were then separated using a separatory funnel. The aqueous phase was titrated with a solution of NaOH (30%) to pH 12 and extracted with 5 X 20 mL portions of dichloromethane. The dichloromethane extracts were then concentrated to approximately 5 mL by rotary evaporation and then concentrated to 0.5 mL using a stream of nitrogen. Pyrazine (10 ng) was added as internal standard and magnesium sulfate was added to serve as a drying agent.

Gas chromatographic analysis

The extracted pyrazines were analyzed using a Varian Model 3700 gas chromatograph equipped with a flame ionization detector. Conditions for separation were as follows: fused silica capillary column (30 m length x 0.32 mm i.d. with a 0.1 micron film thickness) supelcowax 10™ (Supelco, Canada); injector port temperature, 200 ^{O}C; detector temperature, 250 ^{O}C; nitrogen carrier gas flow rate, 1mL/min. Oven temperature was programmed from 40 ^{O}C to 150 ^{O}C at a rate of 1 ^{O}C/min with a 6 min initial temperature hold. Chromatograms were recorded and quantitated using a Hewlett-Packard model HP-3390A integrator. Samples were analyzed in triplacate.

Sensory evaluation of maple syrups

A randomized complete block design experiment was used to evaluate the sensory characteristics of maple syrup samples. The samples were rated using a descriptive sensory test with unstructured scaling. The tests were repeated weekly over a five-week period with each week representing a replicate. The sensory panel was composed of twenty untrained volunteers (twelve females and eight males) ranging in age from 22 to 45 years. The selection of the panelists was based on a pre-screening, which established their capability to discriminate between authentic maple syrup and non-maple syrup. The experiment was performed in a sensory evaluation room maintained at 22^{O}C and illuminated with red lighted to eliminate any bias due to differences in sample color.

A quantity (15 g) of 3 digits coded samples were presented to the panelists in a unbalanced random order. The panelists were instructed to consume at least one third of each sample for the entire test. Mineral water (250 mL) was provided for clearing of taste buds between samples. The panelists rated each sample on an unstructured horizontal line of 10 cm in length. Data was collected by measuring the intensity of the ratings and subjected to statistical analysis using the SAS program (SAS Institute Inc., Cary, North Carolina).

Results and discussion

Figure 1 shows a chromatogram of the pyrazines standards which were selected on the basis of previous identification in maple syrup (Alli et al. 1990). Figure 2 represents the chromatogram obtained from analysis of pyrazines in maple sap. The results indicate that no pyrazine compounds are present in the maple sap extract. The fact that pyrazines are present in maple syrup but not in the sap from which the syrup is prepared indicate that pyrazines in the syrup are formed during processing. Typical chromatograms obtained from analysis of pyrazines in "light", "medium" and "amber" grades are shown in Figures 3, 4 and 5. The identified pyrazines and their concentration in maple syrup are shown in Table 1. The results indicate that 2,6-dimethylpyrazine was the alkylpyrazine present in highest concentration in the three maple syrup samples, and represented as much as 46% of the total pyrazine concentration in the "medium" syrup sample. 2-methylpyrazine, 2,5-dimethylpyrazine and 2,6-dimethylpyrazine were present in comparable quantities in the "light" and "amber" grade syrup samples. These three pyrazine compounds (2-methylpyrazine, 2,5-dimethylpyrazine and 2,6-dimethylpyrazine) as well as 2,3,5-trimethylpyrazine have been reported to be the most abundant and readily formed pyrazine compounds in both model and actual food systems. Koehler and Odell (1970) reported on 2-methylpyrazine and 2,5-dimethylpyrazine from a glucose-asparagine model system. Shibamoto and Bernhard (1976) observed that 2-methylpyrazine represented the principal pyrazine compound (86%) when compared to other alkylpyrazines from glucose-ammonia systems. Chaveron (1989) reported that large quantities of 2,3,5-trimethylpyrazine are formed in roasted cocoa vapor. Ethylpyrazine was detected in trace amount in the three samples of maple syrup; this trace quantity level of 2-ethylpyrazine is similar to that reported by Shibamoto and Bernhard (1976), suggesting that 2-ethylpyrazine could represent a minor component formed during normal heat processing conditions. These results clearly indicate difference in both the total pyrazine concentration and the concentration of individual pyrazines in the different grades of maple syrup.

