THE FORMATION OF PYRAZINE COMPOUNDS AND THEIR CONTRIBUTION TO MAPLE SYRUP FLAVOR

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Short Tittle :

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ABSTRACT

The formation of pyrazines and their contribution to maple syrup flavor were investigated. Maple syrup was prepared in laboratory conditions. The pH of boiling sap increased from 7.2 to 9.2 followed by a decrease to a final pH 7.3. The decrease pH coincided with an increase of total dissolved solids. Gas chromatography/mass spectrometry analysis of extracts from commercial maple syrups, laboratory prepared maple syrup, commercial non-maple syrups, and artificial maple flavorings revealed the presence of 2-methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2-ethylpyrazine, 2,3-dimethylpyrazine, trimethylpyrazine, 2-ethyl-3-methylpyrazine, tetramethypyrazine, 2-ethyl-5-methylpyrazine, 2-ethyl-6-methylpyrazine, 2-ethyl-3,5-dimethylpyrazine and 3-ethyl-2,5-dimethylpyrazine. The number and total concentration of pyrazines in laboratory prepared maple syrup increased with time of processing. A period of induction preceding their formation and their rates of accumulation was observed. Heating of commercial maple syrup resulted in the decrease of pyrazine concentrations. This suggests that the distribution and content of certain pyrazines are affected by processing. A comparative study of the quantities and distribution of pyrazines in various samples was carried out. No pyrazines were found in maple sap. Total pyrazine content of the medium maple syrup was significantly higher ($p < 0.05$) than those of the light and amber syrup. Different pyrazine distribution patterns were observed for the three maple syrups as well as for the non-maple syrup samples. Sensory evaluation of maple syrups was carried out for "maple flavor" and taste preferences by untrained panelists. The results indicated that the taste preference of the amber graded syrup which contained the lowest total pyrazines was significantly higher ($p < 0.05$) than that of the light grade syrup. An inverse relationship between maple syrup flavor and total pyrazine concentration was observed. The results showed that certain pyrazines might contribute to the over all maple syrup flavor character.

RÉSUMÉ

La formation des pyrazines et leur contribution à la saveur du sirop d'érable a été étudiée. A cet effet, du sirop d'érable a été préparé au laboratoire. le pH de la sève en ébullition passait d'une valeur initiale de pH 7.2 à une valeur de pH 9.2, puis diminuait jusqu'à une valeur de pH 7.7. La diminution de pH coïncidait avec une hausse de la teneur en solides dissouts. L'analyse chromatographique en phase gazeuse et la spectrométrie de masse d'extraits de sirops d'érable authentiques, de sirops ne provenant pas de l'érable, et d'échantillons d'arôme artificiel d'érable ont démontré la présence de 2-méthylpyrazine, 2,5-diméthylpyrazine, 2,6-diméthylpyrazine, 2-éthylpyrazine, 2,3-diméthylpyrazine, triméthylpyrazine, 2-éthyl-3-méthylpyrazine, tétraméthylpyrazine, 2-éthyl-5-méthylpyrazine, 2-éthyl-6-méthylpyrazine, 2-éthyl-3,5-diméthylpyrazine et 3-éthyl-2,5-diméthylpyrazine. Le nombre et la concentration en pyrazines dans le sirop fabriqué en laboratoire s'est accru avec le temps de chauffage. Une période d'induction a précédé leur formation. Un chauffage prolongé du sirop entraine une diminution de la concentration en pyrazines. Ceci démontre que la formation et la concentration de certains pyrazines sont influencées par le procédé de fabrication. La comparaison des pyrazines dans les échatillons analysés a démontré l'absence de pyrazine dans la sève d'érable qui sert à la préparation du sirop. La teneur en pyrazines totales du sirop de grade moyen était statistiquement supérieur ($p < 0.05$) à ceux des sirops clair et ambré. Des différences ont été aussi observées dans le cas des autres échantillons. Une évaluation sensorielle a été entreprise pour différencier les sirops du point de vue de leur saveur d'érable et de la préférence des panelistes. L'étude a montré que le sirop ambré avait une saveur d'érable plus prononcé; ce sirop renfermait moins de pyrazines que le sirop clair; ce qui signale la présence d'une relation inverse entre la teneur en pyrazines et la saveur d'érable. Ces résultats montrent que les pyrazines contriburaient à la saveur caractéristique du sirop d'érable.

CLAIMS OF ORIGINAL RESEARCH

1) This study established laboratory conditions that simulate the industrial processing of maple sap to syrup. These conditions included the control of the rate of sap evaporation, time of boiling, adequate volume and rate of addition of fresh sap to achieve a minimum disturbance in the boiling sap in terms of temperature change during processing.

2) These experiments represent the first systematic study directed at investigating the conditions of formation and quantity of pyrazine compounds in maple syrup. This resulted in the identification of six new pyrazine compounds in maple syrup: 2-ethyl-3-methylpyrazine, 2-ethyl-6-methylpyrazine, 2-ethyl-5-methylpyrazine, 2-ethyl-3,5-dimethylpyrazine, 3-ethyl-2,5-dimethylpyrazine and tetramethylpyrazine.

3) This study represent the first study to demonstrate that the pyrazine compounds identified in maple syrup are absent in maple sap from which the syrup is prepared, therefore they are formed during processing.

4) This study has shown, for the first time, that the formation of pyrazines in maple syrup is preceded by an induction period. The pyrazine compounds of maple syrup have different rates of formation that satisfy a *pseudo*-zero order reaction rate.

5) This is the first study directed at investigating the contribution of pyrazines to maple syrup flavor. Total and individual concentrations and distribution of pyrazine compounds in different maple syrup grades have shown to significantly influence the perceived maple flavor and taste of the syrup.

LIST OF PUBLICATIONS

Part of this research has been published as follows:

Akochi-K. E., Alli I., Kermasha S., Yaylayan V. and Dumont J., 1994. Quantitation of alkylpyrazines in maple syrup, maple flavors and non-maple syrups. Food Research International, 27, 451-457.

Akochi-K. E., Alli I. and Kermasha S., 1993. Contribution of alkylpyrazines to the flavor of maple syrup. In *Food Flavors, Ingredients and Composition.* Food Science and Human Nutrition Series, vol. 32. Charalambous G. ed. Elsevier, Amsterdam, pp 729-743.

Copies of these manuscripts are listed respectively as Appendix 3 and Appendix 4 in this thesis.

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*This thesis is dedicated to the memory of **Dr. Ayewa Koblé Lucien**. I miss you, still.*

May you all be blessed.

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I. INTRODUCTION

Maple syrup, the sweet syrup obtained from the sap of maple sugar tree (*Acer saccharum* Marsh) is characterized by its unique flavor also called "maple flavor". The unique character of this product is also reinforced by the limited region in which the maple sugar tree grows. The tree is native to northeastern United States and, Québec and Ontario provinces of Canada. One other factor which expresses the characteristic uniqueness of maple syrup, is that attempts to produce artificial or synthetic maple syrup have not been successful because the subtle and total flavor of authentic maple syrup could not be reproduced.

Traditionally, the conversion of maple sap to syrup involves concentration of dissolved solids by boiling the sap for several hours until a desirable concentration of sugars is achieved. The characteristic flavor of maple syrup, like that of many processed foods, is derived during processing. During this heating process, numerous thermally-induced reactions occur. The products from these reactions can be important contributors to the flavor and aroma of the final syrup. Thermal degradation of sugars present in the sap, under alkaline conditions, can form 3-methyl-2-cyclopentene-2-ol-1-one (cyclotene®), furaneol and related carbonyl compounds of maple syrup. Lignin monomers of maple sap can oxidize by heating under alkaline conditions into vanillin, syringaldehyde, dihydroconferyl alcohol and other related phenolic compounds of maple syrup. Heat induced reactions between amino acids and

reducing sugars (the Maillard reaction) can result in the formation of pyrazine compounds.

Pyrazines (1,4-diazines) are heterocyclic, nitrogen containing compounds found in numerous processed foods and naturally occurring in many plant foods. The presence of alkylpyrazines (substituted alkyl derivatives of 1,4-diazine) in foods contribute characteristic flavor notes such as roasted, nutty, toasted, burned, popcorn and mashed potato. These compounds are regarded as the key flavor components of many fresh foods as well as thermally processed foods based on the evidence accumulated over the past ten to fifteen years. Several researchers have studied the formation of pyrazines from Maillard reactions which involve reducing sugars and amino acids and nitrogen bases such as ammonia. Mechanisms for the formation of pyrazines have been

The objectives of the present study were to investigate the presence and identification of alkylpyrazines in maple syrup and their possible contribution to maple flavor characteristics. In particular, investigations were directed at *1)* the determination of the types and quantities of pyrazine compounds in maple syrup of different grades of quality, *2)* the evaluation of the sensory characteristics of the syrups and determination of the relationship, if any, between the type and quantity of pyrazine compounds and the sensory (flavor and aroma) of the different quality levels of maple syrup. In addition, the effects of the processing conditions (time / temperature of heating of sap) on the types of pyrazine compounds were determined.

The hypothesis of this study is that the types and levels of pyrazines found in maple syrup are determined by the processing conditions of the sap. It is likely, therefore, that the levels of pyrazine compounds could be related to the quality of maple syrup, particularly the flavor quality. If it can be established that pyrazines contribute to maple syrup flavor then quantitative measurements of the types and quantities of the pyrazines could lead to the development of an objective method for assessing the flavor quality of maple syrup.

II. LITERATURE REVIEW

Flavor and aroma are considered to be a critical acceptance characteristics of foods and foodstuffs and affect our daily selections of foods. The importance of flavor in human dietary habits dates back to centuries, when specific spices and fine herbs were selected for conditioning the flavor of meals. Acceptability was based on perceptual information, such as organoleptic evaluation (Berglund *et al.*, 1974). This is still practiced widely. In addition, the importance of flavor in consumer acceptance of food has led to the development of a food flavor industry devoted to studying all aspects of flavor, from basic chemistry to product applications.

Flavor is perceived when a foodstuff taken in the mouth releases volatile compounds which bind to odor receptors and trigger a specific response in the brain (McCord 1949). The terms aroma and flavor will be used interchangeably in this thesis, while recognizing the broader meaning of the latter.

The development of flavor in foods and foodstuffs can result from microbial (biosynthesis and biotransformation) and enzymatic activities or induced during processing (Heath and Reineccius, 1986). Regardless of its origin, the chemical composition of flavor has been shown to be complex. Flavor compounds include alcohols, acids, carbonyls, heterocyclic compounds, terpenoids, aromatic and carbocyclic compounds, to name only a few. These compounds vary tremendously in structures and properties, and their formation is ascribed to the presence of precursors such as lipids, carbohydrates, and proteins present in food systems.

Consumer preferences for natural products along with advances in biotechnology have led to the development of microbially derived flavors. Microbially derived flavors are secondary metabolites and are therefore produced in small quantities (Murray and Duff, 1991). These flavor compounds include esters, alcohols, acids and a large number of others chemical compounds. Low production yields and high cost of flavor recovery are the major obstacles facing microbial flavor production. However, advances in biotechnology (physiological and genetic manipulation, protein engineering) and advances in extraction technology are moderating the high cost of recovery of microbial flavors (Sprecher and Hanssen, 1985).

2.1. Legislation of flavor compounds

Food flavor and many of the chemical based food additives (colorings, sweeteners, flavor potentiators, vitamins) are regulated. The need for regulation is aimed at protecting the consumer from real or potential health risks resulting from the consumption of added substances to processed foods. Legislation is also aimed at preventing fraud in relation to the true nature of the products advertised by the food manufacturer (Hardinge, 1986). From the above, it is understood that the regulation targets only material added to foods. However, consumer demands for natural products and advances in biotechnology have resulted in demands for a redefinition of the term "natural" as to identify the origin of food flavor (Murray and Duff, 1991). In the USA and many other industrial countries, two classes of flavor chemicals exist: "natural" and "artificial". The US Code of Federal Regulations defines as "natural" (Anon, 1988; Welsh *et al.*, 1989): *"The essential oils, oleoresin, essence or extractive, protein hydrolysate, distillate, of any product of roasting, heating or enzymolys (hydrolysis and all enzymatic process with no cell proliferation)., which contains the flavoring constituents derived from a natural source including edible yeast, meat products, dairy products or fermentation products thereof whose significant function in food is flavoring rather than nutrition."*

2.2. Naturally occurring flavor compounds

Enzymatic and microbial activities are responsible for the production of many of the flavor compounds that contribute desirable and undesirable natural

flavors to fresh fruits and vegetables, and processed foodstuff such as cheese and other dairy products, alcoholic beverages such as wine, beer and cider. Many volatiles from fresh fruits and vegetables are not released until the cellular structure of the tissues is disrupted by cutting, blending and chewing. These actions decompartmentalize enzymes which react with substrates.

In the formation of the flavor of onion (*Allium* cepa, L.), the precursor responsible for the flavor, S-(1-propenyl)-L-cysteine sulfoxide, undergoes a rapid hydrolysis by the enzyme allinase to yield sulfenic acid intermediates which yield thiopropanal-S-oxide, a compound with lacrimatory properties, upon further rearrangements (Lindsay, 1985) (Fig. 1). Another example is the formation of green-earthy notes that contribute to the recognition of many fresh vegetables. These notes are developed by methoxy-pyrazines; for instance, 2-methoxy-3-isobutylpyrazine responsible for the characteristic flavor of bell pepper.

Lipoxygenase activities, the oxidative breakdown of unsaturated fatty acid in plants, are associated with the development of flavors reminiscent of ripened fruits (Gardner, 1975, Buttery, 1981). The ripening aromas of fruits such as banana and apple are derived from enzymatic activities involving branched-chain amino acids and the production of esters (Tressl *et al.* 1975). For instance, the chain length of leucine is shortened by decarboxylation

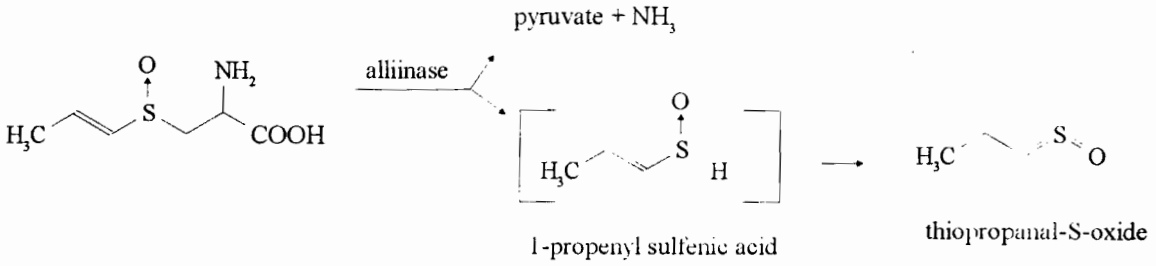

Figure 1: Biosynthesis of fresh onion flavor *(Lindsay, 1985).*

accompanied by the loss of the amino group to yield an aldehyde, which is subjected to further transformations to produce isoamyl acetate and ethyl-3-methylbutyrate, responsible for banana flavor and apple flavor, respectively (Fig. 2). Intermediates (aldehydes, acids, alcohols) produced during this transformation are also important contributors to the overall aroma.

The flavors of citrus fruits and many herbs are due largely to terpenes and sesquiterpenes. These are formed enzymatically through the so called isoprenoid (C5) pathways whereby mevalonic acid pyrophosphate is transformed into *cis* and *trans* terpene isomers with characteristic flavor attributes (Eskin, 1979).

The taste and aroma of various fermented foods are the res0ult of microbial activities. The following is a short description of some important food flavor compounds:

2.2.1. Esters

Esters are constituents of many flavors from fruits and foodstuffs. Esters produced by yeasts, mold and bacteria express fruity or flowery aromas (Bauer and Garbe, 1985). The routes to natural esters are either by alcoholysis of acyl-CoA (Norrstrom, 1963) or by direct esterification of organic acids in the presence of alcohol. Figure 3 shows the general mechanism for the microbial formation of esters (Murray and Duff, 1991). Of the flavor esters, straight chain aliphatics are most common. Acetate esters of alcohols up to 6 carbons express fruity notes, while 8, 10 and 12 carbons acetates express floral notes. Branched

isoamyl acetate
(banana flavor)

ethyl-3-methylbutyrate
(apple flavor)

Figure 2: Structures of frutty flavor esters .
These esters are derived from the degradation of leucine by the actions of S. lactis in plants (Lindsay, 1985).

esters (isoamyl esters) or saturated esters (hexyl esters) are all important flavor sources. Table 1 lists esters found in wine (Murray and Duff, 1991); these compounds have low flavor thresholds therefore their contribution to wine flavor could be appriciable (Berry and Waston, 1987). Aromatic esters: methyl, ethyl and benzyl benzoate, and methyl salycilate are mostly used in perfume manufacturing (Bauer and Garbe, 1985).

Various microorganisms are involved in the formation of esters. *Candida utilitis* and *Hansenula anomala* are reported to convert ethanal to ethyl acetate and ethyl butyrate (Armstrong *et al.*, 1984). Butyl butyrate is largely produced by lipase from *Candida cylindracea*. A patent filed by Farbood *et al.* (1987) describes the use of yeast such as *Geotrichum fragrans* for the production of alkylesters from carboxylic acids in the presence of 5 carbon and 6 carbon amino acids such as valine, isovaline and leucine.

Bacteria and fungi are also known for their ability to be involved in the direct esterification of alcohols and acids (Hosono and Elbiot, 1974; Hosono *et al*, 1974).

2.2.2. Alcohols

Alcohols have high flavor thresholds and therefore are sometimes considered as flavor compounds; for instance, isopropanol has a flavor threshold of 1.5 g/L (Berry and Waston, 1987). Alcohols are formed from α-keto acids or biosynthesized from the breakdown of amino acids and the metabolism of sugars. *Clostridium acetobutylicum* produces propanol and pentanol from

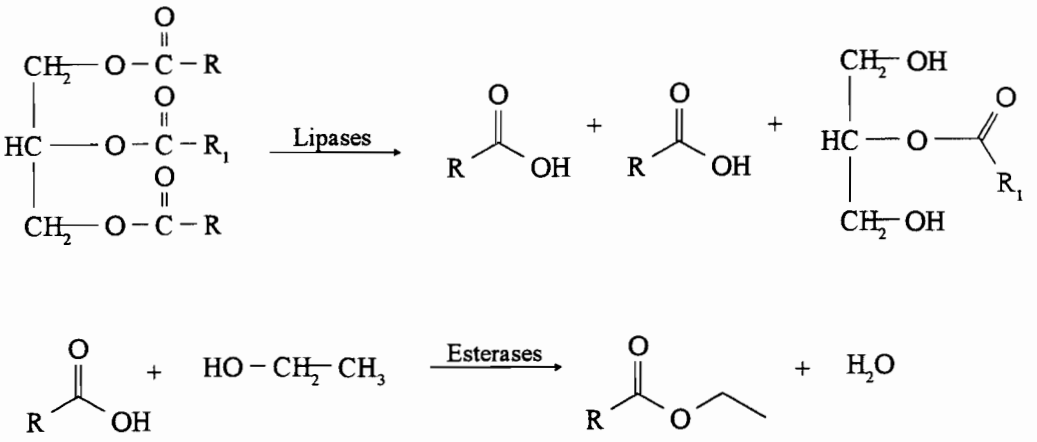

Figure 3: Mechanism for the formation of microbial esters from triglyceride (*Murray and Duff, 1991).*

Table 1: Ester contributing to wine flavor.

Ethyl acetate
Ethyl butyrate
Ethyl hexanoate
Ethyl octanoate
Ethyl decanoate
Propyl acetate
Butyl acetate
Isoamyl acetate
Hexyl acetate
2-Phenyl acetate

Murray and Duff, 1991

propionic and valeric acids. Mushroom flavor (1-octen-3-ol) is produced by *Trichothecium, Penicillium, Aspergillus* and fungus (Bauer and Garbe, 1985). Higher alcohols are essential for the overall flavor quality of alcoholic beverages. As major metabolites of yeast fermentation, alcohols, *i.e.* ethanol, propanol, isobutanol, amyl- iso-amylalcohols and phenyl-ethylalcohol play a major role as precursors in the formation of aldehydes and esters.

2.2.3. Acids

In spite of their low flavor impact, carboxylic acids are important not only as starting material or biotransformation intermediates in the formation of flavor compounds, but also as flavoring materials. The most commonly used acids include acetic, propionic, butyric, isobutyric, valeric, isovaleric and lactic acids. Acetic acid provides or enhances sourness while butyric acid contributes butter-like notes. Sharp unpleasant and pungent odors are associated with short chain fatty acids at high concentration. These characters change to rancid, buttery and cheese notes as the molecular weight increases (Wesh *et al.,* 1989). C3 to C6 acids are reported to accentuate fruity notes while C6 to C10 develop cheese notes.

Microbial formation of carboxylic acids involve *Clostridium butyricum and C. acetobutylicum* (Sharpell and Stegmann, 1981). Boyaval and Corre (1987) reported on the formation of swiss Emental cheese flavor and propionic acid using *Propionibacterium* species. Many other microorganisms have the ability to produce acids either as intermediates or as end-products of their activities.

2.2.4. Lactones

Lactones are cyclic esters formed by cyclization or lactonization of 4- and 5-hydroxy fatty acids following successive β-oxidations of long chain saturated and unsaturated fatty acids or their lipid precursors (Fig. 4) (Welsh *et al.*, 1989); γ-lactones and δ-lactones are the two most widely distributed forms. Lactones are ubiquitous in nature and have been isolated from all major food systems. These include fruits, vegetables, dairy products, fermented foods and alcoholic beverages to which they contribute significant flavors (Maga, 1976; Dufosse *et al.*, 1994). Tressl *et al.* (1978) proposed that lactones may form from oxidative deamination of amino acid such as glutamic acid to yield 2-oxoglutarate which produces 4-oxo-butyrate by decarboxylation and is reduced to 4-hydroxybutyrate; γ-butyrolactone is formed by cyclization of 4-hydroxybutyrate.

Sporobolomyces odorus was reported to produce 4-decanolide using glucose and pentone as substrates (Jourdain *et al.*, 1985). *Candida globiformis*, *Saccharomyces cerevisiae* and *S. fragilis* were also reported to produce 4- and 5-olides.

2.2.5. Carbonyls

Carbonyl compounds, ketones, diketones and aldehydes are potent flavor compounds. Ketones contribute cheese aroma. They are formed from the break down of fatty acids released by lipases. The fatty acids undergo oxidative

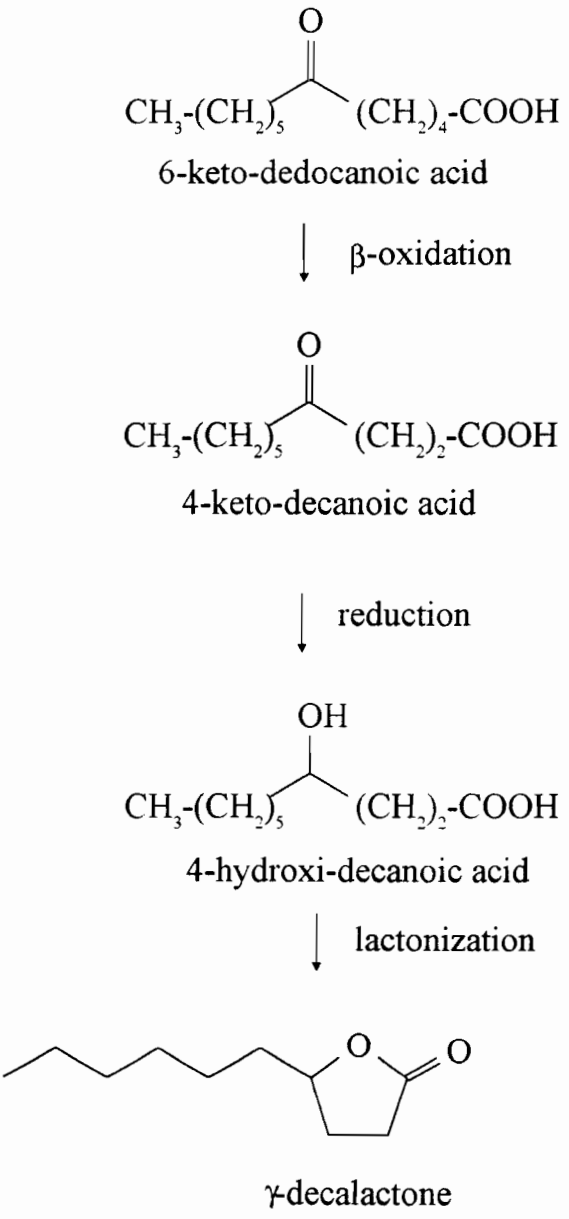

Figure 4: Biosynthesis of lactone from keto acid.
(Welsh et al., 1989)

degradation followed by addition of an acyl group and decarboxylation (Kinsella *et al.*, 1976).

Buttery notes are ascribed to the presence of diketones such as 2,3-butanedione (diacetyl) which are formed by fermentation of citric acid in the presence of lactic streptococci (Welsh *et al.*, 1989). Some carbonyl such as diacetyl have, however, been found to cause flavor defects in fruit juices, wines and beer.

Aliphatic and aromatic aldehydes possess distinct organoleptic qualities. The most important flavor aldehyde is vanillin (Bedoukian, 1967; Welsh *et al.*, 1989). Aldehydes are intermediates in microbial formation process of alcohols through the carboxylation of ketoacids.

2.2.6. Terpenes

Terpenes are responsible for the characteristic aromas of essential oils. This group of compounds, either from resin, essential oils, rubber, squalene or vitamin A, are all formed from the head to tail linkage of the 5 carbon isoprene unit (2-methyl-1,3-butadiene) (Hanssen *et al.*, 1986, Hock *et al.*, 1984). They are synthesized by higher plants and microorganisms.

2.3. Flavors derived from thermal processing of foods and foodstuffs

It is well established that exposing raw food materials to elevated temperatures results in food products with desirable flavor; these include bread, cakes, roasted foods and foodstuffs (nut, cocoa bean, coffee), grilled and boiled

foods. The varied use and the importance of thermally induced flavors include: *a*) the improvement of an existing flavor, *b)* replacement of flavor lost during processing, *c)* masking of undesirable flavor attributes, *d)* maintenance of flavor characteristic which otherwise may change through seasonal variation, and *e)* in cases where natural flavor presents hazardous health risks (Heath and Reineccius, 1986).

When foods and foodstuffs are subjected to heat, thermal degradation of carbohydrates, lipids and amino acids generate fragments that may recombine to form a variety of flavor compounds. Over 600 flavor compounds are generated during the roasting of coffee and over 400 in roasted cocoa (Flament, 1989). These changes are called non-enzymatic browning, amino-carbonyl reactions or Maillard reactions, and may be important to the food processing industry due to their enhancement of organoleptic properties. Some common examples of food processing in which these reactions occur are: coffee roasting (Tressl *et al.* 1981) chocolate manufacturing (Raymond and Rostagno, 1978) bread baking (Folkes and Gramshaw, 1981), maple syrup processing (Danehy, 1986) boiling and broiling of meat, poultry and fish products (Maga, 1982).

The formation of reaction flavors, through the Maillard reaction, requires the presence of precursors such as lipids, carbohydrates, and proteins which undergo a variety of transformations (hydrolysis, decomposition, addition,

cyclization, oxidation, reduction) to yield new chemical compounds, many of which are heterocyclic and have flavor properties.

The Maillard reaction, named after the French scientist Louis-Camille Maillard who first described the formation of a brown pigment from the reaction of glucose and glycine (Maillard, 1912) can be summarized as the interaction between amino and carbonyl compounds (amino-carbonyl reaction) (Namiki, 1988) characterized by the development of flavor and brown color. The mechanism involves a set of reactions leading to a mixture of reactants, intermediates and products. Many of the resulting compounds are unstable species (Nursten and O'Reilly, 1986) with a variety of functional groups, linkages and molecular sizes, and participate in further reactions.

Different aspects of the Maillard reaction have been the subject for numerous studies; the browning reaction pathways proposed by Hodge (1953) have been the basis for research in this field. The proposed mechanism separates the Maillard reaction into three main stages (Fig. 5) which comprise five steps: *(1)* amino-carbonyl reaction to produce N-glycosylamine, *(2)* rearrangement of N-glycosylamine into aldo- or ketosamine also known as Amadori products, *(3)* formation of diketosamine and amino sugar, *(4)* decomposition of the amino sugar and amino compounds through the Strecker degradation, and *(5)* combination and condensation of the resulting compounds into reaction flavor compounds.

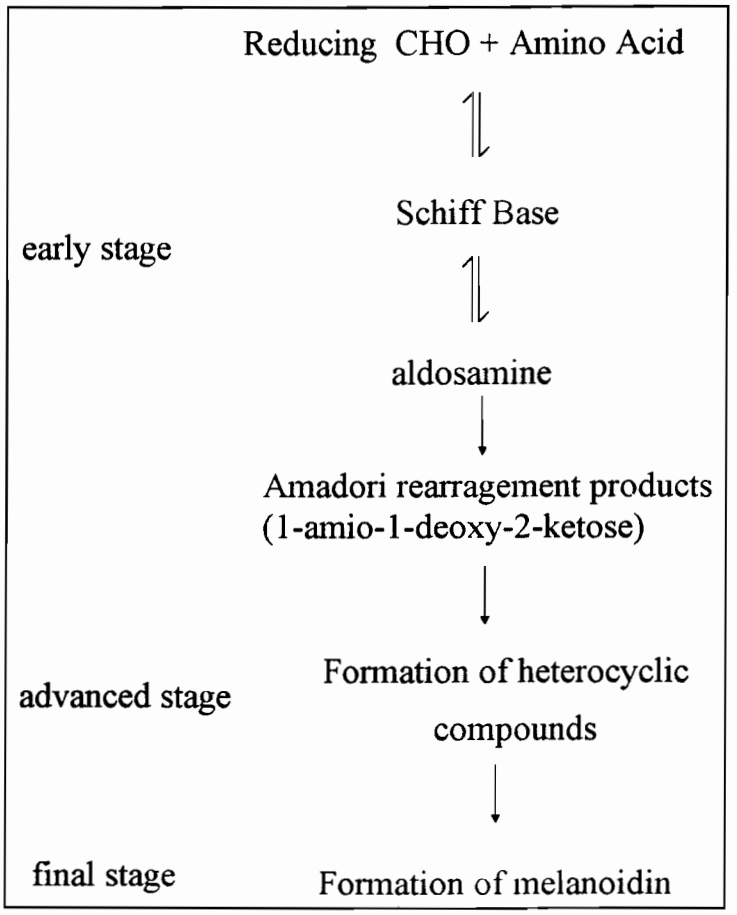

Figure 5: Simplified sequential break down of the Maillard reaction.
(Hodge, 1953)

The flavors and aromas from the Maillard reaction include numerous desirable and undesirable flavors that develop during heat transformation of food material. These aromas are described as toasted, baked, nutty, and roasted and burnt. Volatile flavor compounds formed by Maillard reaction can be classified into the following three groups as proposed by Fors (1983: *(1)* simple sugar dehydration/fragmentation products: cyclopentenes furans, pyrones, carbonyl compounds and acids, *(2)* simple amino acid degradation products: aldehydes and sulfur compounds, *(3)* further reaction products: pyrroles, pyridines, imidazoles, pyrazines, oxazoles and thiazoles.

Research on the color and flavor development aspects of the Maillard reaction now include the nutritional, physiological and physico-chemical properties of Maillard reaction products and their safety aspects (Pintauro *et al.* 1983). Concern regarding the safety of some Maillard reaction products and the lower nutritional value of some browned foods (O'Brien, 1989) have led to considerable research interest in recent decades. In the process of forming appetizing color and flavor and conferring some protection to the food by the formation of compounds such as antioxidants, foods and foodstuffs in which the Maillard reaction occur may loose nutritive value due to the destruction of proteins and the formation of mutagens. Mutagens are chemical compounds which induce biological changes and have been identified in fried, broiled and cooked fish and beef products (Barnes *et al.* 1983). Many mutagens appears to be nitrogen containing heterocyclics, derivatives of carboline or

imidazoquinoline. Their formation may involve the Maillard reaction based on their identification in model systems. Sugimura and Nagao (1979) reported the same mutagens in both model systems and cooked meat; these include Trp-P-1 (3-amino-1,4-dimethyl-5*H*-pyrido [4,3-b] indole), Trp-P-2 (3-amino-1-methyl-5*H*-pyrido [4,3-b] indole), Glu-P-1 (2-amino-6-metyldipyrido [1,2-a:3',2'-d] imidazole) and Glu-P-2 (2-amino-dipyrido [1,2-a:3',2'-d] imidazole. Cooked muscle meat particularly contain mutagenes such as IQ (2-amino-3-methyl-imidazo [4,5-f] quinoline), MeIQ (2-amino-3,4-dimethyl-imidazo [4,5-f] quinoline) and MeIQx (2-amino-3,8-dimethyl-imidazo [4,5-f] quinoxaline) (Wakabayashi *et al.*,1986). MeIQx was isolated from broiled fish, beef, chicken and fried ground beef at levels of 0.3 to 2.1 ng per g and accounted for 21% of the total mutagenicity of these foods (Wakabayashi *et al.* 1986).

2.4. Analysis of flavor compounds

One of the principal reasons for analyzing flavor compounds include the establishment of their chemical nature and the pathways of their formation. Chemicals compounds that contribute flavor act either independently or in combination, to produce characteristic aromas. This type of knowledge is valuable in designing new flavors, facilitating an understanding of the numerous mechanisms of flavor formation and monitoring food flavors during processing and storage.

The problems associated with the analysis of food flavor is reflected in the complex nature of food system and the non-homogenous character of its flavor compounds. Compounds having large differences of polarities and molecular weights as well as different functional groups can contribute to particular flavors (Perkins, 1989). These components may be soluble in water and/or oil within the food system or may be bound to macromolecules (Dumont, 1987). Pyrazines as flavor compounds, have low thresholds, and are present in trace amounts (Koehler *et al.*, 1971; Shibamoto, 1986). Very frequently they act in combination with other volatile compounds to produce a characteristic flavor. For example, although nutty and roasted notes are attributed to the presence of alkylpyrazines, these characteristic flavors of heated foodstuff involve a complex blend of pyrazines and other classes of volatiles such as carbonyls, pyrroles and furans (Bondarovich, 1967), which are formed under similar conditions required for the formation of pyrazines. Total aroma volatiles represent a very minor proportion of the weight of foods (Reineccius *et al.* 1972). Thus, trace constituents in an aroma complex are sometimes of far greater sensory importance than compounds present in larger amounts. The above factors contribute to the difficulties encountered in the isolation, characterization and quantitation of food flavor compounds.

2.4.1. Methods for flavor extraction

The choice of appropriate methods of isolation that will provide useful qualitative and quantitative information is therefore crucial. The efficiency of an

isolation method depends on the composition of the food matrix from which the flavor compounds are to be extracted. Subsequently, grinding, homogenization, centrifugation, pressing and filtration of the food sample may be required (Bemelmaus, 1981). Isolation of aroma from food is basically the separation of the volatile constituents from other food components (proteins, carbohydrates, water, fats, minerals, vitamins) on the basis of volatility (distillation, static and dynamic headspace analysis) and solubility (solvent extraction) (Reineccius, 1993). In terms of volatility, water is the major problem since volatiles of interest may be water soluble. Thus, the water/volatile mixture is extracted followed by subsequent separation of the volatiles from water. In terms of solubility, isolation is achieved in organic solvent. However, fat components, vitamins, chlorophyll and carotenoids, which may prove to be abundant in the food system, are also soluble in organic solvent. Both isolation basis, solubility and volatility, are further complicated by differences in volatility or solubility properties displayed by flavor constituents.

Volatile flavor compounds from foodstuffs can be isolated using the following two types of extraction methods: *1)* High temperature extraction by distillation. This type of extraction is efficient for certain compounds, however artefacts formed from heat induced reactions can be problematic when dealing with thermally labile flavor compounds (Reineccius *et al.* 1972). *2)* Solvent extraction at low temperature such as head space and trapping techniques with cryofocussing for the very volatiles, or a combination of these techniques (Reineccius, 1989) Dialysis is a low temperature extraction method by which

flavor compounds are isolated by diffusion through a membrane (Benkler and Reineccius, 1980; Molimard *et al.*, 1993) may also be used to isolate volatile flavor compounds.

Selective separation of volatiles based on functional groups can also be used. This takes advantage of characteristics such as volatility and/or the presence of reactive functional groups. For instance, pyrazine compounds and other nitrogen containing organic bases can be selectively separated by an aqueous hydrochloric acid extraction resulting in the formation of hydrochloride salts (Akochi-K. *et al*, 1993). Chaveron *et al.* (1989) compared different methods for the extraction of pyrazine compounds, these included column extraction, extraction by shaking and various combinations of these techniques.

2.4.2. Physical and Chemical analysis of flavor

Instrumental methods, mainly high performance liquid chromatography (HPLC), gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) are used in the separation and identification of flavor compounds.

Instrumental analysis are being developed for specific challenges such as the resolution of enantiomeric flavor compounds. Gas chromatography/Fourier transform infrared spectrometry (GC/FTIR) is a useful tool for the analysis of complex mixtures. The capability of infrared spectroscopy in discriminating between isomers makes GC/FTIR particularly well suited for the analysis of natural mixtures such as essential oils. Harres *et al* (1986) investigated terpenes in copaiba balsam and essential oils of parsley and cosmea.

Monoterpenes and sesquiterpenes which often exhibit very similar EI mass spectra were efficiently differentiated using GC/FTIR.

The impact of stereochemical factors in biological activity, such as sensory properties has intensified research in the resolution of stereoisomers. Figure 6 (Emberger *et al.*,1985) shows a series of menthols. The menthol molecule has 3 asymmetric carbons, hence the existence of eight optically active isomers which give four racemates, designated as menthols, neomenthols, isomenthols and neoisomenthols all of which are components of peppermint and other essential oils. Study on the sensory characteristics of these enantiomers (Emberger *et al.*,1985) revealed significant sensory differences between the eight menthols. The enantiomeric separation of the four diastereoisomers of menthol (Fig 6) was accomplished with the aid of multidimensional capillary gas chromatographic analysis using a chiral stationary phase.

2.5. Sensory analysis of food

Sensory evaluation methods can be conveniently divided into two main groups: consumer oriented tests and product oriented tests. These tests are in turn divided into discriminative tests and affective tests; the choice of test depends on the information needed for a particular product. Discriminative tests are used to determine whether or not a difference exist among samples, thus providing information on important characteristics of a product; examples of these tests are triangular, duo and trio tests. Affective test measures how the

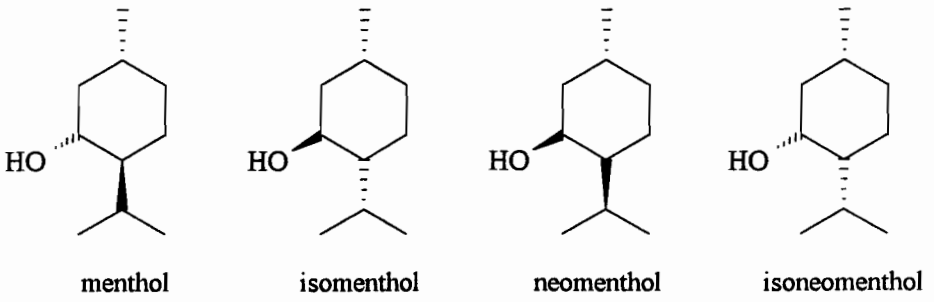

Figure 6: Racemates of menthol. *(Emberger et al., 1985)*

consumer reacts to a product, therefore requires an endorsement. Affective tests are non-parametric because they do not involve the comparison of parameters, instead, products are judged on their performances. Discriminative and affective tests can be combined to provide a more complete information on the consumer as well as the product profile (Stone and Sidel, 1985; Poste *et al.*, 1991).

Despite making use of the best tests possible, the following represent error sources and factors that should be controlled because of their direct influence on human responses to stimulus (Howard, 1972; Poste *et al.*, 1991):

- *i)* expectation error: panelist will find what they expect to find, thus only necessary information on the product should be provided.
- *ii)* stimulus error: this is manifested by the desire to be right; samples therefore must be presented as uniform as possible.
- *iii)* halo effect: this effect arise when a panelist tend to generalize the evaluation due to too many attributes to compare.
- *iv)* central tendency error is due to avoidance by the evaluators of the extremes; samples should then be presented in balanced or randomized orders.
- *v)* motivation will affect the performance and the efficiency of judgment of panelists; the period of the day and the length of the test should be appropriate.

Statistical tests, t-test, F-tests, analysis of variances (ANOVA), LSD and Ducan's multiple tests, are used to draw conclusions such as estimates and inferences based on the responses of the sensory panelists.

2.6. Components of maple sap and syrup

Maple syrup is characterized by a unique "maple" flavor, however; there is presently no definite compound or compounds which make up this "maple" flavor. Nevertheless, numerous flavor compounds have been identified in maple syrup.

Maple syrup is the characteristic product resulting from thermal processing of maple sap, the exudate tapped from the trunk of mature sugar maple trees (*Acer saccharum* MARSH). Birch tree species (*Betula papyrifera* MARSH and *B. alleghaniensis* BRITT) also have the potential of producing sap that can be converted into edible syrup (Kallio *et al.*, 1985; Kallio, 1988; Rowe, 1972).

The sap flow is controlled by a physiological mechanism specific to maple sugar trees (Morselli and Whalen, 1991), and lasts 4 to 8 weeks, towards the end of the winter months. The tapping of maple trees takes place before buds opening and corresponds to a period during which the sap is high in sugar content due to the enzymatic conversion of storage carbohydrate in xylem vessels (Morselli *et al.*, 1986). The sap is converted to syrup by extended heating at elevated temperatures during which the maple syrup flavor develops. The evaporation takes place in flat pans heated, at boiling temperature; when the sap reaches the state of syrup, the temperature reads 105°C and a sugar concentration of 65.5% by weight is obtained.

2.6.1. Grading of maple syrups

Maple syrups are categorized according to their color (Table 2). The color categories made commercially available to consumer are light, medium and amber (USDA, 1979; Dumont, 1995), with an association between color and flavors. The darker syrups are considered to be of low grade while the clearer syrups are considered of higher quality and have high market value (Sendak, 1982). Color grading of maple syrup is used both in Canada and the USA, the only two countries producing syrup from maple sugar trees (Flaherty,1990). The desirable color and specific flavor of each grade may vary widely depending on the sap, year of harvest, processing and storage conditions. Early report by Edson (1912) indicates that poor quality and/or faulty processing techniques could result in darker syrup with stronger maple flavor. Morselli and Whalen (1991) reported on the effects of aseptic tapping on the color of the resulting syrup. The study monitored these influences over six sap flow seasons, and showed that non-aseptic sap contained more than 800 colony forming units of microorganisms per mL, while the aseptic sap contained fewer than 10 colony forming units per mL. The study also showed that aseptic sap produced close to 93% light amber syrup compared to 62% light syrup from the non-aseptic sap. In addition, the non-aseptic produced 12% syrup darker than the category amber (Morselli and Whalen, 1991). Willits and Hills (1976) reported that in traditional sugaring operation, syrup color progresses during the sap season from light to dark.

Table 2: Color grades of maple syrup

Class	Grade	Light Transmission
1	extra light	75% and more
2	light	60.5 - 75%
3	medium	44 - 60%
4	amber	27 - 44%
5	dark	< 27%

USDA, 1979; Dumont, 1995

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.

2.6.2. Chemical compounds of maple sap

In order to determine the origin of some of the flavor compounds reported in maple syrup, it is necessary to look into the components of sap that potentially could contribute to the formation of flavor during the boiling of maple sap. Table 3 lists chemical components which have been reported present in maple sap.

Jones and Alli (1987) reported a sucrose content of 2.5% to 3% along with trace amounts of reducing sugars, mainly glucose and fructose (Leech and Kim, 1990). Organic acids have also been identified by Molica and Morselli (1984). The presence in trace amounts of amino acids such as glutamine, glutamic acid, asparagine and proline have also been reported (Morselli and Whalen, 1986; Kallio, 1988). Filipic *et al.*, (1965) reported the presence of soluble lignins in maple sap.

2.6.3. Flavor compounds of maple syrup

The numerous chemical reactions which occur during the heating of food systems undoubtedly contribute immensely to the development of the characteristic flavors. Very limited studies of these reactions however, have been reported during processing of maple sap to syrup. Although the presence

Table 3 : Identified chemical components of maple sap

Sugars	Nitrogenous compounds
sucrose[1]	aspartic acid[4]
glucose[1]	aspargine[4]
fructose[1]	glutamine[4]
galactose[1]	ammonia[4]
raffinose[2]	proline[4]
	urea[4]
Organic acids	
	Minerals
oxalic acid[3]	
glyconic acid[2]	calcium[2]
fumaric acid[3]	potassium[2]
tartaric acid[3]	sodium[2]
malic acid[3]	magnesium[2]
cis-aconitic acid[3]	manganese[2]
citric acid[3]	
shikimic acid[3]	**Other compounds**
succinic acid[3]	
	soluble lignins[5]

[1]Jones and Alli, 1987; [2]Kallio, 1988; [3]Molica and Morselli, 1984; [4]Morselli and Whalen, 1986; [5]Filipic et al., 1965

of organic acids, sugars, nitrogenous compounds, minerals and soluble lignin (Table 3) can contribute some flavor, maple sap is considered "flavorless".

Although the flavor of maple syrup is very characteristic of the product, the exact chemical compound or compounds which are responsible for this characteristic flavor is yet to be established, desspite the fact that there are commercially available "maple" flavors. The principal reactions which occur during heating are caramelization reactions (Kallio, 1988), reactions between reducing sugars and amino acids or Maillard reaction (Kallio, 1988) and alkaline degradation of lignin derived compounds (Filipic *et al.*, 1965). Table 4 shows a list of volatile compounds which to date, have been reported to have a role in maple flavor. On the basis of their structures, most of these compounds can be grouped as phenolics, carbonyl and pyrazine compounds.

2.6.3.1. Phenols of maple syrup

Phenolic compounds could be derived from the degradation of soluble lignins in maple sap. Lignins are synthesized biologically through the shikimic acid pathway (Fig 7). Shikimic acid from phosphopyruvate produces an enolpyruvate ether which in turn is converted into prephenic acid. The prephenic acid polymerizes to form lignin polymers which under heating would decompose and oxidize to yield phenolic compounds (Table 4) with flavor properties. Vanillin is reported to be a major thermal decomposition product of ferulic acid (Underwood and Fillipic, 1964; Fiddler *et al.*, 1967).

Table 4: Identified flavor compounds of maple syrup

Phenolic compounds	Carbonyl compounds
vanillin[1]	2-hydroxymethylcyclopent-2-en-1-one[1]
syringaldehyde[1]	2-hydroxy-3-methyl-2-cyclopenten-1-one[6]
dehydroconiferyl alcohol[1]	2-methyl-2-cyclopenten-1-one[6]
syringoyl methyl ketone[1]	2-methyl-2,5-cyclohexadien-1,4-dione[6]
2,6-dimethoxyphenol[1]	2,3-dihydro-3,5-dihydroxy-6-ethyl(4*H*)-pyran-4-one[6]
	2,5-dimethyl-4-hydroxy-3(2*H*)-furanone[6]
Pyrazine compounds	3-methyl-3-buten-2-one[6]
	3-methyl-2,5-furandione[2]
methylpyrazine[2]	3-methyl-2-cyclopenten-2-ol-1-one[1]
2,3-dimethylpyrazine[2]	3-hydroxybutanone[2,6]
2,5-dimethylpyrazine[2]	3-hydroxy-2-pyranone[2]
2,6-dimethylpyrazine[2]	3-hydroxy-4-methyl-5-ethyl-2(5*H*)-furan-one[5]
ethylpyrazine[2]	propionaldehyde[5]
trimethylpyrazine[2]	
2-ethyl-6-methylpyrazine[2]	
2,5-dimethyl-3,6-diiso butylpyrazine[3]	
5-isopropyl-2,3-dimethylpyrazine[4*]	
Other compounds	
2-ethyl-1-hexanol[6]	
2-hydroxymethylcyclopenten-2-en-ol[6]	
2-furanmethanol[6]	
2-ethyl-1-hexanoic acid[6]	
n-hexanoic acid[6]	
n-nonanoic acid[6]	

**not actually identified in maple syrup*
[1]Filipic et al., 1965; Potter and Fagerson, 1992; Kermasha et al., 1995; [2]Alli et al., 1990; [3]Winter et al., 1972; [4]Masuda and Mihara, 1986; [5]Bean and Setser, 1992, [6]Kallio, 1988.

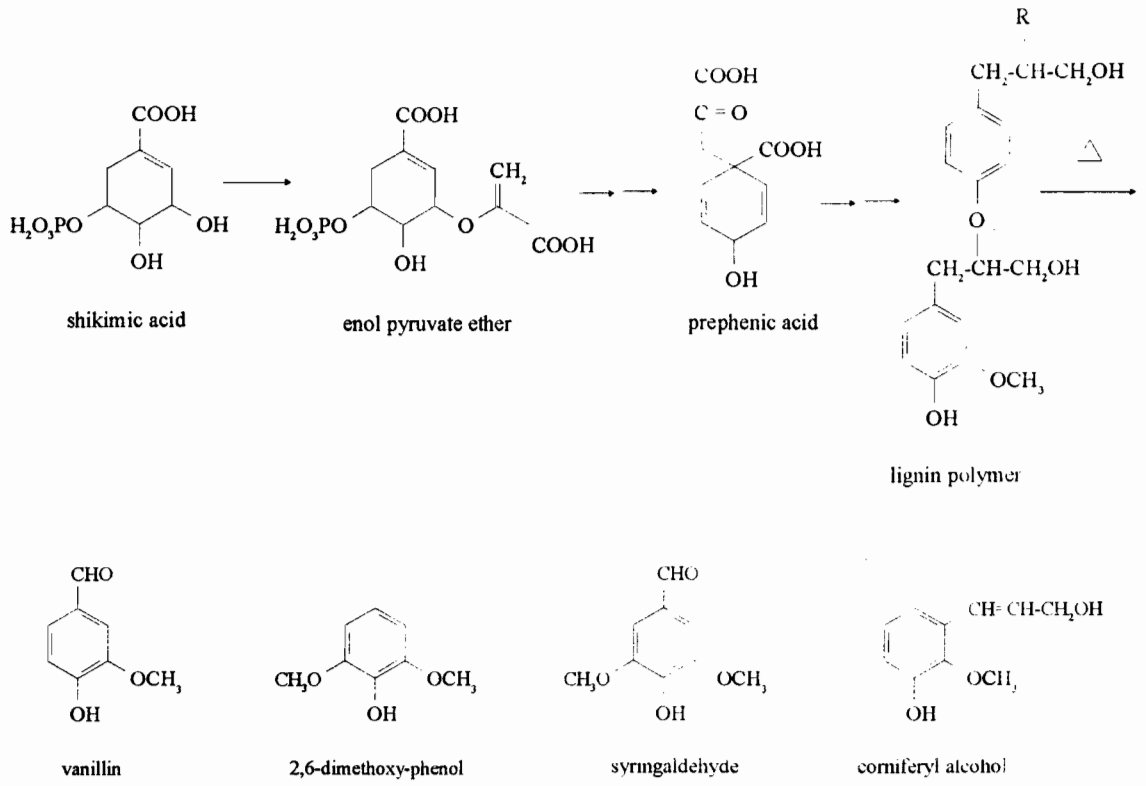

Figure 7: The shikimic acid pathway to phenolics of maple syrup.
(Lindsay, 1985)

Filipic *et al* (1965) demonstrated the presence of several flavor contributing phenolic substances in maple syrup; these include vanillin, syringaldehyde, syringoyl methyl ketone, 2,6-dimethoxyphenol (Table 4). In addition, Potter and Fageron (1992) reported the presence of eugenol, vanillic acid, syringic acid and other phenolic compounds. Kermasha *et al.* (1995) identified the same phenolic compounds in both maple sap and syrup. Despite these reports on the presence of lignin derived phenols in maple syrup, it has still not been established which lignin compounds are present in the initial maple sap

2.6.3.2. Volatile alcohols and carboxylic acids of maple syrup

Several alcohols and volatile acids have been reported in maple syrup (Kallio, 1988). These include: alcohols (2-ethyl-1-hexanol, 2-furanmethanol, 2-hydroxy-methyl-cyclopenten-2-en-ol) and acids (2-ethyl-1-hexanoic acid, n-hexanoic acid, n-nonanoic acid); this author suggested that antifoaming agents used during the processing of maple sap to syrup might be the source of these aliphatic alcohols and carboxylic acids.