Statistical analysis of the results from sensory panelists are shown in Tables 1 and 2. These results indicate that the "amber" maple syrup (darkest color syrup) was judged to have the highest degree of maple flavor attribute, as well as the highest taste preference; this is in agreement with the finding of Belford *et al.*(1991) who also reported that sensory panelists indicated a taste preference for darker colored maple syrup over lighter colored maple syrup. The total pyrazine content (49 ng/g) of the "amber" (darker colored) syrup which was significantly lower ($p < 0.05$) than the total pyrazine content of the lighter (light, medium) syrups was judged to be the preferred syrup with the highest maple flavor attribute. This suggests an inverse relationship between maple syrup flavor and total pyrazine concentration. The "medium" and "amber" syrups (1) were comparable in taste preference and contained comparable quantities of 2,3-dimethyl-, 2,5-dimethyl- and 2-ethyl-3-methyl- pyrazines, and (2) were preferred over the "light" syrup which had a lower quantity of 2,3-dimethyl- and 2-ethyl-3-methyl- pyrazines but higher quantity of 2,5-dimethylpyrazine. This suggests that of the pyrazines identified, 2,3-dimethyl-, 2,5-dimethyl- and 2-ethyl-3-methyl- pyrazines migth play some role in the taste preference of maple syrup; this hypothesis needs to be developed further. Similarly, the "amber" syrup which was significantly different in maple flavor when compared with "light" and "medium" syrups contained significantly lower quantities of trimethylpyrazine, suggesting a possible inverse relation of trimethylpyrazine to maple flavor.

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Table 1: Concentration of pyrazines in maple syrup (ng/g of sample)

	2-met	2,5-dim	2,6-dim	2-eth	2,3-dim	trim	eth-met	Total
light	7.18(0.17)	16.93(0.63)	20.16(0.31)	tr	1.70(0.04)	7.79(0.13)	3.53(0.09)	57.29
(%)*	12.53	29.54	35.20		2.97	13.59	6.17	
medium	12.93(0.71)	10.20(0.97)	32.75(1.05)	tr	3.65(0.39)	6.36(0.22)	4.78(0.91)	70.68
(%)*	18.29	14.43	46.34		5.16	9.00	6.77	
amber	6.91(0.09)	12.19(0.49)	16.82(0.56)	tr	3.62(0.16)	4.23(0.29)	5.09(0.11)	48.89
(%)*	14.14	24.94	34.41		7.42	8.66	10.41	

Results are means of triplicate analysis
Values in ( ) refer to standard deviations
*, percentage of individual pyrazine in the sample
tr, trace amount
2-met = 2-methylpyrazine
2,5-dim = 2,5-dimethylpyrazine
2,6-dim = 2,6-dimethylpyrazine
2-eth = 2-ethylpyrazine
2,3-dim = 2,3-dimethylpyrazine
trim = 2,3,5-trimethylpyrazine
eth-met = 2-ethyl-3-methylpyrazine

Table 2: Flavor quality and preference rating of maple syrup samples

	attribute	
syrup sample	maple flavor	preference
light	5.98[a]	4.53[a]
medium	5.78[b]	4.92[b]
amber	6.74[b]	5.11[b]

Means were compared using the Duncan's new multiple range test
Mean scores with the same letter within the same column are not significantly different at 0.05 level

Appendix 1 : Analysis of variance (ANOVA) for maple syrup flavor

Source	df	Some of squares	Mean square	F-value
samples	2	79.11	39.56	12.00**
judges	16	401.92	25.12	7.62**
experiments	2	3.62	1.81	0.55ns
judges * samples	32	149.97	4.69	1.42ns
Error	58	191.15	3.30	

ns, not significant at 0.05 level
**, significant at 0.01 level

Appendix 2 : Analysis of variance (ANOVA) for maple syrup preference

Source	df	Some of squaress	Mean square	F-value
samples	2	10.13	5.06	2.48ns
judges	18	152.23	8.46	4.15**
experiments	4	3.07	0.77	0.38ns
judges * samples	36	199.47	5.54	2.72**
Error	131	267.01	2.04	