2.6.3.3. Carbonyl compounds of maple syrup

Carbonyl compounds derived from caramelization reactions, which are mainly dehydration and/or fragmentation reactions of sugars, include furans, γ-pyrones and cyclopentenes carbonyl and acid compounds (Bean and Setser, 1992). Caramelization reactions can be expected during the heating of maple

sap which contains sucrose and small amounts of reducing sugars (Jones and Alli, 1987). These reactions occur under both acidic and basic conditions. Acidic conditions promote dehydration reactions which result in the production of furfurals, and alkaline conditions favor isomerization and fragmentation of sucrose (Fig. 8) (Bean and Sester, 1992; Feather, 1982; Monte and Maga, 1981). It is likely that most of the cyclopentene compounds, furan and pyran derivatives, and carbonyl compounds listed in Table 4 result mainly from caramelization reactions. However, it may prove difficult to describe the characteristic maple flavor as resulting from caramelization reactions, since Herz and Shallenberger (1959) describe a "caramel" aroma when glucose only was heated to 180°C. In addition, commercial "caramel" color contain a large number of pyrazine

compounds which are products from reactions between sugars and nitrogen containing compounds (Tsuchida *et al.*, 1986), suggesting that there is much more to the "caramel" reaction attribute than reactions involving sugars only.

Filipic *et al* (1965) reported the presence of 2-hydroxymethylcyclopent-2-en-1-one as a minor component of maple syrup and suggested that this carbonyl compound contributed to the characteristic maple flavor (Table 4). Several other carbonyl compounds (3-hydroxybutanone, 3-hydroxy-2-pyranone, 3-methyl-2,5-furandione) were identified in the dichloromethane extracts of maple syrup (Alli *et al* ,1990). Bean and Setser (1992) reported 3-hydroxy-4-methyl-5-ethyl-2(5*H*)-furanone and propionaldehyde as flavor compounds of

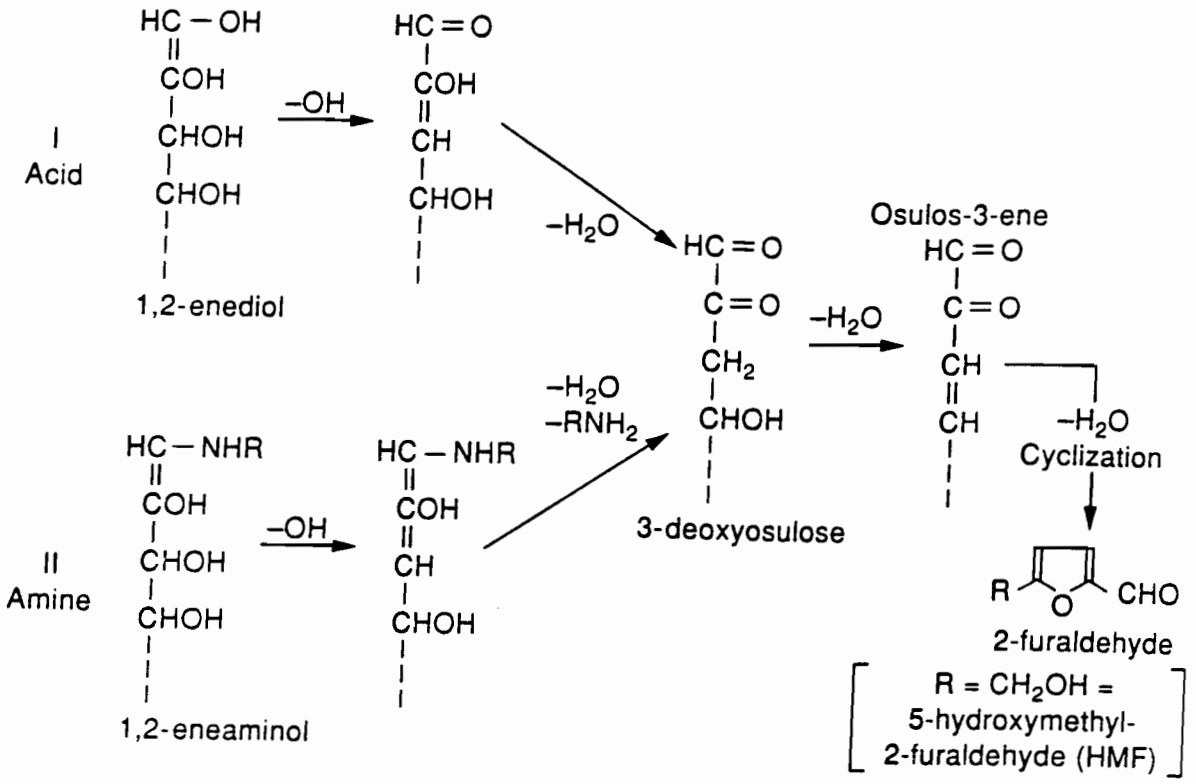

Figure 8: The formation of carbonyl compounds in maple syrup.
(Bean and Sester, 1992)

maple syrup with the maple like syrup flavor; however, these compounds were not actually identified in maple syrup. Kallio (1988) reported the presence of the following carbonyls in the volatiles of maple syrup: 2-hydroxy-3-methyl-2-cyclopenten-1-one, 3-hydroxybutanone, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone, 2,3-dihydro-3,5-dihydroxy-6-methyl(4*H*)-pyran-4-one, 2-methyl-2-cyclopenten-1-one, 2-methyl-2, and cyclohexadien-1,4-dione and 3-methyl-3-buten-2-one (Kallio, 1988; Thomas *et al.*, 1992).

2.6.3.4. Pyrazine compounds of maple syrup:

Pyrazines (Fig. 9) are formed from reactions between reducing sugars and amino acids or nitrogen containing compounds such as ammonia. The presence of both reducing sugars and amino acids in maple sap (Kallio, 1988; Jones and Alli, 1987; Morselli and Whalen, 1986) suggest formation reactions involving these compounds during the thermal transformation of maple sap to syrup. In addition, the identification of various pyrazine compounds in maple syrup (Alli et al, 1990; Kallio, 1988) confirms that these reactions take place during heating of sap. Thus, the pyrazine compounds listed in Table 4 would all be expected to result from thermally induced amino acid/reducing sugar reactions.

There have been sporadic reports either dealing with the presence of pyrazines in maple syrup or associating pyrazines with the flavor of maple syrup (Table 4). Report by Winter *et al.* (1972) suggested that 2,5-dimethyl-3,6-diisobutylpyrazine demonstrated maple-like flavor characteristics. More

recently, Masuda and Mihara (1986) reported that 5-isopropyl-2,3-dimethylpyrazine gave a "sweet, maplelike, brown odor." In these reports however, the pyrazine compounds where not actually identified in maple syrup. In a study of pyrazines in maple syrup, Alli *et al.* (1990) reported the presence of at least seven pyrazine compounds; these included methylpyrazine, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, ethylpyrazine, trimethylpyrazine, and 2-ethyl-6-methylpyrazine. These pyrazine compounds, in addition to a large number of other pyrazines contribute flavor and are present in various thermally processed foods (Maga, 1982), and are therefore not unique to maple syrup. Kallio (1988) reported the presence of the 2,6-dimethylpyrazine, along with several other volatile compounds in the pentane-diethyl ether extract of birch syrup, a product similar to maple syrup but obtained from the sap of birch trees (*Betula pubesceus* Enrh).

2.7. Physical and chemical properties of pyrazines

Pyrazines are heterocyclic compounds with two nitrogen atoms at *para* position within a six membered aromatic ring (Fig 9). They are basic compounds due to the free electrons of the nitrogen atoms and undergo basic nucleophilic reaction much like primary and secondary amines. The molecule is fairly stable (ring energy = 105 Kj/mole) due to ring resonance with a zero dipole moment. This weak base (pKa_1 = 0.6, pKa_2 = -6.2) can be alkylated by

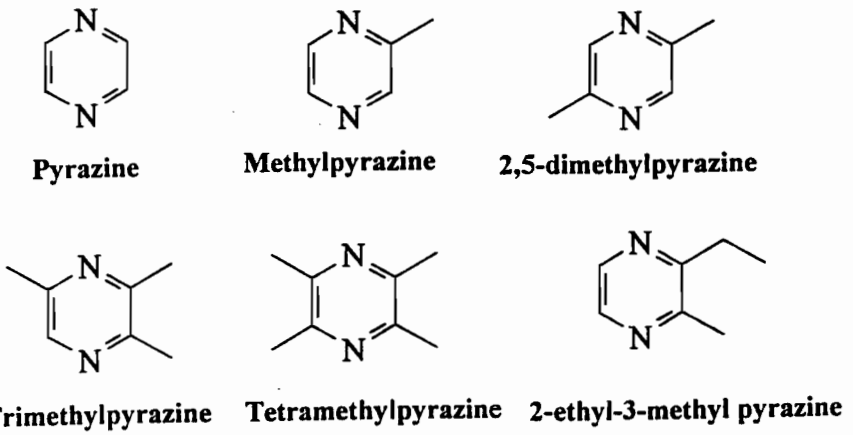

Figure 9: Structure of commonly found pyrazines in heated foods.
The flavor character of any pyrazine compound depends strongly upon concentration and its chemical structure (Fors, 1983).

the conventional alkylation methods to yield alkylpyrazines (Paquette, 1968). Pyrazines found in heat treated foods are products of the Maillard reaction, whereas those from vegetables, fruits, fermented foods and beverages are biosynthesized by enzymes and microorganisms (Maga, 1982).

2.7.1. Flavor characteristic of some pyrazines

The flavor character of any given pyrazine depends upon its concentration and chemical structure; as a rule, alkyl- and acetyl- pyrazines produce roasted-nutlike sensory impression, while higher substitution of the ring produces raw vegetable, hearthy, green notes and floral notes (Fors, 1983). Pyrazines elicit potent organoleptic characteristics even at extremely low concentrations. Heath and Reineccius (1986) reported the sensory threshold of 2-methoxy-3-isopropylpyrazine at 0.001 ppb (Table 5).

2.7.2. Biosynthesis of pyrazines

Pyrazine compounds in fruits, vegetable and fermented foods and foodstuffs is produced by enzymatically or microbial mediated routes. The characteristic bell pepper flavor, 2-methoxy-3-isobutyl pyrazine (Buttery, 1981) is derived from the action of strains of *Pseudomonas prolens* and *Pseudomonas tetrolens*, using branched-chain amino acid such as leucine as substrate (Murray and Whitfield, 1975). The enzymatic scheme proposed for this reaction is shown on Figure 10. In this mechanism, an amino group is added to the carbon atom bearing the hydroxy group of leucine; this is followed

Table 5: Flavor characteristics of some pyrazines.

	Flavor description	Threshold
pyrazine	pungent, floral on dilution	100 ppm
2-methylpyrazine	roasted nuts, chocolate grilled chicken	100 ppb
ethylpyrazine	roasted nuts, buttery, rum notes	10 ppm
2,3-dimethylpyrazine	chocolate on dilution nutty, green, pungent	50 ppb
2,5-dimethylpyrazine	potato chips, chocolate, roasted peanuts	25 ppm
2-acetyl-3-methylpyrazine	popcorn, cereal	4 ppb
2-isopropyl-3-methoxy-	bell pepper,	0.016 ppb
2-ethoxy-6-methylpyrazine	pineapple	11 ppb
5-methylcyclopentapyrazine	grilled meat, sweet tobacco	150 ppm

Fors, 1983

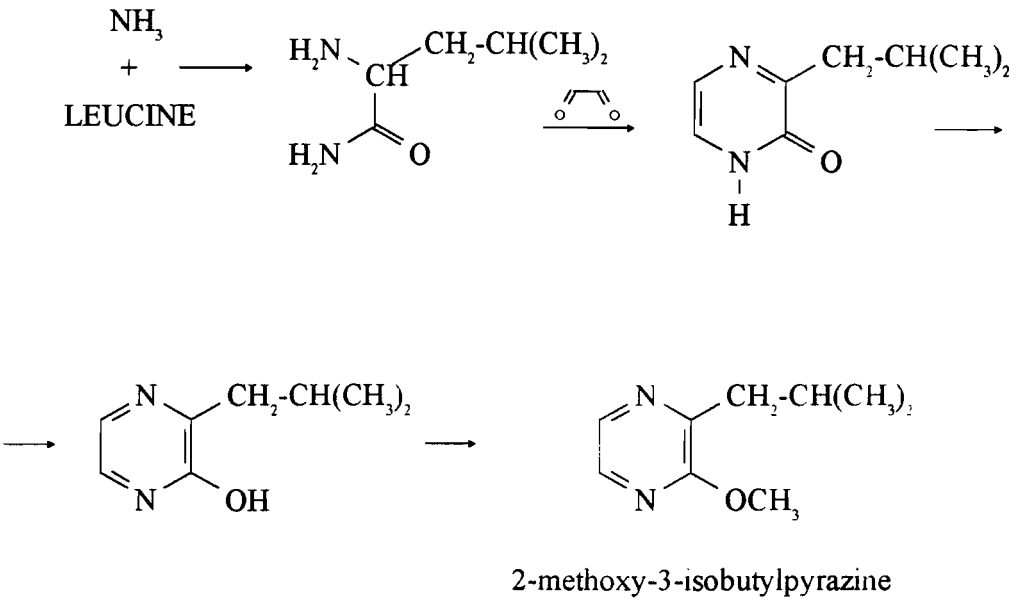

Figure 10: Biosynthesic route for the formation of methoxypyrazine.
Methoxypyrazine formed in plants elicit green-hearthy aromas. The characteristic bell pepper aroma is produced by 2-methoxy-3-isobutyl pyrazine (Murray et al., 1970).

by the addition of an α-dicarbonyl and formation of the methoxy by addition of a methyl group. Morgan *et al.* (1972) reported the involvement of *Pseudomonas tetrolens* in the formation of 2-methoxy-3- isopropyl pyrazine responsible for the musty flavor defect in milk and eggs. Devys *et al.* (1992) reported on the formation of methoxy-pyrazines by the action of *Septoria nodorum.*

2.7.3. Heat induced formation of pyrazines

Theoretically, self-condensation of α-amino-carbonyls or the condensation of 1,2-dicarbonyl with 1,2-diamine, followed by an oxidation reaction would explain the formation of pyrazine compounds. However, foods contain numerous amino acids and carbohydrates which when heated, undergo a variety of reactions and combinations such that products are derived from different mechanisms with each mechanism occurring through different steps and intermediates. This has given rise to numerous reports on the mechanisms involved in the formation of pyrazine compounds (Walradt, 1971; Rizzi, 1972; Shibamoto and Bernhard, 1977; and 1978). Mechanisticaly, it is known that reactions which favor the formation of pyrazines involve the combination of two fragments and in many cases condensation reactions between α-dicarbonyl fragments and amino compounds or their fragments bearing the amino group.

The most widely accepted mechanism for heat induced formation of pyrazines is through the Strecker degradation (Fig. 11): α-diketones react with amino acids to form α-amino ketones. These may condense with other α-amino

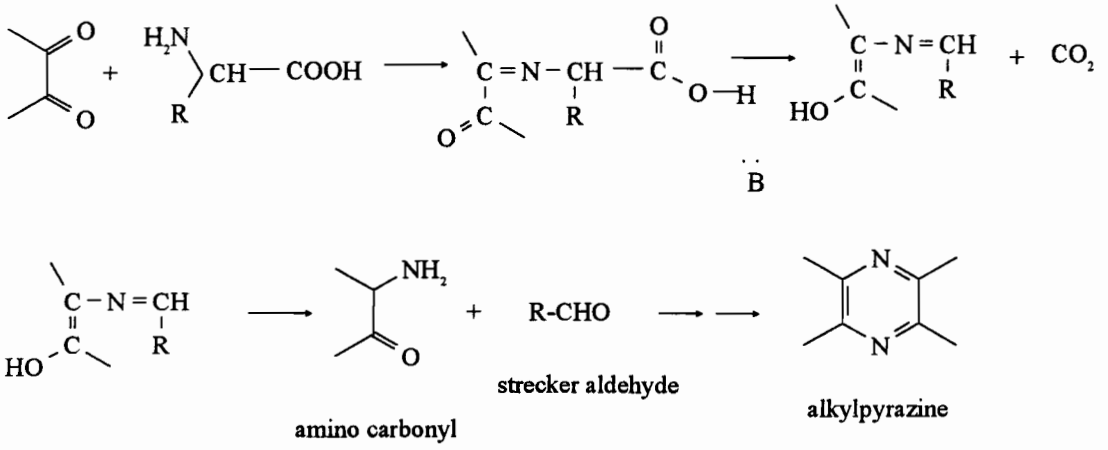

Figure 11: The Strecker degradation of amino acids.
Amino carbonyl formed from this reaction leads to pyrazines (Bean and Sester, 1992).

ketones to form a dihydropyrazine, which may undergo oxidation to form pyrazine(Hodge *et al.*, 1972).

Dawes and Edwards (1966) proposed that pyruvaldehyde formed during sugar fragmentation could react with amino acids to form amino-propanal and yield dimethyl-dihydropyrazines by condensation (Fig. 12). Shibamoto and Bernhard (1977; 1978) proposed that the reaction of sugars and amines results in α-amino-carbonyl intermediates which condense to form a broad range of pyrazines; these authors proposed the formation pathways for ten α-amino-carbonyl intermediates from the fragmentation of rhamnose. The α-amino-carbonyl intermediates are used here to illustrate the formation of methylpyrazine (Fig. 13), 2,3-dimethylpyrazine (Fig. 14), 2,5-dimethylpyrazine (Fig. 15), 2,6-dimethylpyrazine (Fig. 16), ethylpyrazine (Fig. 17), trimethylpyrazine (Fig. 18), and 2-ethyl-3-methylpyrazine (Fig. 19). Rizzi (1972) proposed the condensation of amino acetones to form a range of acyclic amino acetones, which undergo various reactions (dehydration, isomerization, aldolization, retro-Manich condensation) to finally cyclize to yield different alkylpyrazines. Shibamoto *et al.* (1979) proposed pathways for the formation of alkylpyrazines from 2,3,5-trimethyl-5,6-dihydropyrazine through the rearrangement of dihydropyrazine isomers under basic conditions. The dihydropyrazines undergo rapid oxidation to form trimethylpyrazine (Fig. 20). Isomers that are less sensitive to oxidation hydrolyze into α-amino carbonyls, dicarbonyls and diamino compounds, which recombine to yield alkylpyrazines.

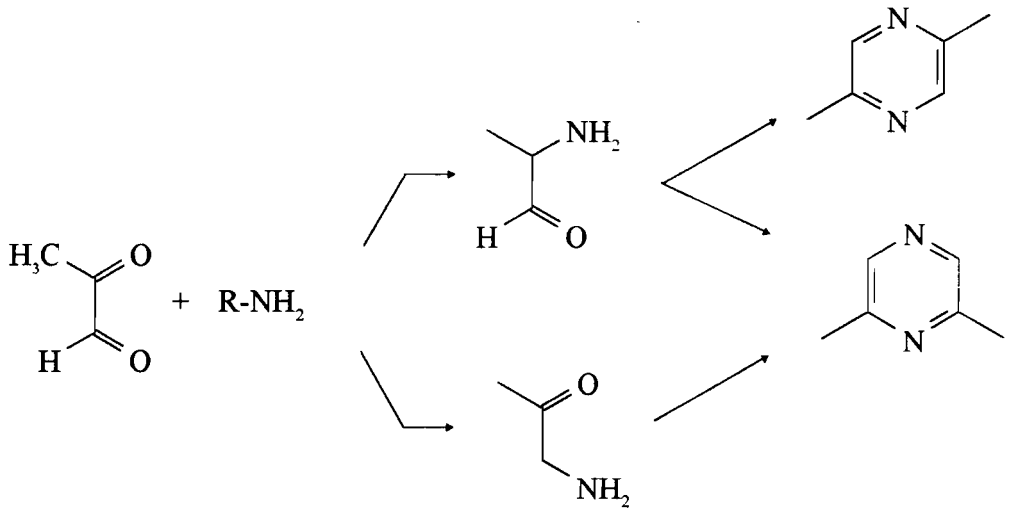

Figure 12: Formation of alkylpyrazines from pyruvaldehyde.
(Dawes and Edwards, 1966)

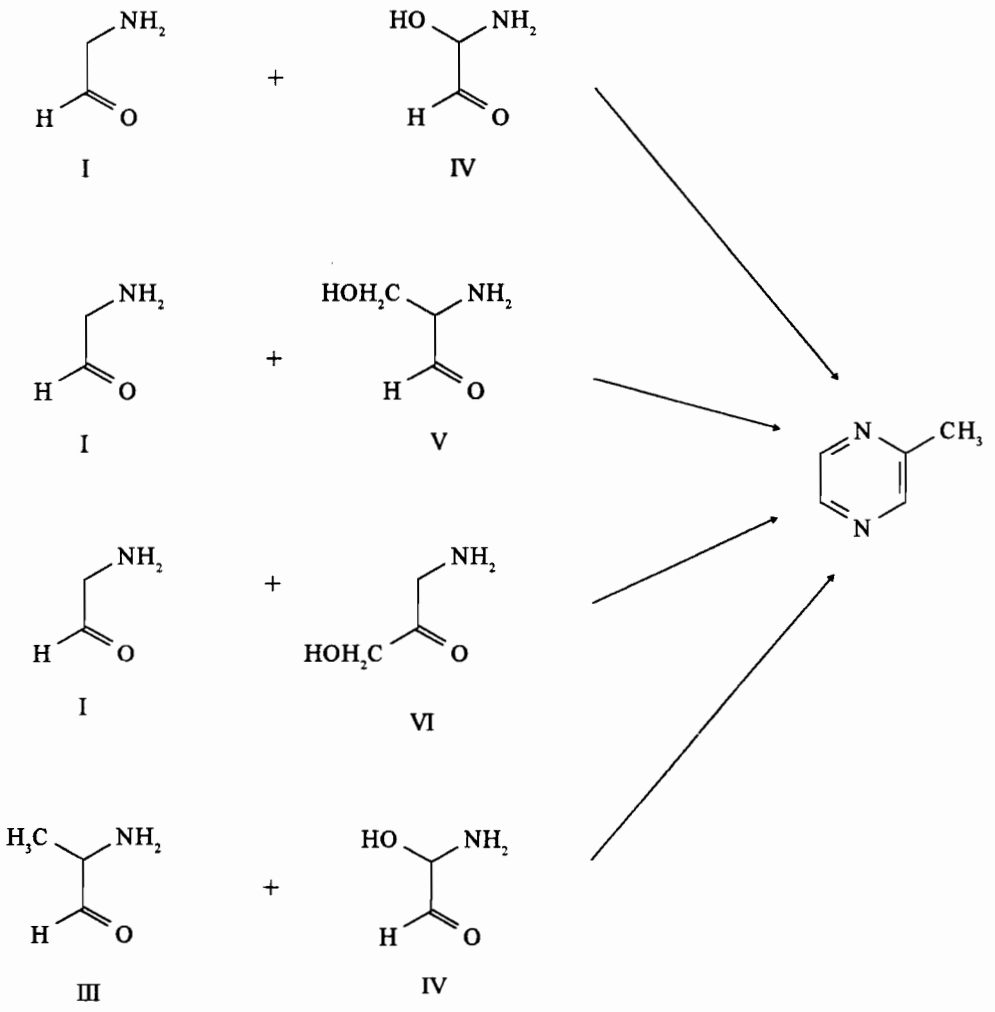

Figure 13: Formation of methylpyrazine from amino carbonyl pairs.
(Shibamoto and Bernhard, 1977)

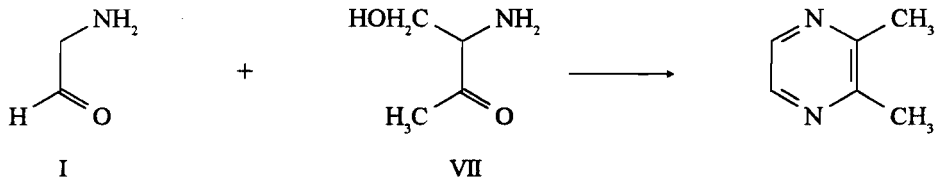

Figure 14: Formation of 2,3-dimethylpyrazine from amino carbonyl pairs.
(Shibamoto and Bernhard, 1977)

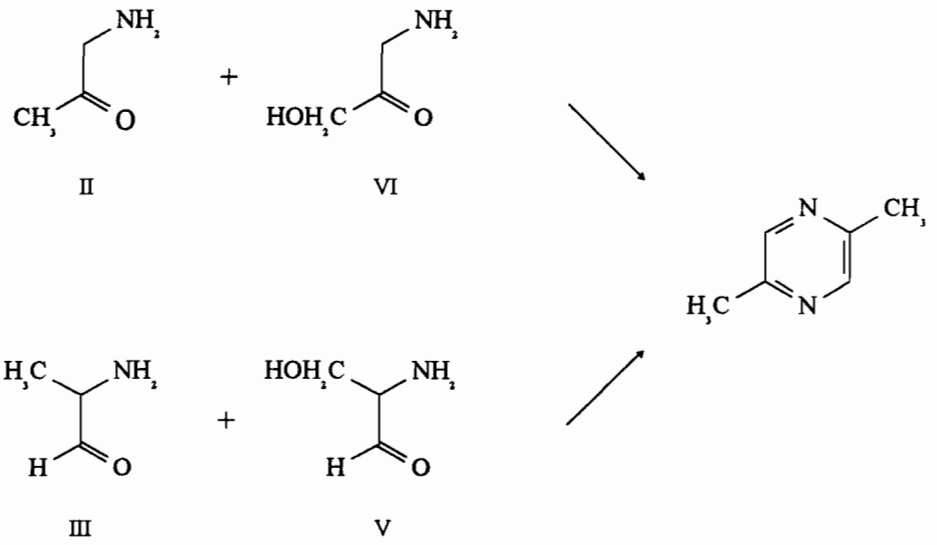

Figure 15: Formation of 2,5-dimethylpyrazine from amino carbonyl pairs.
(Shibamoto and Bernhard, 1977)

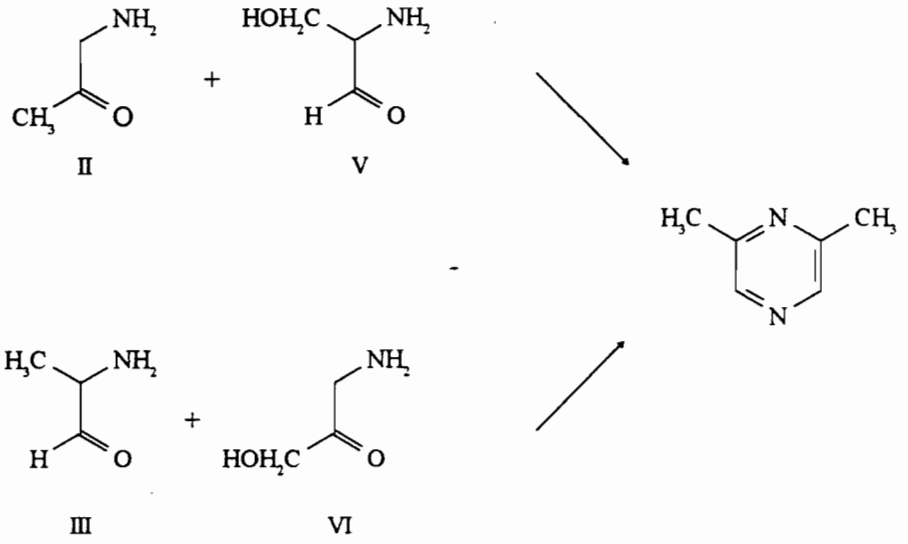

Figure 16: Formation of 2,6-dimethylpyrazine from amino carbonyl pairs.
(Shibamoto and Bernhard, 1977)

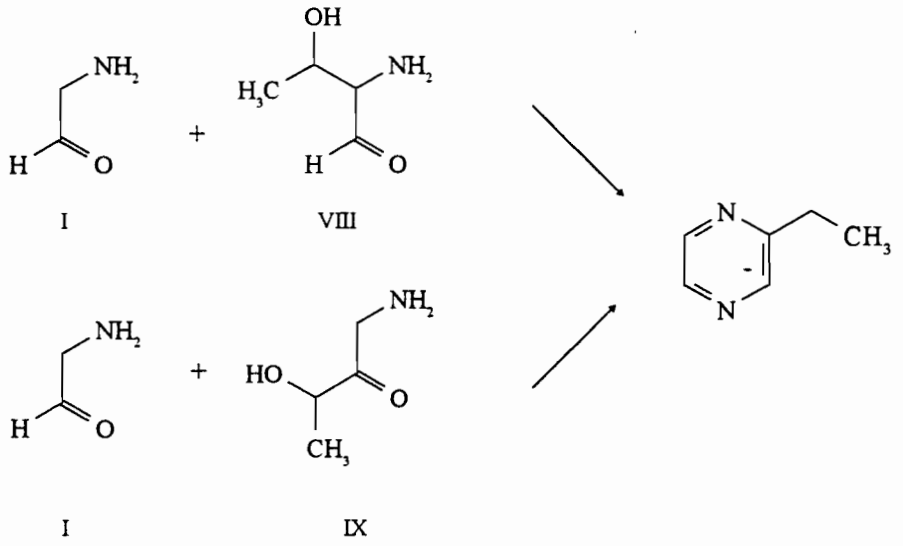

Figure 17: Formation of ethylpyrazine from amino carbonyl pairs.
(Shibamoto and Bernhard, 1977)

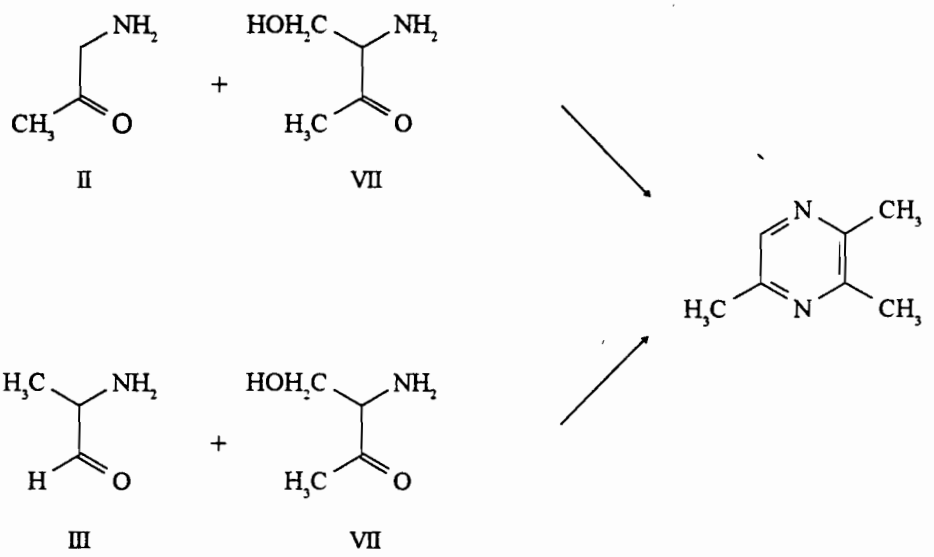

Figure 18: Formation of trimethylpyrazine from amino carbonyl pairs.
(Shibamoto and Bernhard, 1977)

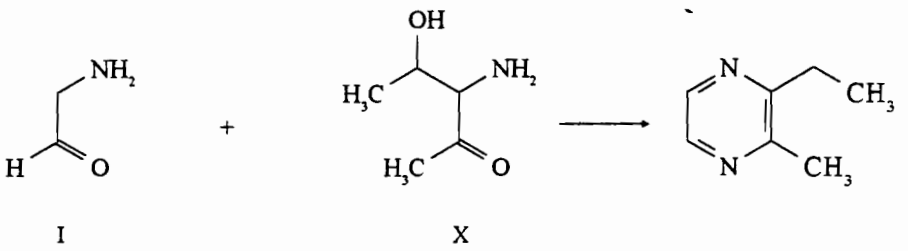

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Figure 19: Formation of 2-ethyl-3-methylpyrazine from amino carbonyl pairs. *(Shibamoto and Bernhard, 1977)*

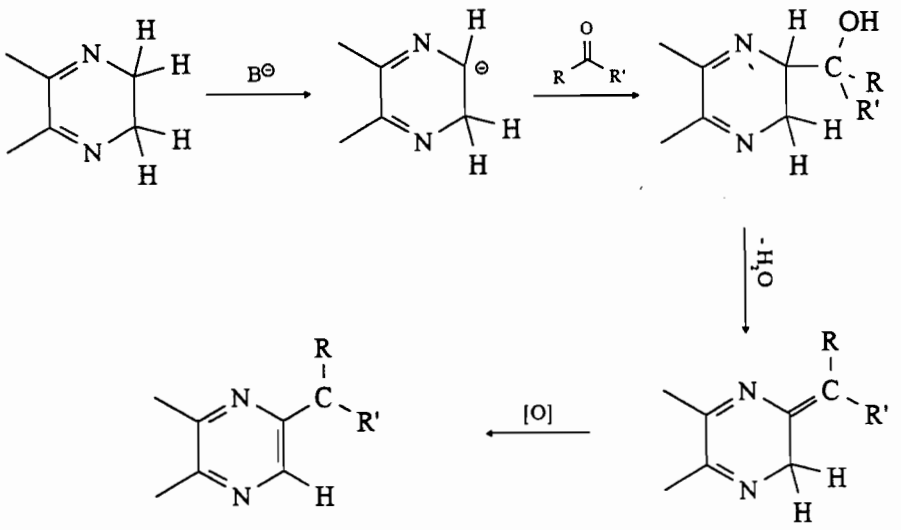


Figure 20: Reactive dihydropyrazine. *(Shibamoto et al., 1979)*

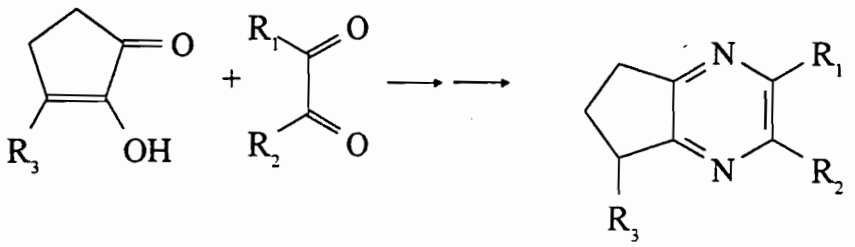
The addition of an aldehyde to a pyrazine carbanion yield larger pyrazines or unsaturated side chains substitution. Newell *et al.* (1967) investigated pyrazine formation in peanut and concluded that the nitrogen atom from the amino acid is introduced into the pyrazine ring through the Strecker degradation; these authors attributed the variable distribution of pyrazines to the differing rate at which different amino groups react with various two-, three-, and four-carbon fragments depending on the ease of nucleophilic attack on the sugar fragments. Addition to carbonyl groups involves attack of a nucleophile at the carbonyl carbon due to the combined resonance effect and the electro affinity of the oxygen molecule leaving the carbon with a partial positive charge.

Figure 21 illustrates the formation of more complex pyrazine compounds such as fused ring pyrazines (Manley *et al.*, 1974).

Newell *et al.* (1967) discussed the formation of dimethylpyrazine from the Amadori 1,2-enaminol which produces a Schiff's base cation and yields an imine upon subsequent decarboxylation (Fig. 22). Hydrolysis of the imine results in a dienamine. Enolization of the 1,2-double bond and migration of the 3,4-double bond yields a ketosamine. Through reto-aldolization the ketosamine would yield aminoacetones which would condense to form dimethylpyrazine.

Namiki *et al* (1983) found free radical activities in the reaction mixture during the early stage of the Maillard reaction and postulated the formation of pyrazine compounds via a hydropyrazine radical.

Carbon atoms in the structure of pyrazine compounds are introduced from α-dicarbonyl fragments of sugars, and nitrogen atoms from amino compounds (Koehler *et al.*, 1969). Figure 23 illustrates the participation of amino groups of amino acids to the formation of pyrazine compounds. In the case of lysine, Hwang *et al.* (1994) proposed that both amino groups are involved in the formation of ten pyrazine compounds in dry and aqueous model systems and that the nitrogen atoms from α-amino groups react more readily with dicarbonyls than the nitrogens from ε-amino groups.



6,7-dihydro-5H-cyclopentapyrazine

Figure 21: Mechanism for the formation of bicyclic pyrazines.
Dihydropyrazines are formed from beta-unsaturated carbonyls in the presence of amino acids after successive nucleoplic, 1,4-addition and condensation reactions (Manley et al., 1974)

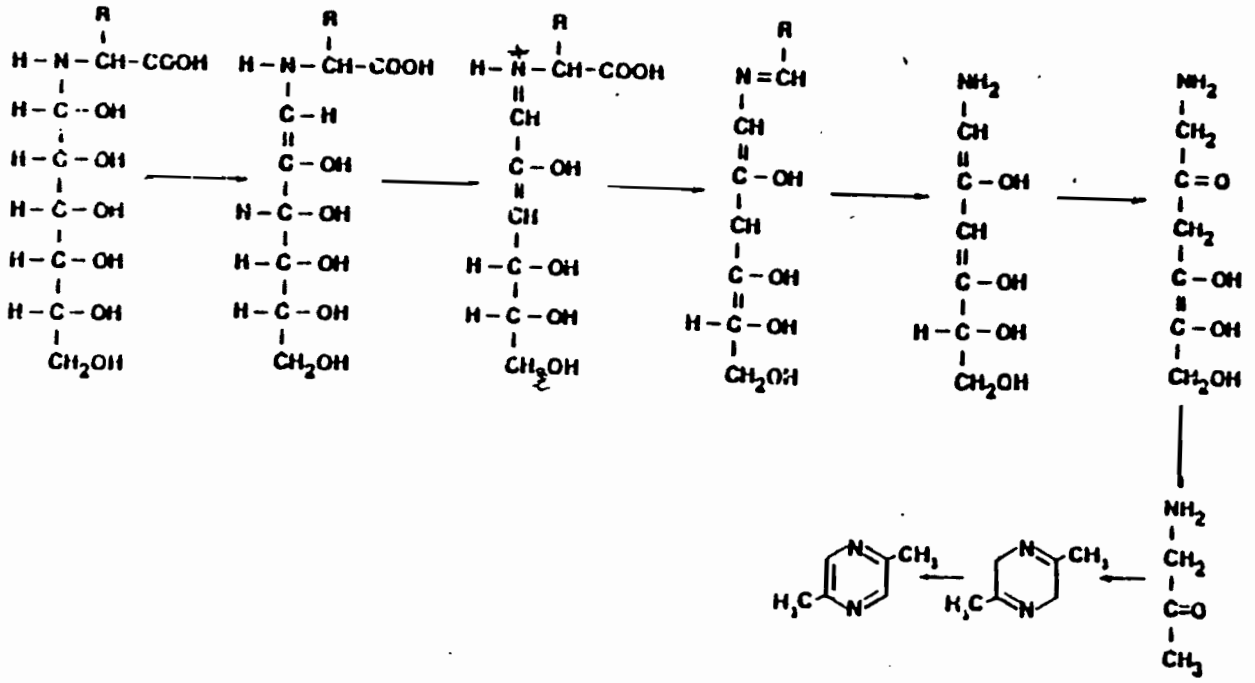


Figure 22: Formation of pyrazines from Amadori rearrangement products.
The ketosamine undergo retro-aldol condensation to yield amino-acetones intermediates which condense to form pyrazine (Newell et al., 1967).

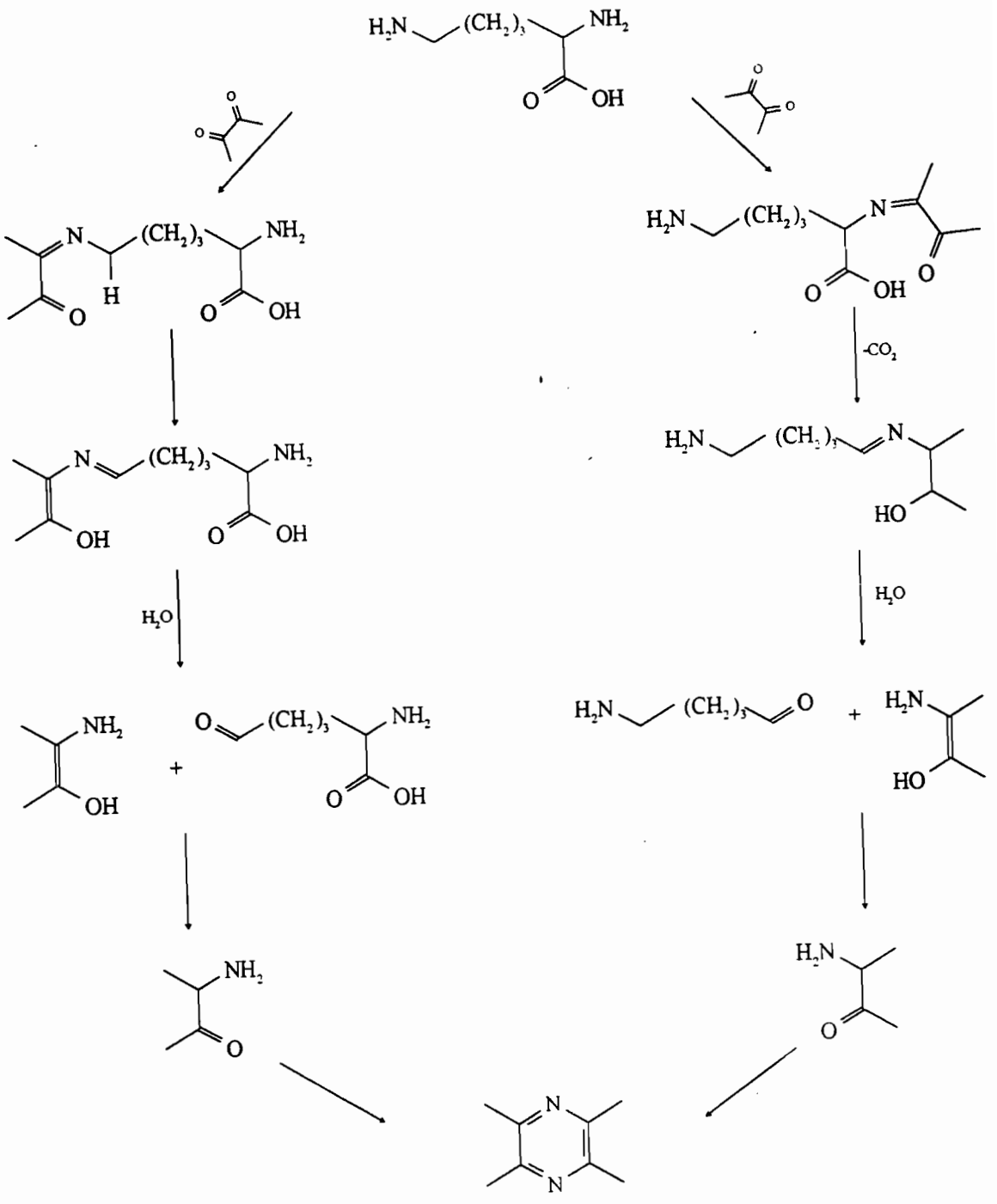


Figure 23: Pyrazine from α and ε amino groups of lysine. *(Hwang et al., 1994)*

III. MATERIAL AND METHODS

Maple sap from the 1992 and 1993 harvests was obtained from the Morgan Arboretum (Macdonald Campus of McGill University, Québec). The sample was kept frozen at -30°C and thawed just before analysis or processing.

Commercial pure maple syrup 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).

Three commercial non-maple syrups i.e.. Aunt Jamima™ (The Quacker Oats Company), Crown Corn Syrup™ and Old Tyme™ (Best Food Canada) were purchased from a local supermarket.

Artificial maple flavorings: LD595, LD596, LD597 and LD598 were obtained from Quest International (Montréal, Canada). Pyrazine standards were purchased from Aldrich Chemical Co. (Milwaukee, WI.).

Double distilled chromatographic grades of diethylether and dichloromethane were purchased from BDH (Montréal, Canada).

3.1. Determination of pH and total dissolved solids

The pH of samples was measured using a multi channel pH-meter (Accumet, model 610, Fisher Chemicals Co.).

The total dissolved solids (TS) of sap and syrup samples was determined by measurement of refractive index (Ri) using an Abbé refractometer (Morselli and Whalen, 1991). The refractometer was standardized using known concentrations of sucrose. A standard curve was drawn from the data (Fig. 24) and used for conversion of refractometer readings to % sucrose. The quantity of

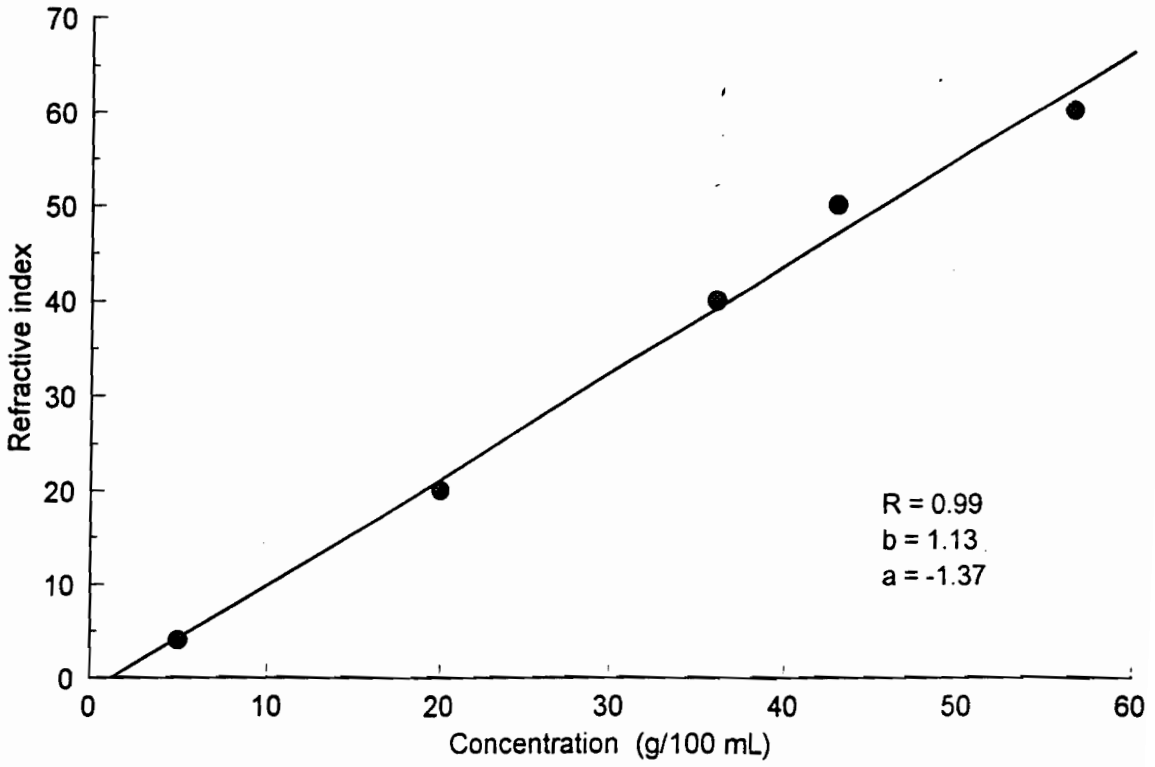


Figure 24: Standard curve for relationship between refractive index and sucrose concentration

Table 6: Reference sugars and their retention time

Sugars	Rt (min)
fructose	19.94
sorbitol*	22.67
glucose	24.57
sucrose	38.21

*Rt, retention time, *, internal standard*

modified by Veeraragavan *et al.* (1990). Lyophilized maple sap (150 mg) was dissolved in 1 mL of Na_2S, and 50 µL was injected. Analysis was performed on a Beckman System 6300 high-performance amino acids analyzer.

3.4. Processing of maple sap

3.4.1. Preliminary experiments

Conditions for laboratory processing of maple sap to syrup were established by preliminary experiments. Volumes of 200 mL, 400 mL 600 mL and 800 mL sucrose solutions (3%) were boiled in glass beakers (1000 mL) and the process was monitored. Direct heating resulted in burnt sucrose for volume of 400 mL and less; boiling of the larger volumes was not homogeneous. Large temperature variations ranging from 5 to 10°C differences occurred, and the time of evaporation for a given volume of sucrose solution was not reproducible. To achieve uniformity in boiling, a sand bed was used as heat transfer between the heat source and the beakers containing the sucrose solutions. Differences between consecutive heatings of same volume were reduced (1 to 2 min). Subsequent adjustments of the burners allowed the reduction of differences in temperature and boiling period of simultaneous evaporation of replicates while maintaining a 105°C temperature. Following this, it was established that a starting volume of 600 mL was adequate and sucrose solution additions (90 to 100 mL) every 15 to 20 min maintained a homogeneous boiling. Portions of

solution were added over a period of 2 min so that variations of temperature did not exceed 2 to 3°C. Rate of evaporation, time of boiling, adequate volume for an uniform boiling, and adequate volumes and rates of addition of fresh solution to achieve a minimum perturbation of the boiling system in terms of temperature change were then established using actual maple sap.

3.4.2. Maple sap evaporation process

On the basis of the preliminary trials, an evaporating process in which glass beakers (1000 mL capacity) were placed on a sand bed within a stainless steel pan was designed (Fig. 25). The system was heated with burners adjusted such that *(1)* an average processing temperature of 105°C was maintained *(2)* the six beakers in the stainless steel pan started boiling simultaneously and *(3)* the rate of evaporation remained the same (an average of 6 mL/min), for all beakers. This design allowed for uniform evaporation rate which is considered important for replication. Temperature of boiling sap samples was measured simultaneously using a multi-channel tele-thermometer (YSI model 42SC; Yellow Spring Instrument Co., Ohio).

Using the conditions established from the preliminary experiments, maple sap samples were processed in triplicate by a single and a continuous evaporating cycle:

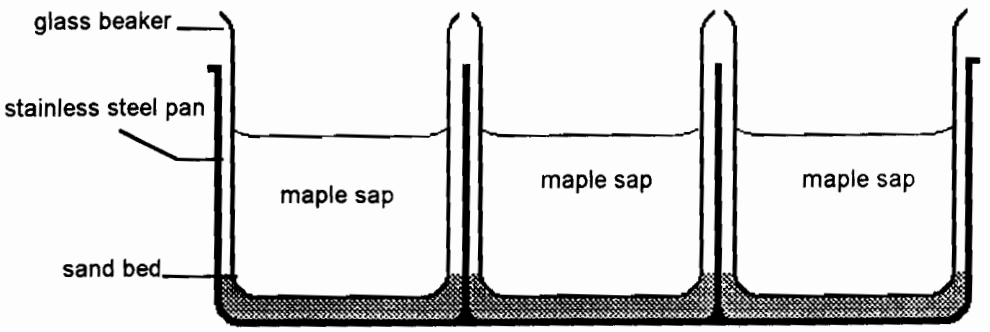


Figure 25: Sectional view of a laboratory maple sap evaporator

3.4.3. Single evaporating cycle of maple sap

The sample of sap was boiled in batches of 600 mL at a constant temperature of 105^{o}C. As the temperature was kept constant, the time of heating, which influence the formation of pyrazine compounds, was varied. Analysis of pyrazine compounds were carried out at 0, 30, 60, 90, 120 min of boiling.

3.4.4. Continuous boiling of maple sap

In this process the volume of the boiling sap (starting with 600 mL) was maintained constant by periodic additions of fresh sap (95 mL at 15 min intervals) to an equivalent total volume of 1500 mL or more. In contrast with the single cycle boiling, continuous boiling allowed for longer boiling period (3 to 4 h) of larger volume of sap and simulated commercial processing of sap to syrup.

3.4.5. Extended heating of maple syrup

Commercial maple syrup (500 g) was heated in beakers using the laboratory evaporator. The heating was extended to 30 min and 50 min at 105^{o}C and the samples were kept for subsequent analysis.

3.5. Gas liquid chromatographic analysis

3.5.1. Gas chromatographic analysis of individual sugars

The analysis was performed using a Varian gas chromatograph (model 3700) equipped with a flame ionization detector, after the sugars were converted to their corresponding silyl-derivatives (Jones and Alli, 1987). Separation was achieved using a DB 1701 (14 %-cyanopropylphenyl silicone bonded phase) fused silica capillary column (30 m length x 0.25 mm i.d. with a 0.25 m film thickness; J&W Scientific, Canada). The conditions for separation were as follows: injector port temperature, 230°C; detector temperature, 280°C; nitrogen carrier gas flow rate, 10 mL/min, oven temperature was programmed from 120°C to 250°C at a rate of 4°C/min with a 6 min initial temperature hold.

All chromatograms were recorded and integration was done using a Hewlett-Packard model HP-3390A integrator, which was programmed, using the internal standard method, to calculate the concentration of each standard or reference compound based on its absolute response factor (Grob and Karrer, 1985; Huang *et al.,* 1990).

3.5.2. Gas chromatographic analysis of pyrazine compounds

Pyrazine compounds in maple sap and syrup, commercial non-maple syrups and artificial maple flavoring were identified and qantitated using pyrazines previously reported in maple syrup as reference (Alli *et al.*, 1990; Akochi-K. *et al.*, 1994). Retention times (gas chromatography) and electron

impact (EI) ionization mass fragments (gas chromatography/mass spectrometry) of the reference pyrazines were compared to those of the samples.