ns, not significant at 0.05 level
**, significant at 0.01 level

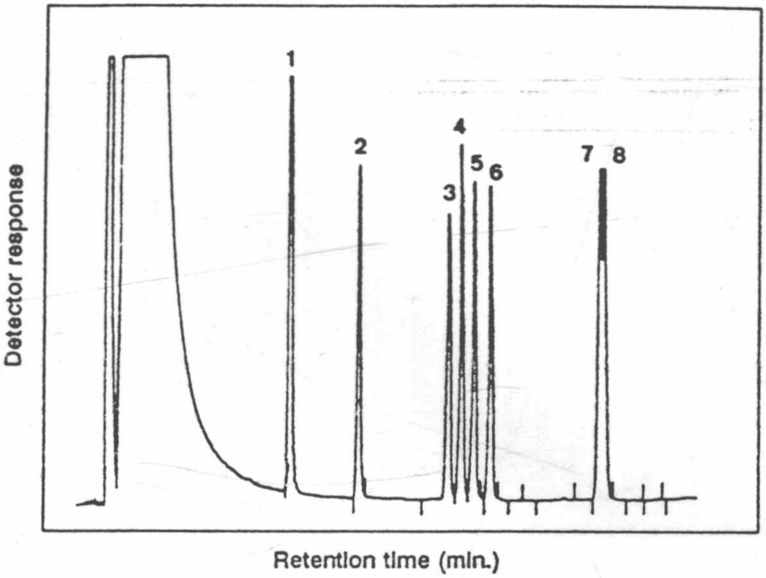

Figure 1: Chromatogram of pyrazine standards

1 = pyrazine
2 = 2-methylpyrazine
3 = 2,5-dimethylpyrazine
4 = 2,6-dimethylpyrazine
5 = 2-ethylpyrazine
6 = 2,3-dimethylpyrazine
7 = 2,3,5-triméthylpyrazine
8 = 2-ethyl-3-methylpyrazine

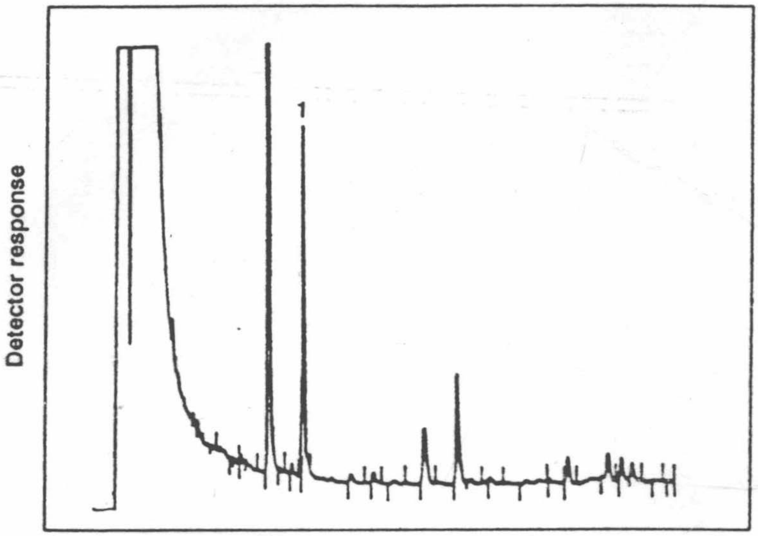

Figure 2: Chromatogram of pyrazines in maple sap

1 = pyrazine	5 = 2-ethylpyrazine
2 = 2-methylpyrazine	6 = 2,3-dimethylpyrazine
3 = 2,5-dimethylpyrazine	7 = 2,3,5-triméthylpyrazine
4 = 2,6-dimethylpyrazine	8 = 2-ethyl-3-methylpyrazine

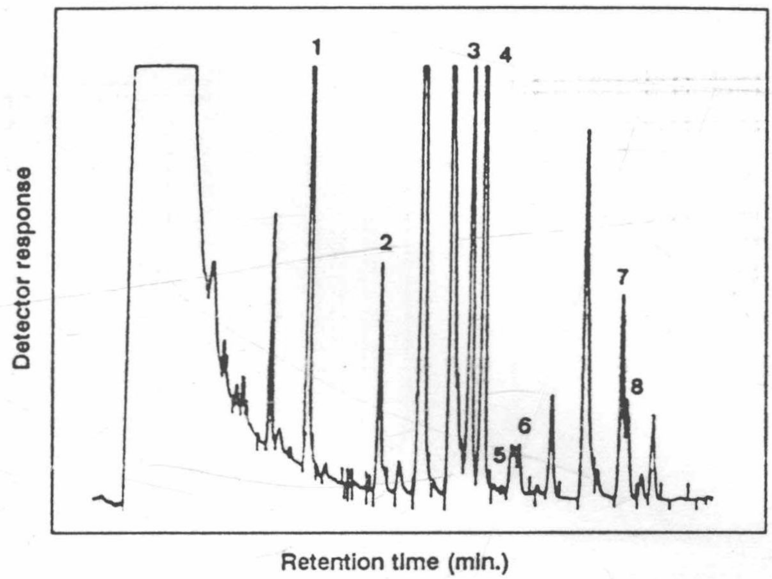

Figure 3: Chromatogram of pyrazines in "light" maple syrup

1 = pyrazine	5 = 2-ethylpyrazine
2 = 2-methylpyrazine	6 = 2,3-dimethylpyrazine
3 = 2,5-dimethylpyrazine	7 = 2,3,5-triméthylpyrazine
4 = 2,6-dimethylpyrazine	8 = 2-ethyl-3-methylpyrazine