3.5.3. Preparation of pyrazine standard solutions

A stock solution of the mixture of pyrazine standards was prepared by dissolving known weights (Table 7) of each of the reference pyrazine compounds in 50 mL of dichloromethane: pyrazine (as internal standard), methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, ethylpyrazine, 2,3-dimethylpyrazine, trimethylpyrazine and 2-ethyl-3-methylpyrazine. Stock solution (2 μL) was diluted to 500 μL with dichloromethane; 2 μL were injected into the gas chromatograph. Solutions of each individual standard were also prepared using the procedure described above.

3.5.4. Extraction of pyrazine compounds

A method used previously for the extraction and identification of pyrazines in maple syrup (Alli *et al.*, 1990) (Fig. 26) was evaluated for extraction efficiency; a series of three internal standards was used (Table 9). A quantity (approximately 0.1 g; exact quantity determined) of each internal standard was added to the sample to be extracted, as indicated in Figure 26 and 27. The recovery of each internal standard was determined at the end of the extraction procedure. Pyrazine (1,4-diazine) which was not previously identified in maple syrup was used as internal standard (IS) #1 to determine the recovery

Table 7: Reference pyrazines and their retention time

Pyrazines	Rt (min)
pyrazine	11.04
2-methylpyrazine	14.94
2,5-dimethylpyrazine	19.78
2,6-dimethylpyrazine	20.17
ethylpyrazine	21.18
2,3-dimethylpyrazine	22.05
trimethylpyrazine	28.08
2-ethyl-3-methylpyrazine	28.31

Rt, retention time

of pyrazines during the entire extraction procedure (Fig. 26, steps 1 to 4); Reineccius *et al.* (1972) used the parent pyrazine as IS for the quantitation of pyrazine substituted compounds in cocoa beans. Undecanone was used as IS #2 to determine the recovery of step 3 of the extraction procedure; Huang *et al.* (1989) used undecanone as internal standard in the quantitation of pyrazines formed from amino acid-glucose model systems. Dodecane was used as IS #3 to determine the extraction efficiencies of step 4 of the procedure; Hunziker (1989) used dodecane as IS for measurement of recovery of flavor components. The results of these recovery experiments (Table 9) indicate that improvement in the extraction efficiencies is required in the procedure except for the final step (step 4, Fig. 26). To improve the recoveries of the internal standards, the extraction procedure was modified (Fig. 27) as follows: syrup sample (100 g) were diluted with distilled water (100 mL), and pH of the adjusted to pH 3 by dropewise addition of HCl (11% v/v) and stirred at medium speed with a magnetic stirrer (10 min). Sodium chloride (30 g) was added and the mixture was extracted with diethylether (150 mL) with stirring for a further 30 min. The aqueous phase was separated and adjusted to pH 11 and extracted with dichloromethane (5x20 mL). The dichloromethane extract was concentrated to 5 mL with a rotary evaporator and then to a final volume of 0.5 mL using a stream of nitrogen.

The modified extraction procedure was used to isolate pyrazine compounds from commercial non-maple syrups, artificial maple flavor as well as lyophilized maple sap. The quantities of these samples used in the extraction procedure were as follows: non-maple syrup, 100 g; artificial maple flavor, 5 g; freeze dried maple sap, 30 g.

Pyrazines were subsequently determined by gas liquid chromatography using a Varian gas chromatograph (model 3700) equipped with a flame

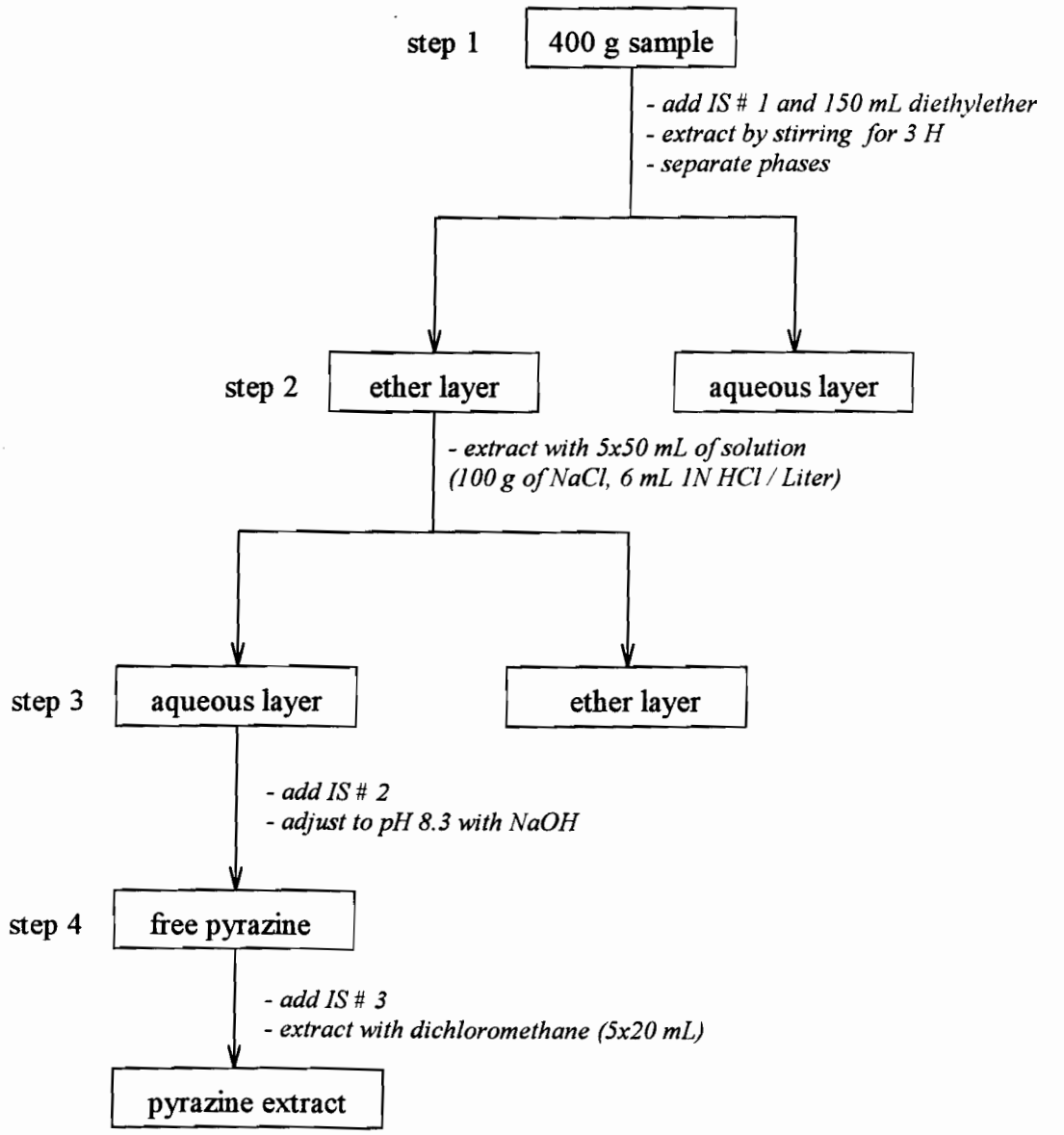


Figure 26: Method for the extraction of pyrazines (Alli *et al.*, 1990)

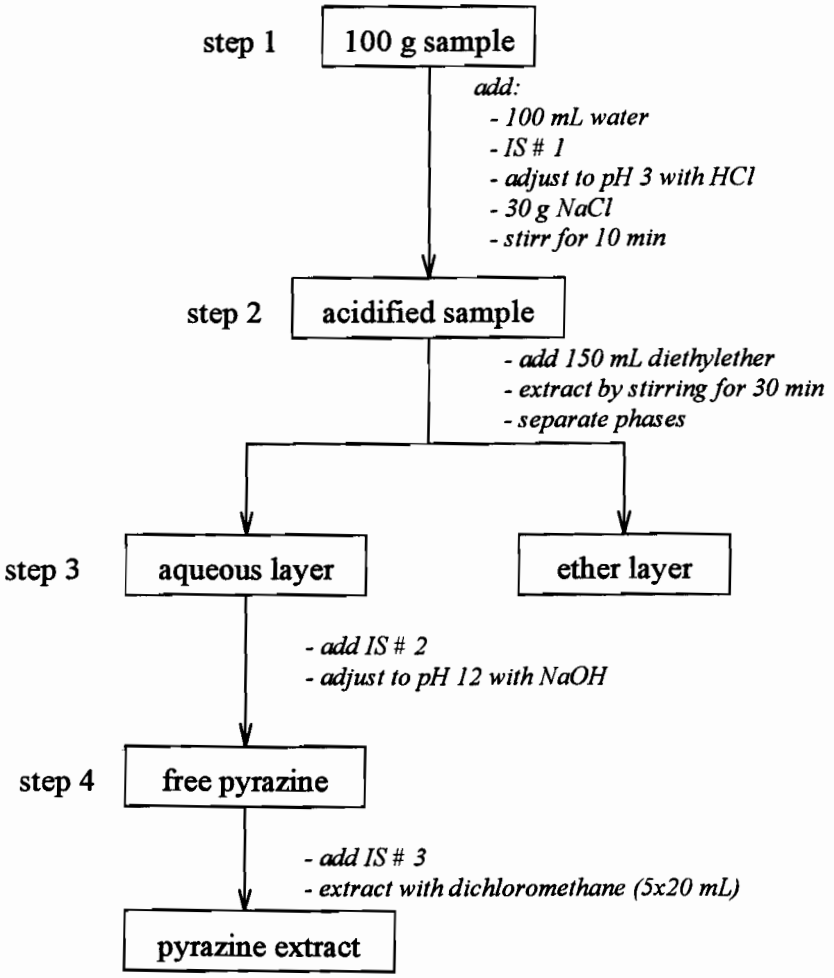


Figure 27: Modified method for the extraction of pyrazines

ionization detector was used. In the qualitative determination, splitless injection mode was used for samples of non-maple syrup which contained low quantities of pyrazines, while split injection mode was used when the quantities were high as in the case of the artificial maple flavors. On-column injection mode was used for all quantitative analysis. The following conditions were used for the separation: Supelcowax 10 (polyethylene glycol polar bonded phase) fused silica capillary column (30 m length x 0.32 mm i.d. with a 0.1 μ film thickness; Supelco, Canada); injector port temperature, 200°C; detector temperature, 250°C; nitrogen carrier gas flow rate, 1 mL/min. Oven temperature was programmed from 40°C to 150°C at a rate of 1°C/min with a 6 min initial temperature hold.

All chromatograms were recorded and integration was done using a Hewlett-Packard model HP-3390A integrator, which was programmed, using the internal standard method, to calculate the concentration of each standard or reference compound based on its absolute response factor (Grob and Karrer, 1985; Huang *et al.,* 1990).

3.5.5. Gas chromatography/Mass spectrometry analysis of pyrazines

Gas chromatography/mass spectrometry (GC/MS) analyses were performed using a gas chromatograph (Hewlett Packard HP Model 5890 series II) coupled to a mass spectrometer (HP 5971B series). Ionization was achieved in the electron impact (EI) mode with an energy level of 70 eV. Helium was used as carrier gas. Analytical conditions were similar to those used for gas

chromatography. Pyrazines were identified based on their EI fragmentation data compared to data in the Wiley library.

3.5.6. Estimation of rate constant for pyrazine formation in maple syrup

Rate constants for the formation of pyrazine compounds in maple sap during processing to syrup were obtained assuming a *pseudo*-zero order reaction, defined as follows (Stamp and Labuza, 1983):

$$B = B_0 + k_z t$$

Whereby B (ng/g) is the concentration of pyrazines measured at t (min), B_0 (ng/g) is the concentration of pyrazines measured at t = 0, k_z (ng/min) is the pseudo-zero order rate constant of formation.

3.6. Sensory evaluation of maple syrups

3.6.1. Prescreening of panelists

A group of 20 untrained, volunteers participated in the sensory evaluation of maple syrups. The selection of panelists was based on availability, motivation and affection of the product (Moskowitz, 1984). The pre-screening, which established the capabilities of the panelist to discriminate between authentic maple syrup and non-maple syrup, was conducted using 56 potential evaluators. During the first session, the subjects were presented with one maple syrup and one non-maple syrup, and were asked to identify the samples, by a direct comparison of taste and flavor. From these tests, 35

panelists, who were consistently accurate in their identification, were retained for a second stage of selection. At this stage, the panelists were asked to identify maple syrups samples in a triangle test (Larmond, 1987) based on "maple flavor" attribute. The test samples consisted of two identical samples and one different from the two others. Twenty evaluators were retained following this test on the basis of their ability to show consistency in correctly identifying maple syrups. This working panel was composed of 20 volunteers (12 females and 8 males) ranging in age from 22 to 45 years. Additional sessions were conducted to identify the suitable time period of the day for the tests; a period of 30 to 45 min was retained for the evaluation of 3 samples. The time of the testing was either 11 am (before lunch) or 2 p.m. after lunch (Larmond, 1987; Poste *et al.*, 1991).

3.6.2. Methods of laboratory sensory analysis

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 (Larmond, 1987; Poste *et al.*, 1991). The tests were repeated weekly over a five-week period with each week representing a replicate. The experiment was performed in a sensory evaluation room maintained at 22°C and red lighted to eliminate any bias due to differences in sample color. A quantity (15 g) of 3 digits coded to represent the 3 maple syrup samples (light, medium, and dark amber) were presented to the panelists in a unbalanced random order. The digits codes were

generated with the SAS program (SAS Institute Inc., Cary, North Carolina) and were changed from one session to an other. 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 (Fig. 28). A second part of the test consisted in indicating the level of preference of the samples with qualifying terms from "like very much" to “dislike very much” (Fig. 28) including a neutral point "no preference". Data was collected by measuring the intensity of the ratings and subjected to statistical analysis using the SAS program .

[ ]

NAME_____ DATE_____

Please evaluate these maple syrup samples as indicated bellow :

For each attribute, make a vertical line on the horizontal grading line to indicate your rating.

The end marks on the horizontal grading lines represent the minimum (left) and the maximum (right) of the attribute being tested.

Label each vertical line with the code number of the sample it represents.

Please taste the samples in the following order :

I- Indicate the intensity of the following attributes for each sample

MINIMUM MAXIMUM

taste _____

maple flavor _____

II- Overall rating of each sample using the same order as above

#.....	#.....	#.....
__ like very much	__ like very much	__ like very much
__ like moderately	__ like moderately	__ like moderately
__ like slightly	__ like slightly	__ like slightly
__ no preference	__ no preference	__ no preference
__ dislike slightly	__ dislike slightly	__ dislike slightly
__ dislike moderately	__ dislike moderately	__ dislike moderately
__ dislike very much	__ dislike very much	__ dislike very much

#.....	#.....	#.....
__ like very much	__ like very much	__ like very much
__ like moderately	__ like moderately	__ like moderately
__ like slightly	__ like slightly	__ like slightly
__ no preference	__ no preference	__ no preference
__ dislike slightly	__ dislike slightly	__ dislike slightly
__ dislike moderately	__ dislike moderately	__ dislike moderately
__ dislike very much	__ dislike very much	__ dislike very much

General comments : .....

.....

.....

Figure 28: Sample of a sensory evaluation sheet

IV. RESULTS AND DISCUSSION

4.1. Preliminary experiment

A suitable maple sap evaporator was needed in the laboratory to ensure that evaporation and replication could be compared directly with the industrial process. Variations in maple syrup quality and constituents are attributed, in part, to the thermal conditions of processing (Dumont, 1995). Other important factors which lead to changes in maple syrup quality are aseptic conditions of sap harvesting, and differences in sap quality within a season (Morselli and Whalen, 1991). Therefore, it was necessary to control parameters such as temperature, time of boiling and volume of sap. The evaporator and determined conditions of heating permitted this type of control. The size of the beakers permitted the use of volumes of sap which proved convenient for homogeneous boiling without burning the syrup. The design allowed the monitoring of the process in terms of textural and color changes, and also facilitated determination of syrup yields.

4.2. Individual sugars in maple sap and syrup

Figures 29, 30 and 31 show typical chromatograms from the gas chromatographic analysis of standard reference sugars, individual sugars of maple sap and individual sugars of maple syrup samples, respectively. Table 8

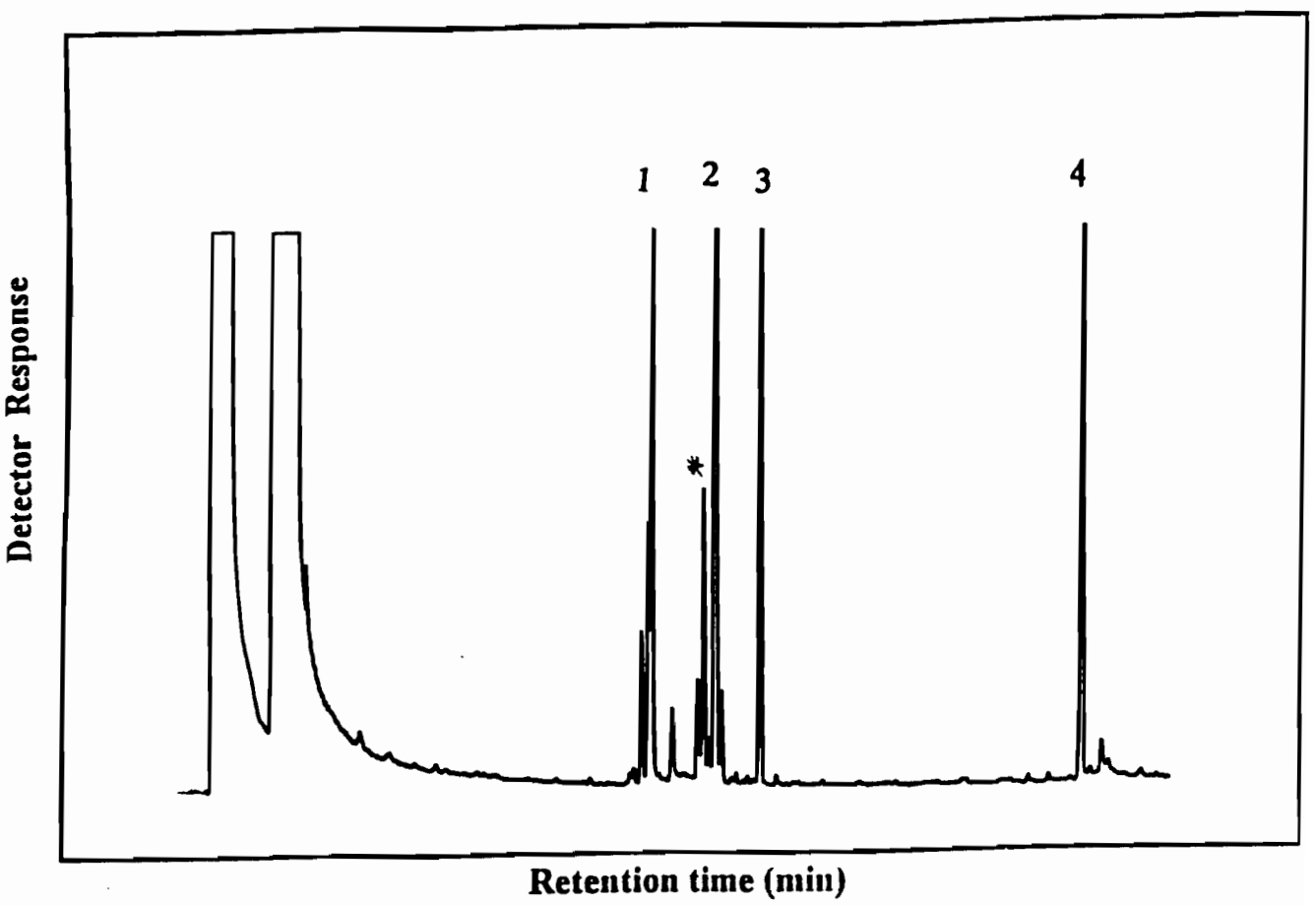


Figure29: Gas Chromatogram of trimethyl silyl derivatives of reference sugars. *1, fructose; 2, sorbitol (internal standard); 3, glucose;4, sucrose * glucose anomer.*

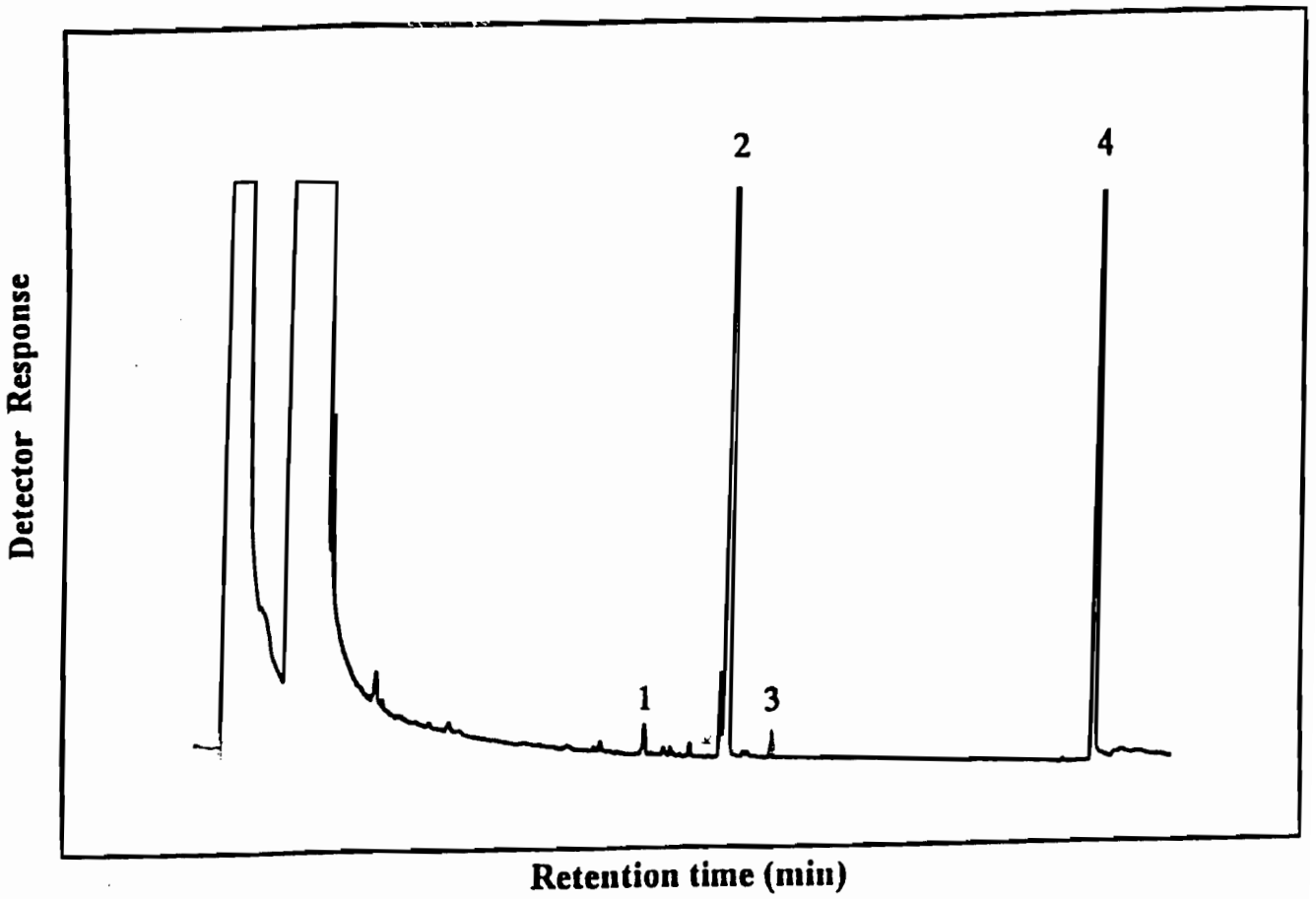


Figure30: Gas Chromatogram of trimethyl silyl derivatives of maple sap sugars. *1, fructose; 2, sorbitol (internal standard); 3, glucose;4, sucrose * glucose anomer.*

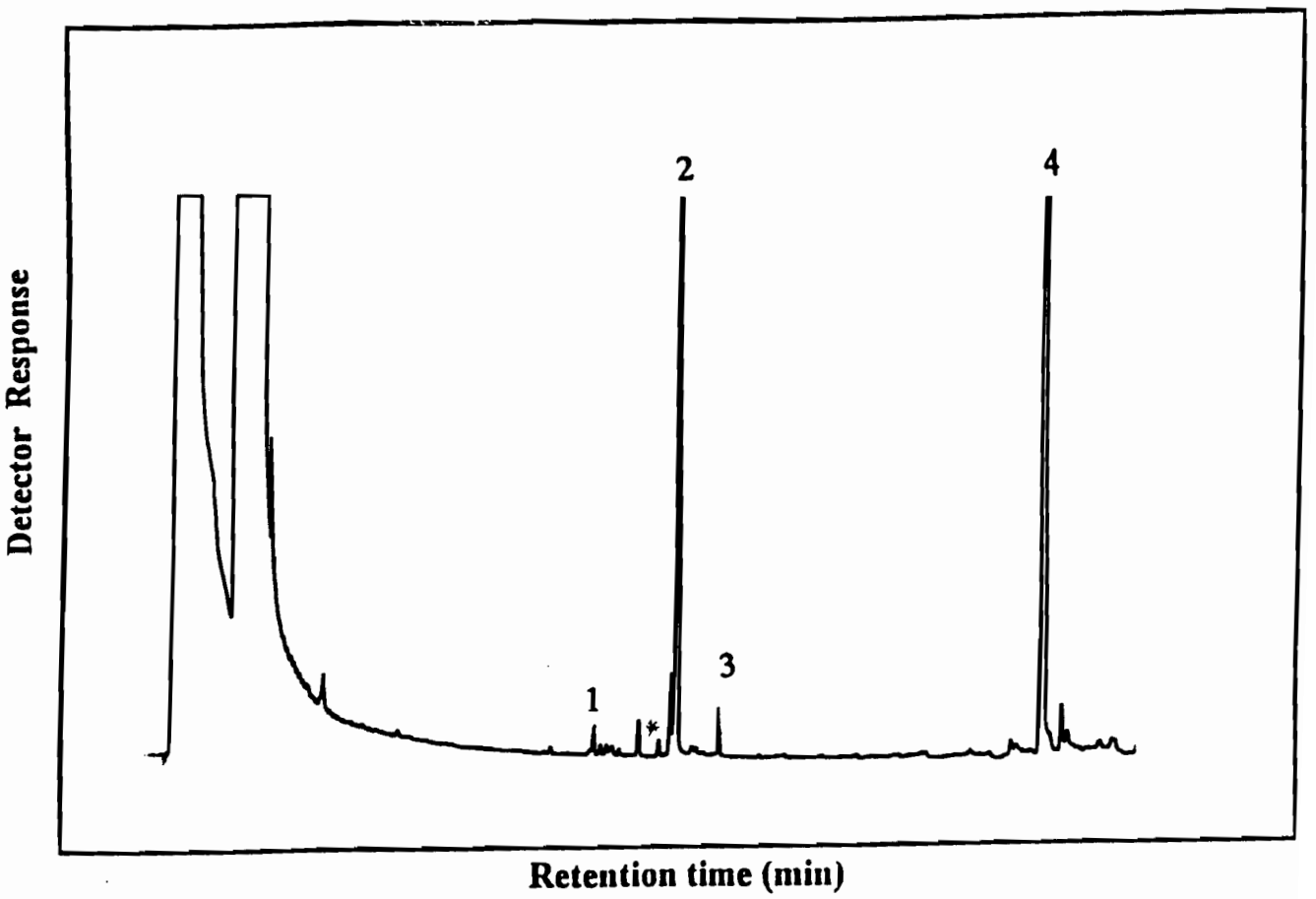


Figure31: Gas Chromatogram of trimethyl silyl derivatives of maple syrup sugars. *1, fructose; 2, sorbitol (internal standard); 3, glucose;4, sucrose * glucose anomer.*

Table 8 Content of fructose, glucose and sucrose in maple sap, heated maple sap and maple syrup

Samples	Sugars (mg/g)		
	Fructose	Glucose	Sucrose
Unheated maple sap	0.09 (0.01)	0.09 (0.00)	23.21 (0.02)
[1]%	*0.38*	*0.38*	*99.27*
Heated maple sap to 9% TS	0.19 (0.02)	0.38 (0.01)	58.98 (1.15)
%	*0.32*	*0.64*	*99.04*
Heated maple sap to 16% TS	0.24 (0.00)	0.50 (0.01)	109.34 (1.02)
%	*0.22*	*0.45*	*99.33*
Laboratory syrup (65%) TS)	1.82 (0.32)	3.25 (0.07)	416.97 (1.28)
%	*0.43*	*0.77*	*98.80*
Maple syrup (65.5% TS)	1.64 (0.22)	2.77 (0.13)	417.63 (0.17)
%	*0.39*	*0.66*	*98.96*

Results are means (standard deviation) of triplicate determinations

[1]% refers to individual sugar as a percentage of total carbohydrates

TS = Total dissolved solids

shows the distribution of individual sugars in maple sap, maple sap boiled to 9% and 16% total dissolved solids, in laboratory prepared maple syrup (65.5% total dissolved solids) and in commercial maple syrup. Fructose, glucose and sucrose were identified. These sugars were previously identified in maple sap (Jones and Alli, 1986; Kallio, 1988; Leech and Kim, 1990). Sucrose represented more than 99% of the total sugar content of maple sap; the contents of fructose and glucose were 0.38%, 0.38%, respectively; representing a total of 23.39 g of total sugars/L of sap. Glucose showed an increase in the intermediate heated maple saps with sucrose remaining in highest concentration. The concentrations of fructose increased from 0.09 mg/g in the sap to 1.82 mg/g in laboratory prepared syrup, 0.09 mg/g in the sap to 3.25 mg/g for glucose and sucrose increased from 23.21 mg/g in the sap to 416.97 mg/g in laboratory prepared maple syrup; these increases are the result of water evaporation. Heat degradation of sucrose may have contributed to the increase of fructose and glucose. The slow increasing rate and low level of fructose in maple syrup may be due to its degradation at elevated temperatures. The sugars of commercial maple syrup were 0.39% fructose, 0.66% glucose and 98.96% sucrose. The sugar contents of laboratory prepared maple syrup were similar to that of the commercial syrup sample; this suggests that laboratory processing conditions simulated very well the industrial method for the concentration of sugars in maple syrup. The high sucrose content of maple syrup is responsible for both

the sweetness and the viscosity characters. Although glucose and fructose are present in small quantities, they are important flavor precursors.

4.3. Individual free amino acids

Analysis of maple sap for individual free amino acids showed the presence of trace quantities of aspartic acid, serine, glycine, alanine, valine and lysine (Fig. 32). 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 amino-carbonyl fragments, which combine with other amino-carbonyl fragments to form pyrazines; with glycine and lysine being highly reactive and serine and alanine moderately reactive in the formation of N-heterocyclic compounds (Baltes, 1990).

4.4. Changes in pH and total solids during processing of sap to syrup

The use of heat to convert maple sap to syrup results in the development of the texture, the color and the flavor characteristics of the final product. While textural characteristics are attributed to the concentration of sugars (65% sucrose) in the syrup, the color and maple flavor, which serve as basis for grading the syrup, are induced by caramelization and Maillard type reactions.

Figure 33 shows the change in pH during the processing of maple sap. The pH of boiling sap increased from 7.2 to pH 9.2 after 30 min of boiling at 105°C then decreased to pH 7.3 (Fig. 33a). The initial increase in pH could be

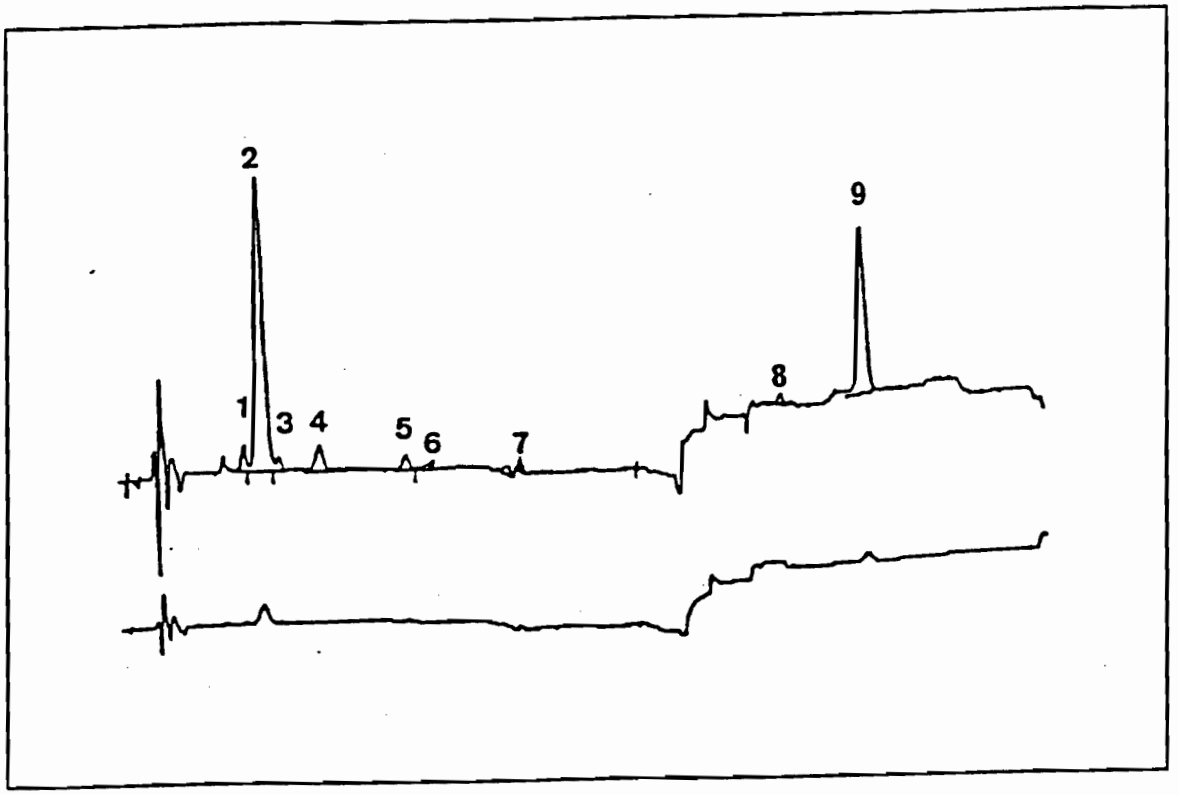


Figure 32: HPLC analysis of individual free amino acids of maple sap
aspartic acid (1), unidentified (2), serine (3), unidentified (4), glycine (5), alanine (6), valine (7), lysine (8) and ammonia (9)

due in part to *(1)* the formation of Amadori rearrangement products since these secondary and tertiary amines are more basic than amino acids, and *(2)* the loss of organic acids present in maple sap (Kallio, 1988; Mollica and Morselli, 1984) due to decarboxylation. 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 of the pH. The second phase of the pH change, from pH 9.2 to pH 7.3, may be due to the decomposition of the Amadori products which are implicated in further reactions (Namiki, 1988), concentration of organic acids (Nelson, 1928) and the presence of CO_2 produced during the Strecker degradation. The pH change during heating of maple sap under refluxing conditions followed closely the changes observed for the open heating (Fig. 33). This confirms that chemical reactions during heating contributed to the observed pH changes rather than losses through evaporation. Figure 33 also shows change in total dissolved solids (TS) during heating of maple sap. The increase in TS was gradual during the first 40 min of boiling. This was followed by a sharp increase during a 20 min period from 5% TS to 65% TS. The rapid increase of TS could be attributed to an enhanced evaporation of water due to solids concentration. The increase coincided with the decrease of pH. It should be pointed out that the above observations on pH and total dissolved solids changes were monitored only during single cycle boiling experiments but not during the continuous boiling experiments because the system was disturbed by the frequent additions of fresh sap.

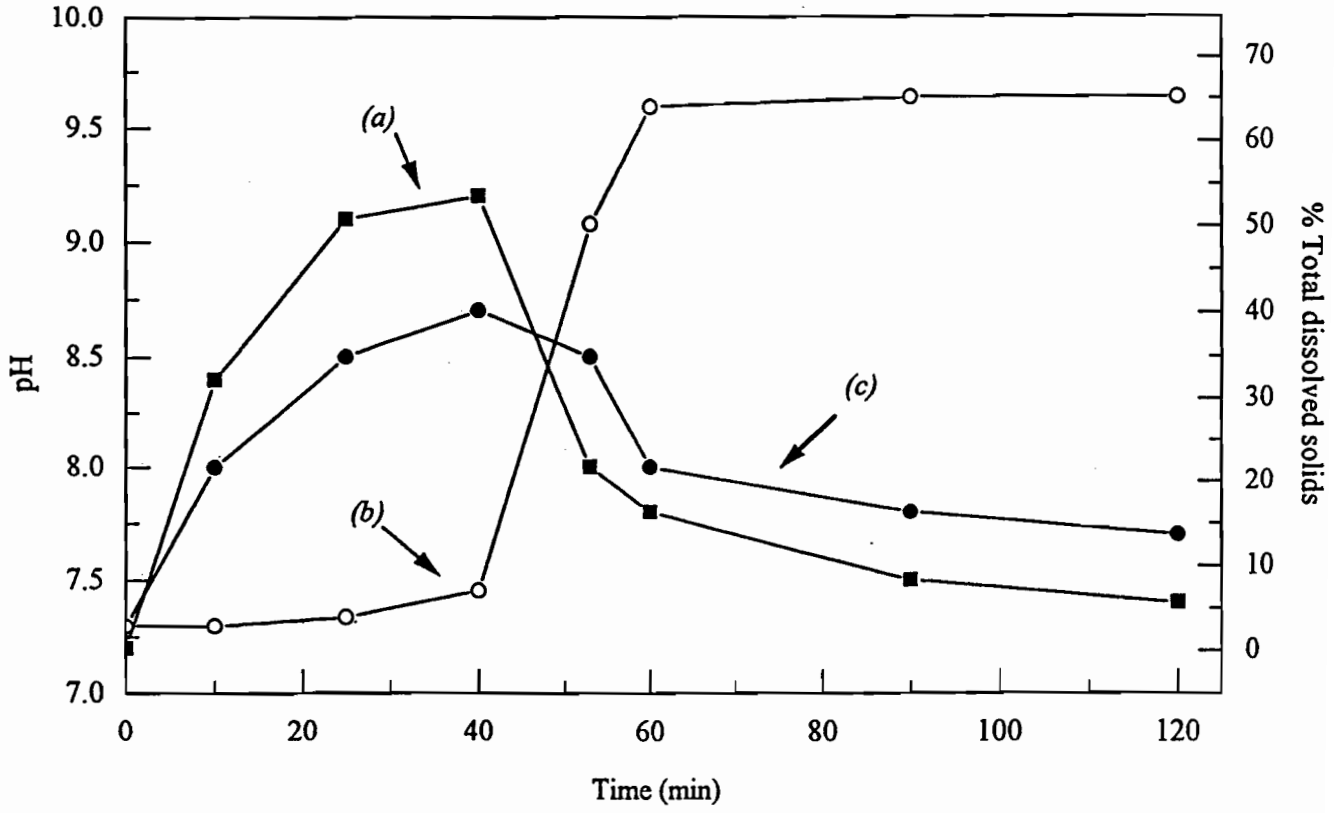


Figure 33: Changes of pH *(a)* total solids *(b)* and pH under reflux *(c)* during heating of maple sap

4.5. Isolation of pyrazine compounds from maple syrup

Extraction of pyrazine compounds in the presence of large quantities of other volatile compounds represents a challenge. The relatively low amounts of pyrazines present in maple syrup along with the large sample size required for reproducible extraction as described in the method of Alli *et al.* (1990) led to efforts to improve and optimize the extraction method for the quantitation of pyrazine compounds in maple syrup.

Table 9 shows the recoveries of the three internal standards (IS) used in the evaluation of the extraction method (section 3.3.2, Material and Methods). The recoveries of the internal standards pyrazine, undecanone and dodecane were 77%, 87% and 96%, respectively, when the extraction procedure described by Alli *et al.* (1990) for the identification of pyrazine was used (Figure 26). Modification of the extraction procedure as shown in Figure 27 resulted in recoveries of 97%, 95% and 97% for pyrazine, undecanone and dodecane, respectively (Table 9). The recovery of IS pyrazine improved from 77% to 97% and that of undecanone was from 87% to 95%, suggesting that the extraction efficiency was improved between step 1 and step 4 of the modified method. A likely explanation for the improved recovery of IS pyrazine is that addition of hydrochloric acid resulted in the conversion of pyrazines to their hydrochloride salts. This is brought about by the formation of di-quartenary pyrazine salts, the resonance sequence within the pyrazine ring which could lead to the di-quarternary pyrazine compounds (Fig. 34). Electron pairs of the nitrogen atoms within the ring do not participate in the resonance and therefore

Table 9: Recoveries of internal standards and reference pyrazines

	Recovery (%)	
	Original method[1]	Modified method[2]
Internal standards		
Pyrazine	77 (2.1)	97 (3.2)
Undecanone	87 (1.5)	95 (0.8)
Dodecane	96 (0.3)	97 (1.1)
Reference pyrazines		
2-methylpyrazine	77 (1.9)	90 (1.2)
2,5-dimethylpyrazine	80 (1.3)	93 (1.8)
2,6-dimethylpyrazine	82 (1.7)	91 (1.2)
2-ethylpyrazine	63 (1.1)	61 (2.3)
2,3-dimethylpyrazine	89 (2.5)	92 (0.5)
trimethylpyrazine	83 (1.1)	95 (1.1)
2-ethyl-3-methylpyrazine	87 (2.0)	91 (1.2)

Results are means (standard deviation) of triplicate analysis

[1] *Figure 26 (Alli et al., 1990)*

[2] *Figure 27 (developed in present work, Akochi-K. et al., 1994)*

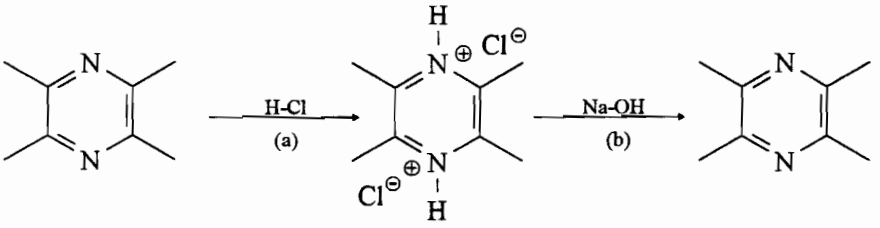


Figure 34: Formation of di-quaternary salts of pyrazine

are available for bonding to other groups. Curphey (1965) reported the formation of pyrazine quartenary salts when 1,4-diazine and ethoxy-borotetra-fluoride were reacted in the presence of 1,2-dichloroethane. More recently, Namiki and Hayashi (1983) reported on the presence of N,N'-disubstituted pyrazine cation in the early stage of the Maillard reaction. The non-volatile di-quarternary salts which are formed (Fig. 34), solubilize in the aqueous phase, therefore, the likelihood of loss by volatilization is greatly reduced. The subsequent "salting out" by addition of NaCl serves to enhance the extraction of volatile, non-nitrogenous compounds by diethylether in the next step of the extraction procedure.

The improved recovery of internal standard undecanone suggests that adjustment to pH 11 instead of pH 8 resulted in more efficient release of nitrogen containing compounds from the aqueous to the subsequent dichloromethane phase (Figure 27).

No significant improvement was achieved in the recovery of internal standard dodecane, suggesting that the transfer of nitrogenous flavor compounds from the aqueous to the dichloromethane phase was not a critical step in the initial extraction procedure.

Table 9 also shows the recoveries of the reference pyrazines using the original procedure developed by Alli *et al.* (1990) (Fig. 26) and the modified

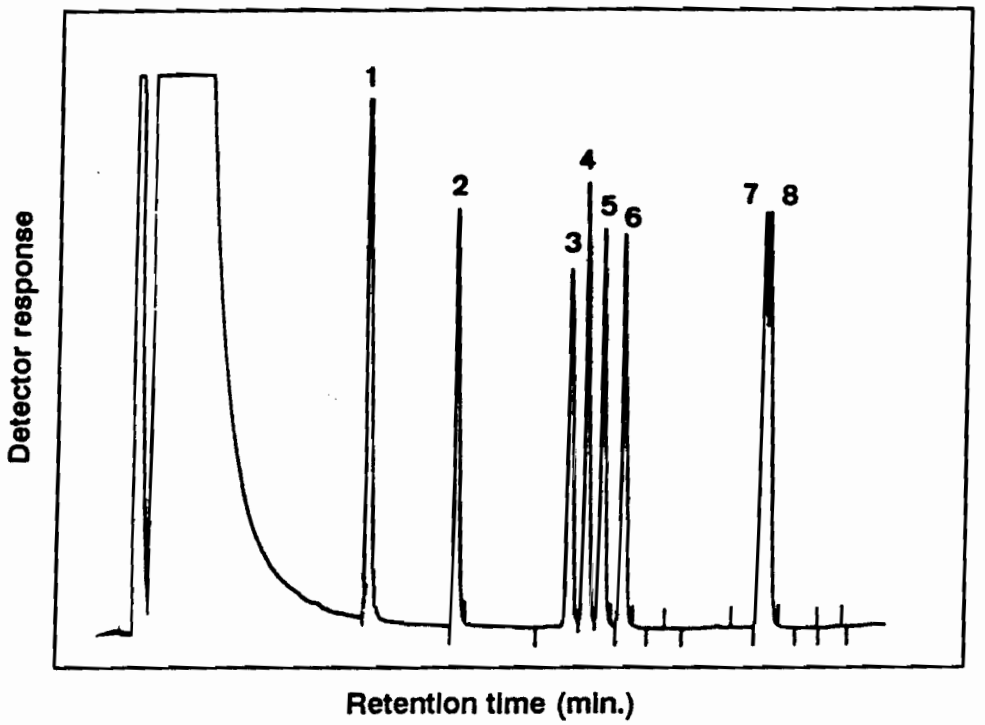


Figure 35 Gas chromatogram of reference pyrazine standards.
1, *pyrazine (1,4-diazine);* **2**, *methylpyrazine;* **3**, *2,5-dimethylpyrazine;* **4**, *2,6-dimethylpyrazine;* **5**, *2-ethylpyrazine;* **6**, 2,3-*dimethylpyrazine;* **7**, *trimethylpyrazine;* **8**, *2-ethyl-3-methylpyrazine.*

procedure developed in this study (Akochi-K. *et al.,* 1994) (Fig. 27). With the exception of 2-ethylpyrazine, the recoveries of pyrazines were increased to above 90%. These increased recoveries confirm the enhanced extraction of pyrazines for quantitation as a result of the modification of the method in the present study.

4.6. Gas-liquid chromatographic analysis of pyrazine standards

Figure 35 shows a typical chromatographic separation of the mixture of reference pyrazine standards. The peaks were identified using a combination of methods: *1)* retention time comparison with authentic and *2)*, confirmation by GC/MS. The parent pyrazine (unsubstituted 1,4-diazine) was used as the internal standard in the quantitative analysis because of its absence in maple syrup.

4.7. Pyrazine compounds in commercial maple syrups

Figure 36 shows the chromatogram obtained from gas chromatographic analysis of pyrazines in maple sap. The results indicate that no pyrazine compounds are present in maple sap.

Typical chromatograms obtained from analysis of pyrazines in light, medium and dark grades of commercial maple syrup are shown in Figures 37, 38 and 39, respectively. Identified pyrazines and their concentration in these

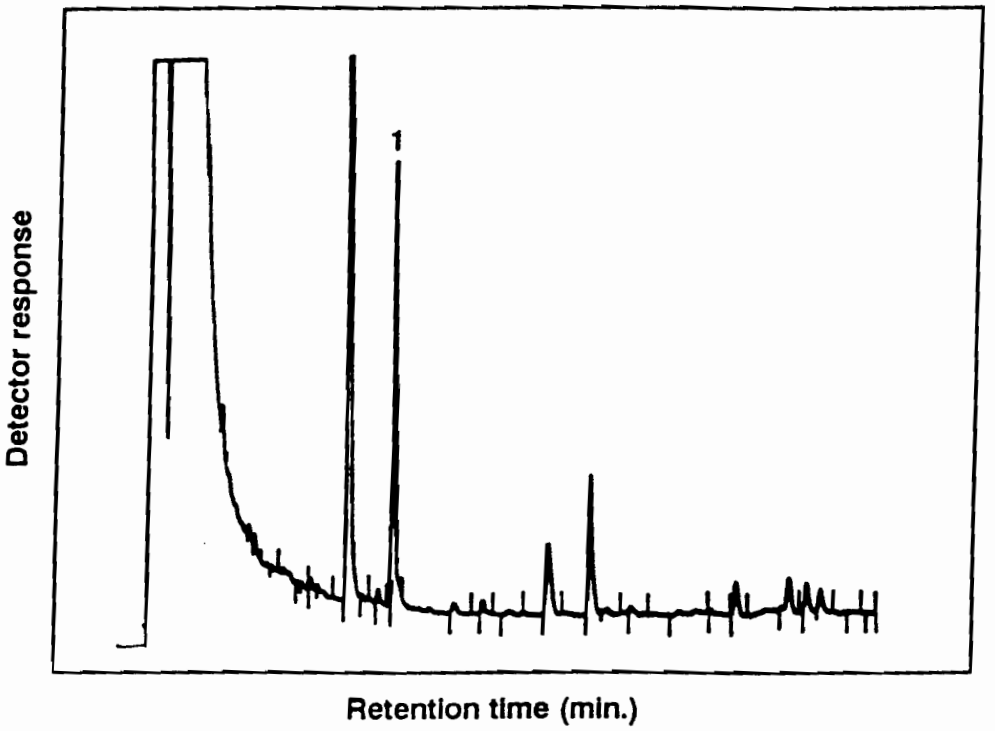


Figure 36 Gas chromatogram of maple sap analyzed for pyrazines.
1, *pyrazine (1,4-diazine)*

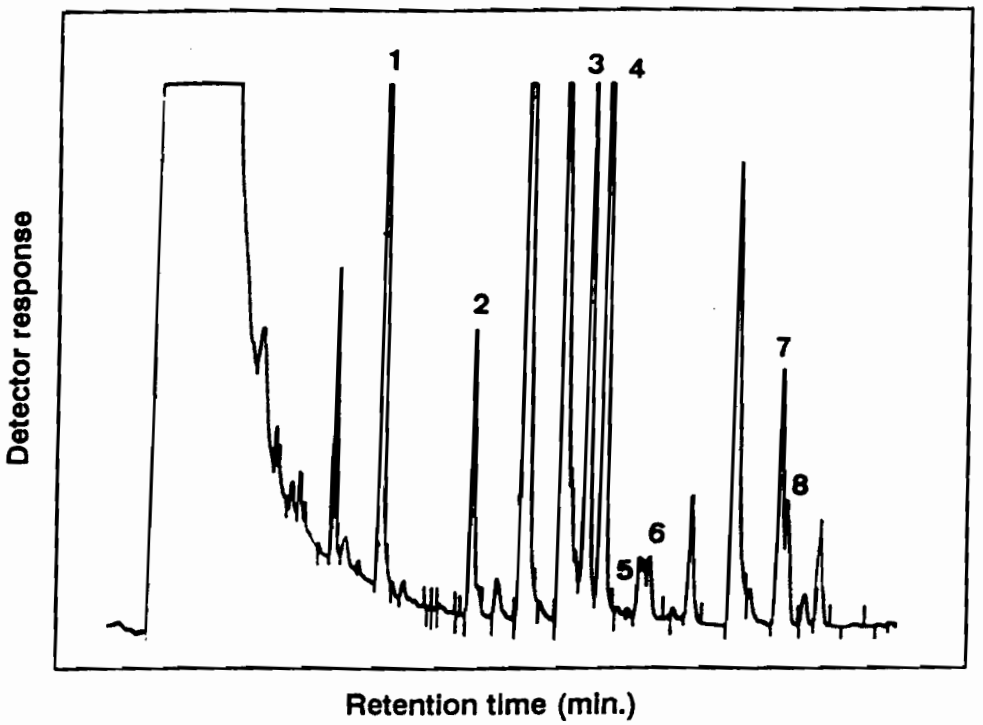


Figure 37 Gas chromatogram of pyrazines in light maple syrup.
1, *pyrazine (1,4-diazine);* **2**, *methylpyrazine;* **3**, *2,5-dimethylpyrazine;* **4**, *2,6-dimethylpyrazine;* **5**, *2-ethylpyrazine;* **6**, 2,3-*dimethylpyrazine;* **7**, *trimethylpyrazine;* **8**, *2-ethyl-3-methylpyrazine.*