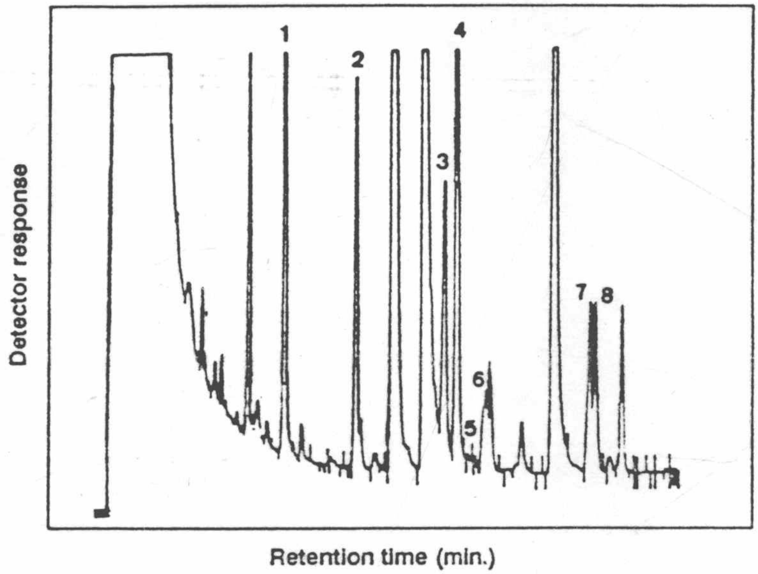

Figure 4: Chromatogram of pyrazines in "medium" maple syrup

1 = pyrazine	5 = 2-ethylpyrazine
2 = 2-methylpyrazine	6 = 2,3-dimethylpyrazine
3 = 2,5-dimethylpyrazine	7 = 2,3,5-triméthylpyrazine
4 = 2,6-dimethylpyrazine	8 = 2-ethyl-3-methylpyrazine

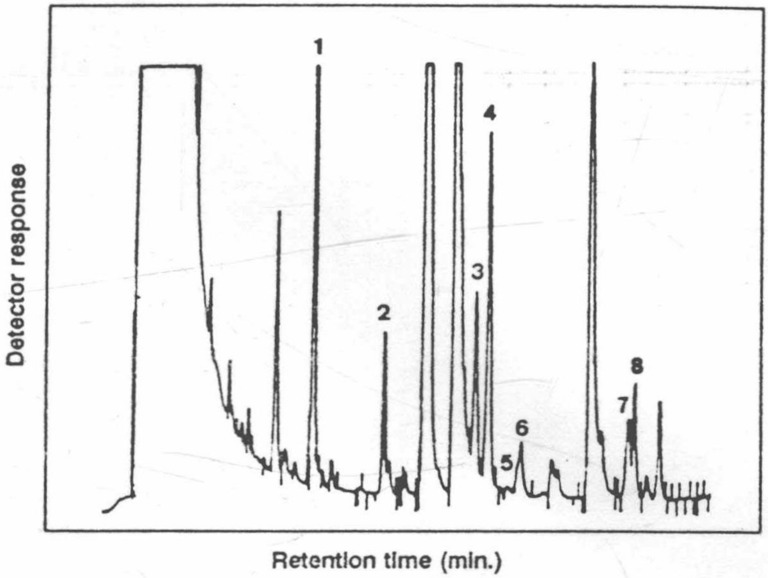

Figure 5: Chromatogram of pyrazines in "amber" maple syrup

1 = pyrazine	5 = 2-ethylpyrazine
2 = 2-methylpyrazine	6 = 2,3-dimethylpyrazine
3 = 2,5-dimethylpyrazine	7 = 2,3,5-triméthylpyrazine
4 = 2,6-dimethylpyrazine	8 = 2-ethyl-3-methylpyrazine

Figure 6: Percent of total identified pyrazines in maple syrups