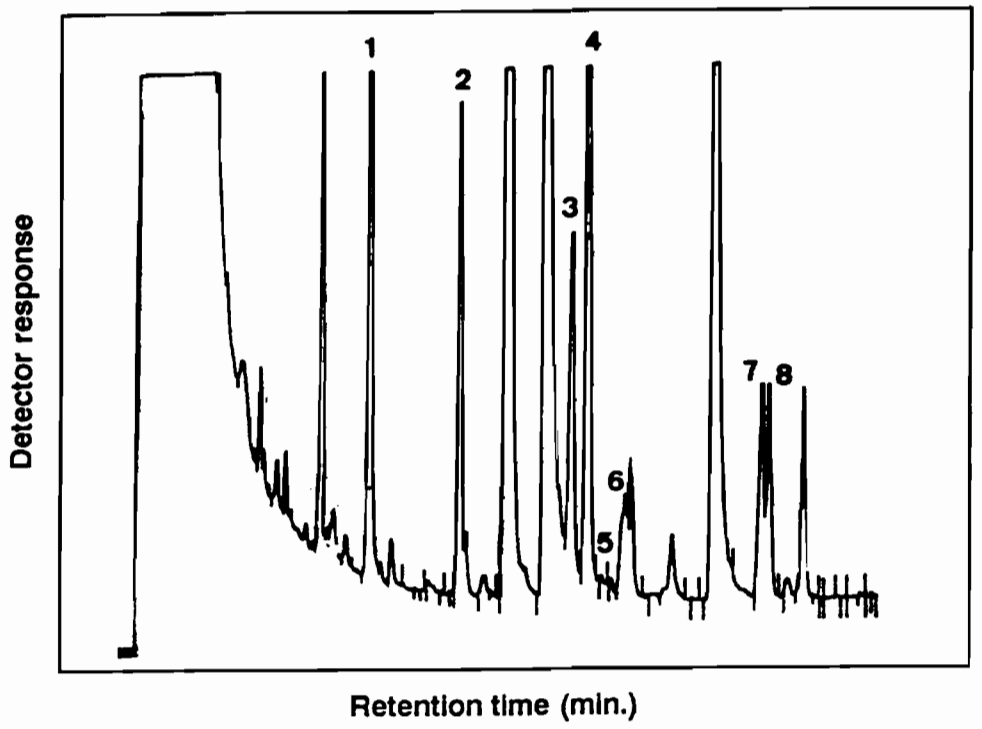


Figure 38 Gas chromatogram of pyrazines in medium maple syrup.
1, *pyrazine (1,4-diazine);* **2**, *methylpyrazine;* **3**, *2,5-dimethylpyrazine;* **4**, *2,6-dimethylpyrazine;* **5**, *2-ethylpyrazine;* **6**, 2,3-*dimethylpyrazine;* **7**, *trimethylpyrazine;* **8**, *2-ethyl-3-methylpyrazine.*

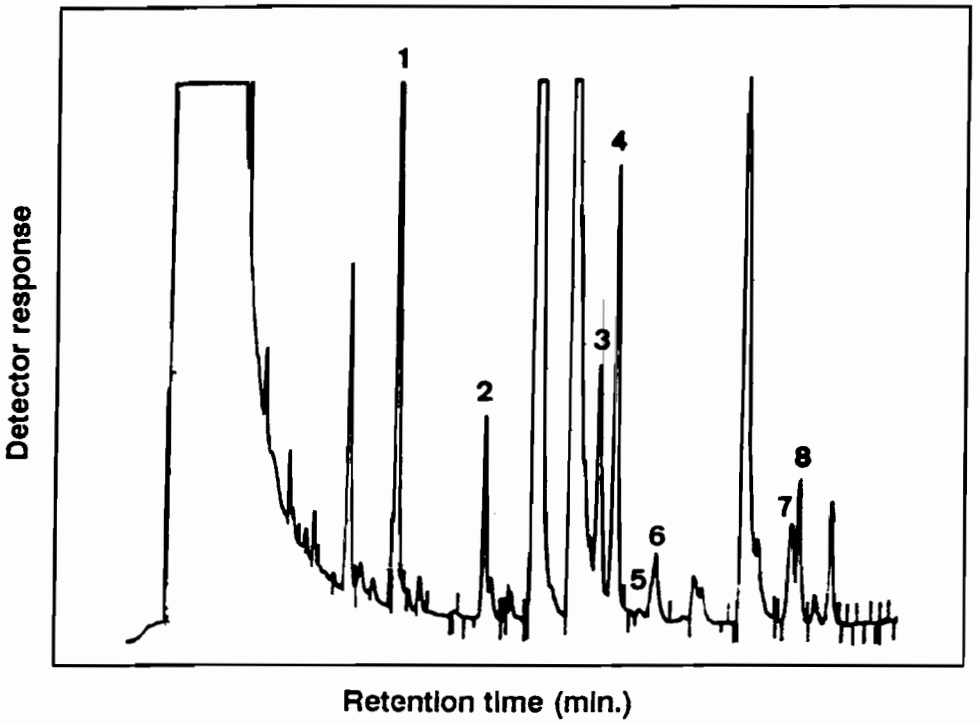


Figure 39 Gas chromatogram of pyrazines in amber maple syrup.

1, *pyrazine (1,4-diazine);* **2**, *methylpyrazine;* **3**, *2,5-dimethylpyrazine;* **4**, *2,6-dimethylpyrazine;* **5**, *2-ethylpyrazine;* **6**, 2,3-*dimethylpyrazine;* **7**, *trimethylpyrazine;* **8**, *2-ethyl-3-methylpyrazine.*

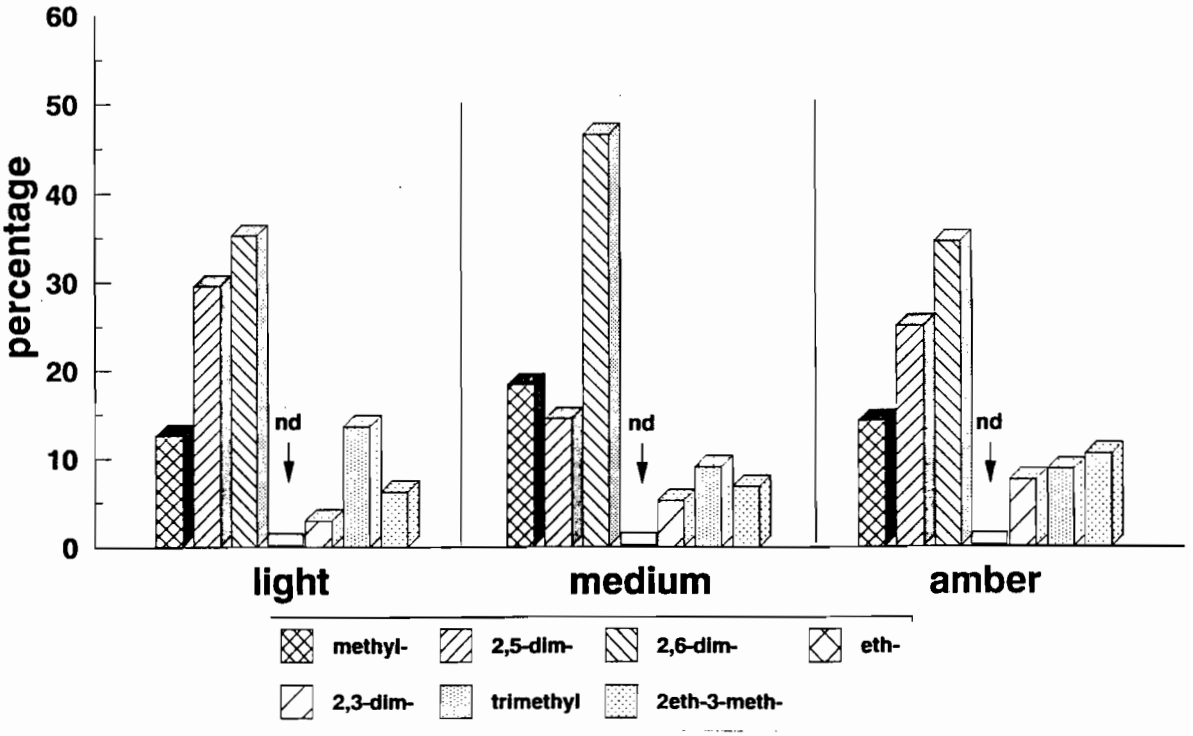


Figure 40: Comparison of identified pyrazines in commercial maple syrups

Table 10: Concentration of of identified pyrazines in commercial maple syrups, non-maple syrups and artificial maple flavorings

	Maple Syrups			Non-Maple Syrups			Artificial Maple Flavors			
Pyrazine	light	medium	amber	aunt J[1].	O. Tyme[2]	corn s.[3]	LD595	LD596	LD597	LD598
2-methyl- (ng/g)	7.18[a]	12.93[b]	6.91[a]	4.72[d]	3.04[d]	11.25[e]	626.98[h]	1762.23[i]	936.05[h]	244.60[g]
%[4]	*12.53*	*18.29*	*14.14*	*22.96*	*21.35*	*60.75*	*17.29*	*35.75*	*33.63*	*20.87*
2,5-dimethyl-	16.93[b]	10.20[a]	12.19[a]	5.26[e]	2.71[d]	2.42[d]	628.95[h]	1325.85[i]	750.85[h]	256.13[g]
%[4]	*29.54*	*14.43*	*24.94*	*25.58*	*19.03*	*13.07*	*17.88*	*26.89*	*26.97*	*21.86*
2,6-dimethyl-	20.16[a]	32.75[b]	16.82[b]	1.55[d]	4.97[e]	tr	849.80[j]	701.50[i]	422.10[h]	262.59[g]
%[4]	*35.20*	*46.34*	*34.41*	*7.54*	*34.90*		*24.16*	*14.23*	*15.16*	*22.41*
2-ethyl-	tr	tr	tr	0.43	tr	tr	537.53	tr	tr	tr
%[4]				*2.09*			*15.29*			
2,3-dimethyl-	1.70[a]	3.65[b]	3.62[b]	1.07[d]	0.50[d]	0.44[d]	256.28[i]	327.78[j]	193.98[h]	75.42[g]
%[4]	*2.97*	*5.16*	*7.42*	*5.20*	*3.51*	*2.38*	*7.29*	*6.65*	*6.97*	*6.44*
trimethyl-	7.79[c]	6.36[b]	4.23[a]	tr	tr	tr	213.15[h]	381.48[i]	240.68[i]	92.84[g]
%[4]	*13.59*	*9.00*	*8.66*				*6.06*	*7.74*	*8.65*	*7.92*
2-ethyl-3-met-	3.53[a]	4.78[b]	5.09[b]	7.53[e]	3.02[d]	4.41[d]	403.98[h]	431.03[h]	239.95[g]	240.25[g]
%[4]	*6.17*	*6.77*	*10.41*	*36.62*	*21.21*	*23.81*	*11.49*	*8.74*	*8.62*	*20.50*
Total (ng/g)	**57.29[b]**	**70.68[c]**	**48.89[a]**	**20.56[e]**	**14.24[d]**	**18.52[e]**	**3516.67[h]**	**4929.87[i]**	**2783.61[h]**	**1171.83[g]**

Means within the same row having the same letter superscript are not significantly different ($p<0.05$)

[1]*Aunt Jamima syrup,* [2]*Old Tyme syrup,* [3]*Crown Corn syrup,* [4]*Percent of total identified pyrazine.*

syrups are shown in Table 10. Histograms showing comparative percentages of the pyrazines are shown in Figure 40.

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. This is in agreement with numerous studies on the formation of pyrazine compounds. Pyrazines of heated foods are the results of amino-carbonyl reactions catalyzed by heat (Shibamotoand Bernhard, 1977; Maga, 1982; Akochi-K. *et al.*, 1995).

The results indicate that 2,6-dimethylpyrazine was present in highest concentration in the three commercial maple syrup samples of different grades, and represented as much as 46% of the total pyrazine concentration in the medium syrup grade 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 trimethylpyrazine have been reported to be the most abundant and readily formed pyrazine compounds in both model system and actual food systems. Koehler and Odell (1970) reported on the formation of 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 trimethylpyrazine are formed

in the vapor from roasted cocoa beans. 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 differences in both total pyrazine and individual concentration of pyrazines in the different grades of maple syrup. The total pyrazine concentration among the three maple syrup samples of different grades were significantly different ($p<0.05$) (Table 10) with the syrup grade "medium" having the highest quantity of total pyrazines and the "amber" grade having the lowest quantity.

The fact that time and temperature of heating have an affect on both the color of maple syrup (Dumont, 1995; Morselli and Whalen, 1991) and the formation of pyrazine compounds (Akochi-K. *et al.*, 1995; Chaveron, 1989; Shibamoto and Bernhard, 1976), leads to the suggestion that the different amounts of total pyrazines in light, medium and amber grades of maple syrup could be related to the fact that these samples had been exposed to different time / temperature processing. Results obtained when commercial light maple syrup was subjected to extended periods of heating show a decrease in the total concentration of pyrazines after 30 and 50 min of heating at 105°C (Table 14).

4.8. Newly identified pyrazines of maple syrup

In addition to the six previously identified pyrazines (2-methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2-ethylpyrazine, 2,3-dimethylpyrazine and trimethylpyrazine) in maple syrup (Alli *et al.*, 1990), six new pyrazine compounds were identified by GC-MS analysis. Table 11 shows the EI fragmentation data for the following pyrazines: 2-ethyl-3-methylpyrazine, 2-ethyl-6-methylpyrazine, 2-ethyl-5-methylpyrazine, 2-ethyl-3,5-dimethylpyrazine, 3-ethyl-2,5-dimethylpyrazine and tetramethylpyrazine (peaks #8, #9, #10 #11, #12 and #13, respectively, Fig. 41) that have not been previously identified in maple syrup. 2-ethyl-3-methylpyrazine and tetramethylpyrazine were positively identified by their retention times in comparison to those of the standards.

These pyrazines have been reported as important contributors of flavor. 2-ethyl-3-methylpyrazine was reported to contribute nutty, roasted and raw potato aromas, at thresholds of 2 ppm in beer (Fors, 1983). 2-ethyl-6-methylpyrazine, 2-ethyl-5-methylpyrazine and 3-ethyl-2,5-dimethylpyrazine have been reported in coffee; tetramethylpyrazine has been isolated from cocoa products (Maga and Sizer, 1973).

Table 11: EI mas fragments of newly identified pyrazines in maple syrup.

razines	Source	RT[a]	Peaks in the EI Spectra of Pyrazines (*m/z*)
ethyl-6-methyl-	syrup	25.18	123(6.4) 122(55.1) **121(100)** 95(3.8) 94(15.4) 67 (2.6) 66(7.7) 56(16.7) 53(7.7) 42(13.9)
	reference[b]	NA	123(6.3) 122(64.6) **121(100)** 120(2.5) 108(2.5) 95(3.8) 94(17.7) 80(1.3) 67(3.8) 66(8.9) 56(16.5) 53(8.9) 42(13.9)
-ethyl-3-methyl-	syrup	25.63	123(5.1) 122(52.0) **121(100)** 94(12.7) 93(3.8) 66(5.1) 56(16.5) 54(6.3) 53(5.1) 39(13.9)
	reference[c]	26.11	123(5.1) 122(58.2) **121(100)** 94(13.9) 93(3.8) 66(6.3) 56(15.2) 54(7.6) 53(8.9) 39(29.1)
-ethyl-5-methyl-	syrup	25.33	123(3.8) 122(60.8) **121(100)** 120(2.5) 107(2.5) 95(2.5) 94(12.7) 93(6.3) 80(3.8) 66(3.8) 56(16.5) 54(6.3) 53(5.1) 52(7.6) 2(10.1)
	reference[b]	NA	123(6.3) 122(68.4) **121(100)** 120(2.5) 107(5.1) 95(2.5) 94(15.2) 93(3.8) 80(3.8) 66(3.8) 56(20.3) 54(8.9) 53(7.6) 52(6.3) 42(12.7)
-ethyl-3,5-dimethyl-	syrup	32.73	137(7.6) 136(72.2) **135(100)** 109(5.1) 108(15.2) 107(6.3) 96(11.4) 80(6.3) 56(17.7) 54(11.4) 53(12.7) 42(20.3)
	reference[b]	NA	137(6.3) 136(77.2) **135(100)** 121(5.1) 109(3.8) 108(16.5) 107(3.8) 96(3.8) 80(5.1) 56(16.5) 54(20.3) 53(13.9) 42(35.4)
l-ethyl-2,5-dimethyl-	syrup	32.80	137(5.1) 136(69.6) **135(100)** 121(3.8) 109(2.5) 108(13.9) 94(2.5) 80(5.1) 61(1.3) 56(17.7) 54(13.9) 53(18.9) 42(20.3)
	reference[b]	NA	137(6.4) 136(71.8) **135(100)** 121(3.8) 109(2.6) 108(15.4) 94(2.5) 80(1.3) 61(1.3) 56(5.1) 54(34.6) 53(16.7) 42(38.5) 27(11.5)
Tetramethyl-	syrup	34.06	137(8.9) **136(100)** 135(11.4) 121(19.0) 95(5.1) 94(6.3) 54(88.6) 53(16.5) 52(8.9) 42(50.6)
	reference[c]	34.10	137(10.3) **136(100)** 135(6.4) 121(3.9) 110(1.3) 95(6.4) 94(6.4) 80(3.9) 66(1.3) 54(88.6) 53(17.7) 52(10.1) 42(49.4) 27(15.2)

[a]*Retention time in min;* [b]*EI from reference pyrazines of the data library coupled to the mass spectrometer;* [c]*EI from authentic pyrazine.*

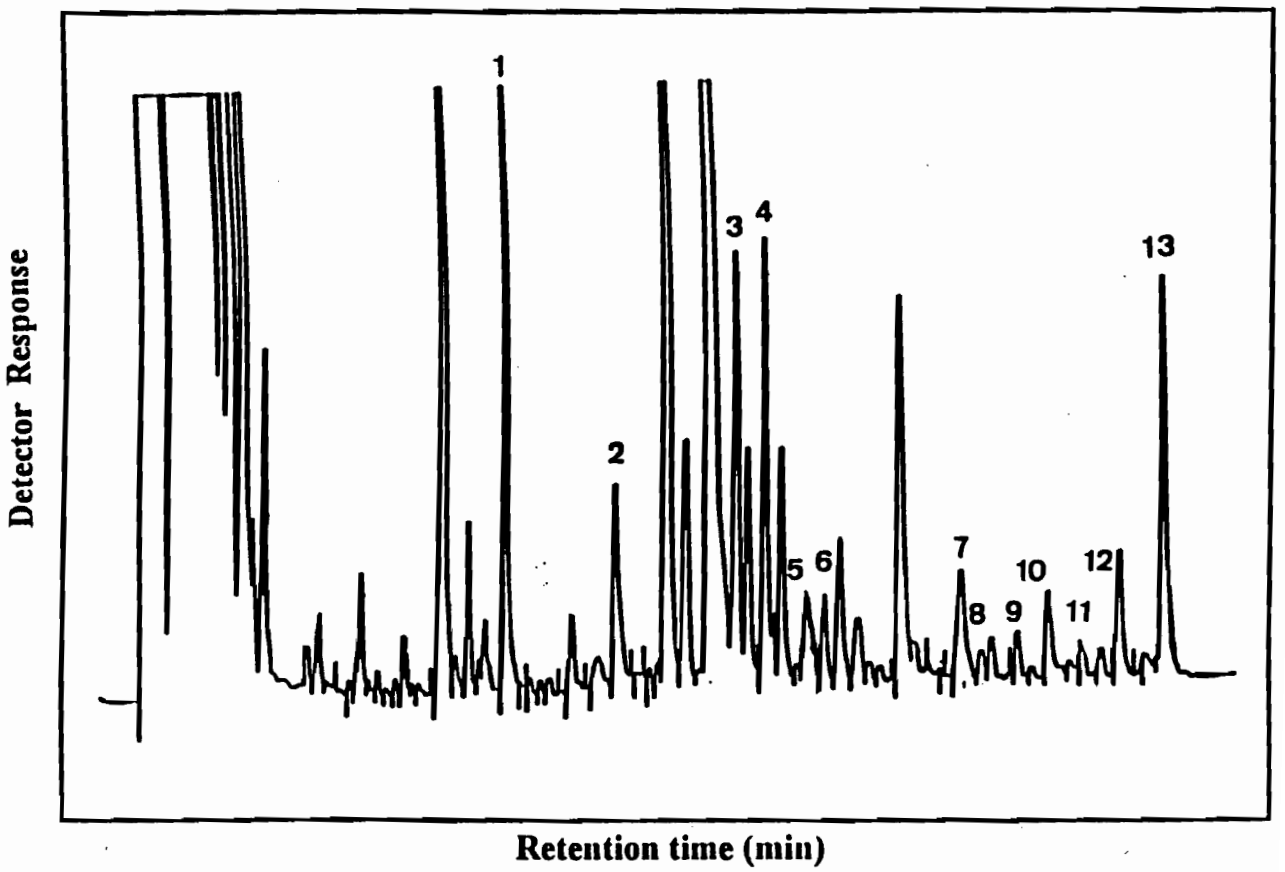


Figure 41: Chromatogram of newly identified pyrazines in maple syrup.
1, *pyrazine (1,4-diazine);* **2**, *methylpyrazine;* **3**, *2,5-dimethylpyrazine;* **4**, *2,6-dimethylpyrazine;* **5**, *2-ethylpyrazine;* **6**, 2,3-*dimethylpyrazine;* **7**, *trimethylpyrazine;* **8**, *2-ethyl-3-methylpyrazine;* **9**, *2-ethyl-6-methylpyrazine;* **10**, *2-ethyl-5-methylpyrazine;* **11**, *2-ethyl-3,5-dimethylpyrazine;* **12**, *3-ethyl-2,5-methylpyrazine;* **13**, *tetramethylpyrazine.*

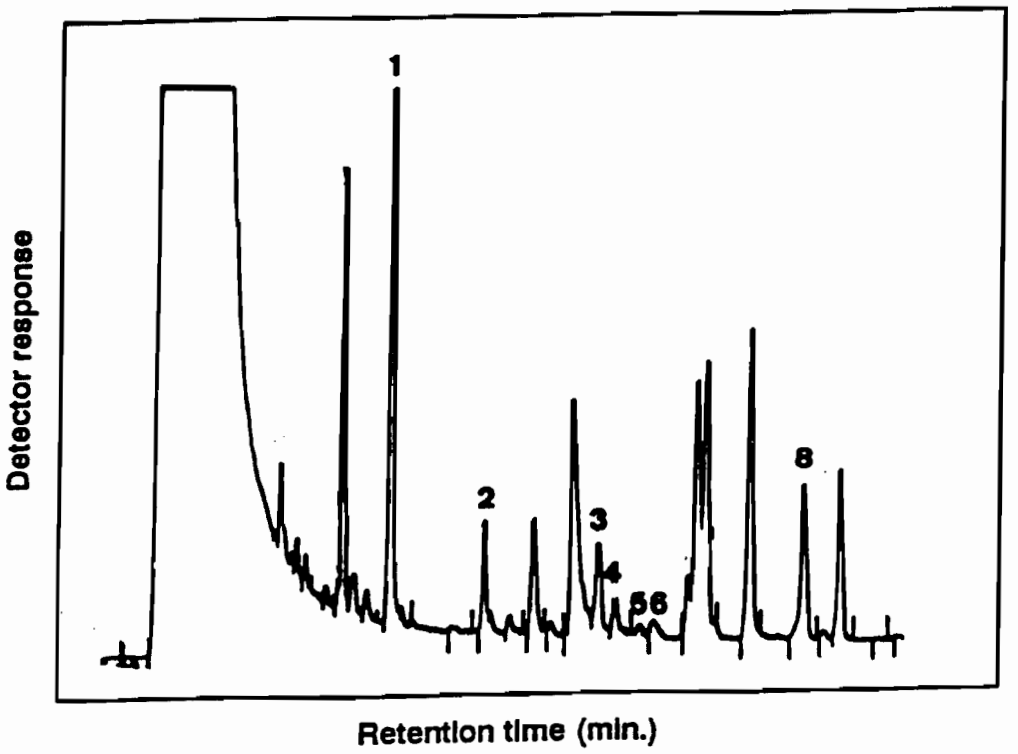


Figure 42 Gas chromatogram of pyrazine in aunt jamima syrup.

1, *pyrazine (1,4-diazine);* **2**, *methylpyrazine;* **3**, *2,5-dimethylpyrazine;* **4**, *2,6-dimethylpyrazine;* **5**, *2-ethylpyrazine;* **6**, 2,3-*dimethylpyrazine;* **7**, *trimethylpyrazine;* **8**, *2-ethyl-3-methylpyrazine.*

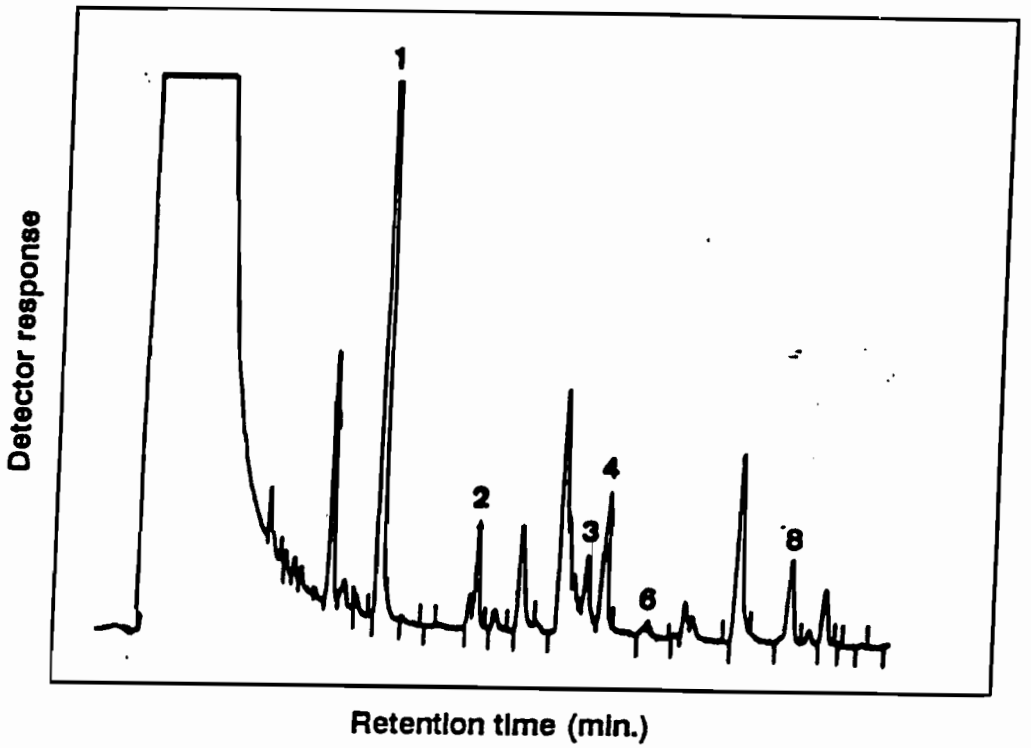


Figure 43 Gas chromatogram of pyrazine in old tyme syrup.

1, *pyrazine (1,4-diazine);* **2**, *methylpyrazine;* **3**, *2,5-dimethylpyrazine;* **4**, *2,6-dimethylpyrazine;* **5**, *2-ethylpyrazine;* **6**, 2,3-*dimethylpyrazine;* **7**, *trimethylpyrazine;* **8**, *2-ethyl-3-methylpyrazine.*

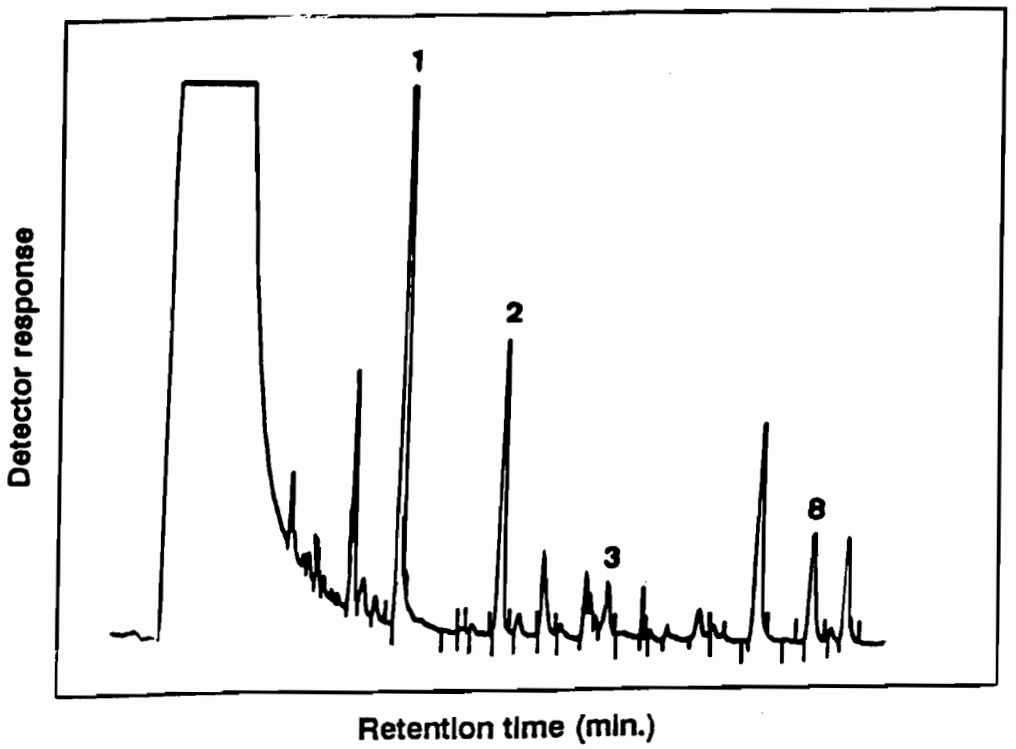


Figure 44 Gas chromatogram of pyrazine in crown corn syrup.
1, *pyrazine (1,4-diazine);* **2**, *methylpyrazine;* **3**, *2,5-dimethylpyrazine;* **4**, *2,6-dimethylpyrazine;* **5**, *2-ethylpyrazine;* **6**, 2,3-*dimethylpyrazine;* **7**, *trimethylpyrazine;* **8**, *2-ethyl-3-methylpyrazine.*

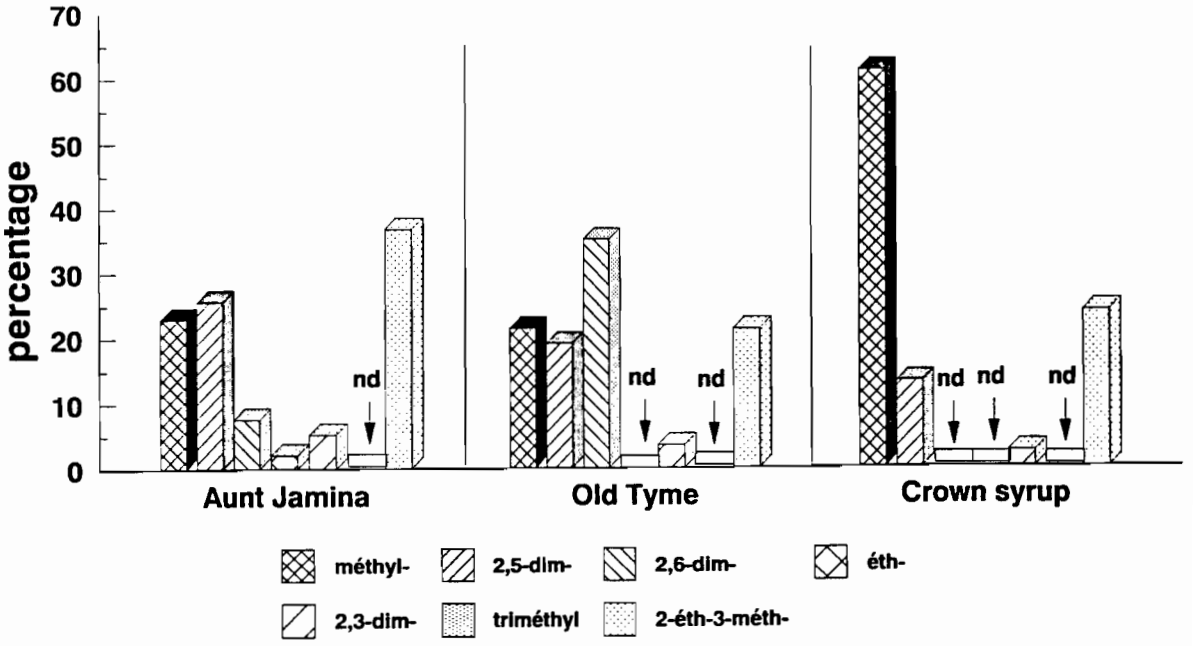


Figure 45: Comparison of identified pyrazines in non-maple syrups.

4.9. Pyrazines in commercial non-maple syrups

Figures 42, 43 and 44 show typical chromatograms of non-maple syrup extracts of Aunt Jamima, Old tyme and Crown corn syrup, respectively. Identified pyrazines from these samples are presented in Table 10. Histograms comparing the relative percentages is shown in Figure 45. In these samples, there were wide variations in the distribution and concentrations of pyrazines. 2-methylpyrazine represent more than 60% of the total pyrazines in Crown corn syrup for 21% and 23% in Old tyme and Aunt jamima syrups, respectively. The quantity of 2-ethyl-3-methylpyrazine was relatively high in all three samples, and was the predominant pyrazine in Aunt jamima syrup (36%). 2,6-dimethylpyrazine was the most abundant pyrazine in Old tyme syrup (34%). Trace amounts of pyrazines were also identified in non-maple syrups; these include trimethylpyrazine for all three samples, 2,6-dimethylpyrazine for Crown corn syrup and Old tyme syrup, and 2,3-dimethylpyrazine for Crown corn syrup only. In addition to differences in individual pyrazines, total pyrazine concentrations among the non-maple syrup samples were significantly different ($p<0.05$) (Table 10).

4.10. Pyrazines in artificial maple flavorings

Figures 46, 47, 48 and 49 show typical chromatograms of artificial maple flavorings; the identified pyrazines are presented in Table 10, and Figure 50 shows comparative histograms of the pyrazines. The two flavoring samples

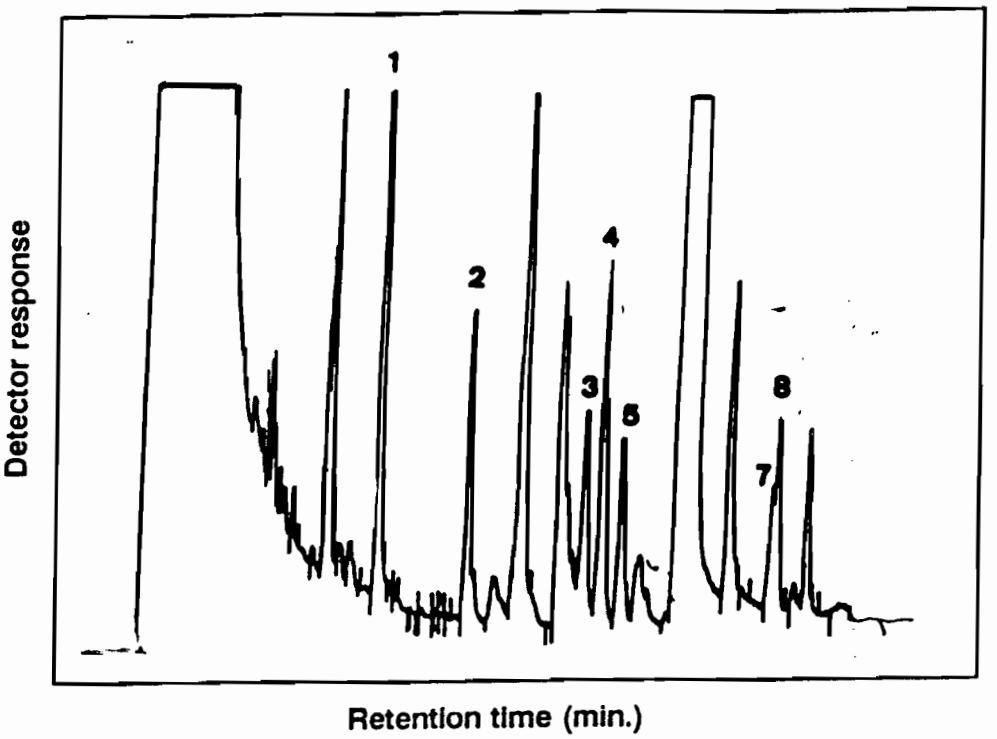


Figure 46: Gas chromatogram of pyrazine in artificial maple flavor LD595.
1, *pyrazine (1,4-diazine);* **2**, *methylpyrazine;* **3**, *2,5-dimethylpyrazine;* **4**, *2,6-dimethylpyrazine;* **5**, *2-ethylpyrazine;* **6**, 2,3-*dimethylpyrazine;* **7**, *trimethylpyrazine;* **8**, *2-ethyl-3-methylpyrazine.*

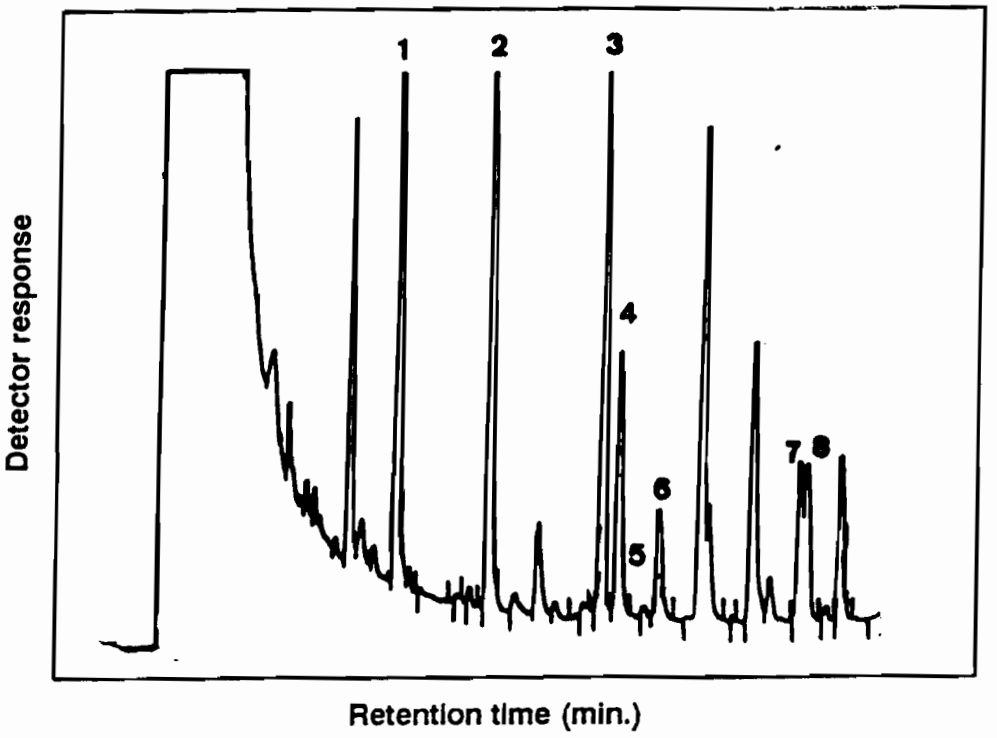


Figure 47: Gas chromatogram of pyrazine in artificial maple flavor LD596.
1, *pyrazine (1,4-diazine);* **2**, *methylpyrazine;* **3**, *2,5-dimethylpyrazine;* **4**, *2,6-dimethylpyrazine;* **5**, *2-ethylpyrazine;* **6**, 2,3-*dimethylpyrazine;* **7**, *trimethylpyrazine;* **8**, *2-ethyl-3-methylpyrazine.*

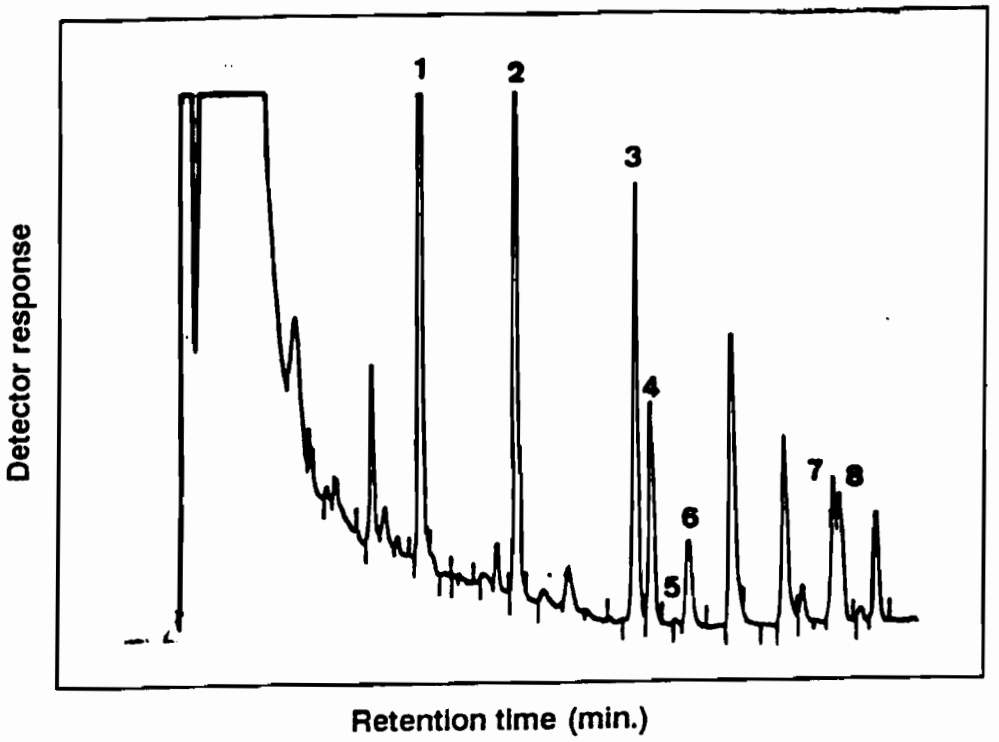


Figure 48: Gas chromatogram of pyrazine in artificial maple flavor LD597.
1, *pyrazine (1,4-diazine);* **2**, *methylpyrazine;* **3**, *2,5-dimethylpyrazine;* **4**, *2,6-dimethylpyrazine;* **5**, *2-ethylpyrazine;* **6**, 2,3-*dimethylpyrazine;* **7**, *trimethylpyrazine;* **8**, *2-ethyl-3-methylpyrazine.*

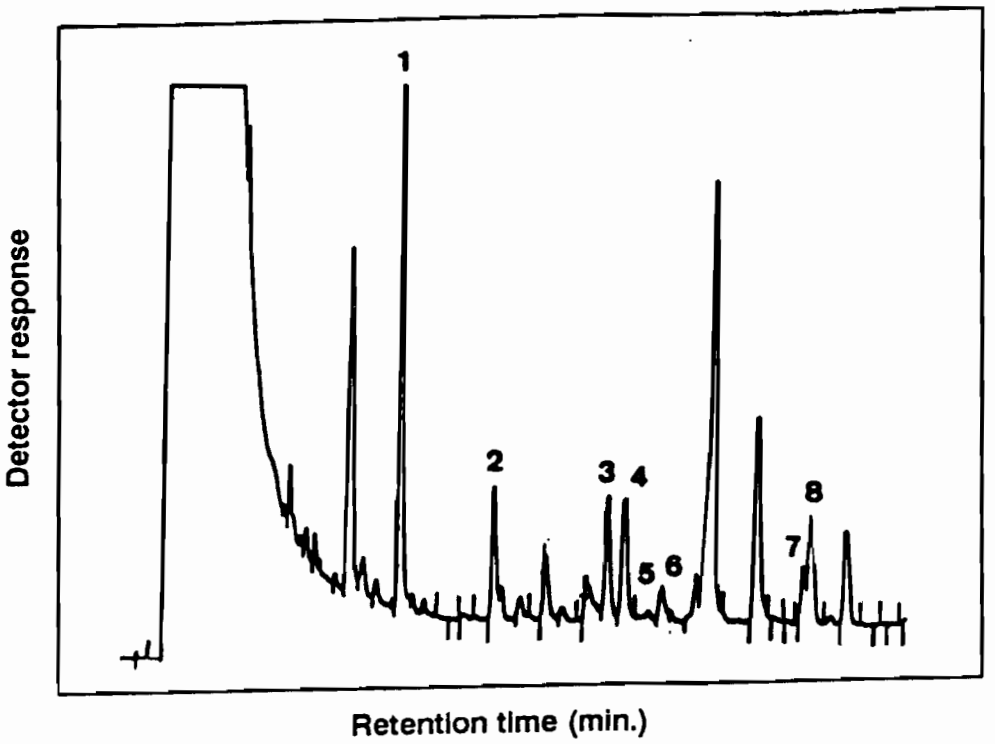


Figure 49: Gas chromatogram of pyrazines in artificial maple flavor LD598.
1, *pyrazine (1,4-diazine);* **2**, *methylpyrazine;* **3**, *2,5-dimethylpyrazine;* **4**, *2,6-dimethylpyrazine;* **5**, *2-ethylpyrazine;* **6**, 2,3-*dimethylpyrazine;* **7**, *trimethylpyrazine;* **8**, *2-ethyl-3-methylpyrazine.*

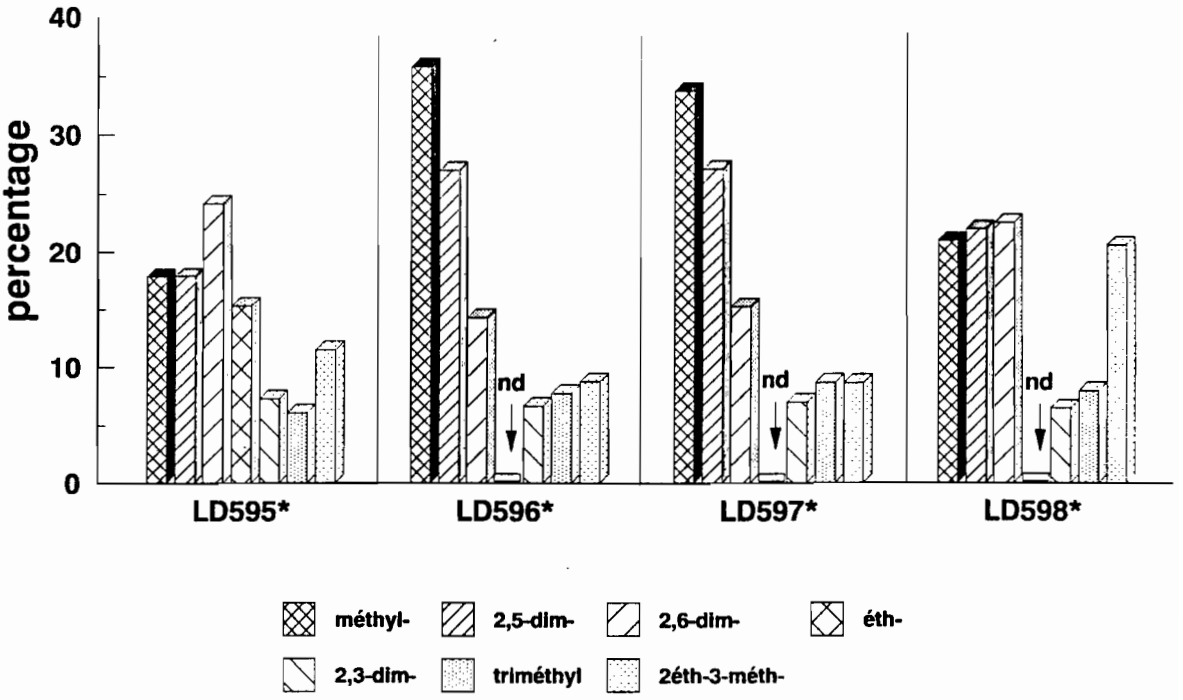


Figure 50: Comparison of identified pyrazines in artificial flavorings.

LD596 and LD597, both contained relatively large amounts of 2-methylpyrazine, 2,5-dimethylpyrazine and 2,6-dimethylpyrazine; 2,6-dimethylpyrazine was predominant in LD595, representing 24% of the total pyrazine. A high concentration of 2-methyl pyrazine (15%) was present in the sample LD595; the same pyrazine was only in trace amount in samples LD596, LD597 and LD598. LD598 contained a relatively high quantity of 2-ethyl-3-methylpyrazine (20%), however, this pyrazine was less than 12% in the other artificial flavoring samples. Total pyrazine contents of LD595 and LD597 were not significantly different ($p<0.05$) (Table 10), while from those of LD596 and LD598l were significantly different ($p<0.05$).

4.11. Comparison of pyrazines from commercial maple and non-maple syrups and artificial maple flavorings

A total of thirteen alkylpyrazines were identified in the three commercial maple syrup samples, and the levels of seven of these pyrazines characterized quantitatively ranged from 49 ng/g to 71 ng/g (Table 10). Commercial non-maple syrups contained substantially lower quantities of total pyrazines than maple syrups; the total pyrazine concentration of the six identified pyrazines in the three samples of non-maple syrups ranged from 14.2 ng/g to 20.6 ng/g (Table 10) and were lower than that of authentic maple syrups. Artificial maple flavorings contained substantially higher total quantities of the seven identified pyrazines with concentrations ranging from 1.17 μg/g to 4.93 μg/g. Total concentration for a group of nine pyrazines identified in roasted cocoa bean

ranged from 1.36 μg/g to 8.5 μg/g (Reineccius *et al.*, 1972).

The principal pyrazine in the maple syrups was 2,6-dimethylpyrazine representing 34% to 43% of the total identified pyrazines in these syrups. 2,6-dimethylpyrazine was also the principal pyrazine in flavoring samples LD595 and in Old tyme non-maple syrup. Other pyrazines which were present in relatively high concentrations in all samples were 2,5-dimethylpyrazine and 2-methylpyrazine. Reineccius *et al.* (1972) who did not succeed in separating 2-methylpyrazine, 2,5-dimethylpyrazine and 2,6-dimethylpyrazine, found that, in general, a mixture of these pyrazines represented a substantial proportion of identified pyrazines in roasted cocoa beans. Tetramethylpyrazine and trimethylpyrazine were also found to be present in large quantities in roasted cocoa beans. Trimethylpyrazine was present in the maple syrups (9% to 14% of total pyrazines) and artificial maple flavors (6% to 9% of total pyrazines) but present in only trace quantities in the non-maple syrups. This leads to the speculation that trimethylpyrazine could play a role in imparting the characteristic maple flavor. 2-ethyl-3-methylpyrazine represented a major component (21% to 37% of the total pyrazines) in non-maple syrups but a relatively minor component in the maple syrups (6% to 10% of total pyrazines) and the artificial maple flavor (8% to 11% of total pyrazines). 2,3-Dimethylpyrazine was a relatively minor component in all samples, representing 3% to 7% of the total pyrazines in the maple syrups, 2% to 5% in the non-maple syrups and 6% to 7% in the artificial maple flavor.

4.12. Pyrazine compounds in laboratory prepared maple syrup

Table 12 shows pyrazine compounds in maple syrup obtained from single cycle boiling of maple sap in the laboratory (Section 3.4.3., Material and Methods). 2,5-dimethylpyrazine and trimethylpyrazine were detected. Boiling the sap for 60 min at 105°C resulted in 1.68 ng/g of 2,5-dimethylpyrazine and 2.54 ng/g of trimethylpyrazine; after 90 min, 2,5-dimethylpyrazine increased to 4.76 ng/g while trimethylpyrazine decreased to 0.96 ng/g. A decrease was observed for 2,5-dimethylpyrazine (to 2.86 ng/g) and, a further decrease for trimethylpyrazine (to 0.67 ng/g) after 120 min. These changes reflect the net effect of formation, volatilization and/or decomposition of these compounds. In their study of a rhamnose-ammonia model system, Shibamoto and Bernhard (1977) proposed fragmentation pathways of sugar and amino acids, leading to α-amino carbonyl fragments that result in the formation of pyrazines including 2,5-dimethylpyrazine and of trimethylpyrazine. The formation of 2,5-dimethylpyrazine and trimethylpyrazine have been reported during the early stages of roasting of cocoa beans (Chaveron *et al.*, 1989). It has been suggested that after the formation of heterocyclic compounds such as pyrazines in the Maillard reaction, follows their decomposition leading eventually to the formation of melanoidin (Hodge, 1953; Reineccius *et al.,* 1972). It is possible

Table 12: Pyrazines formed when maple sap is subjected to a single heating cycle

			Pyrazine compounds (ng/g)	
Time (min)	pH	TS (%)	2,5-dimethylpyrazine	trimethylpyrazine
0	7.2	3.0	ND	ND
40	9.2	5.0	ND	ND
60	7.8	63.0	1.68 (0.05)	2.54 (0.01)
90	7.5	65.0	4.76 (0.02)	0.96 (0.00)
120	7.3	65.0	2.86 (0.11)	0.67 (0.00)

Results are means (standard deviation) of triplicate analysis
TS = total dissolved solids
ND = not detected

that some degree of decomposition of 2,5-dimethylpyrazine and trimethylpyrazine may have occurred during boiling of maple sap. The formation of pyrazine compounds in cocoa beans (Chaveron *et al*, 1989), and in amino acid-glucose model systems has been reported to increase linearly with time of heating. Huang *et al.* (1989) reported a linear pattern for the formation of pyrazine, 2-methylpyrazine, 2,5-dimethylpyrazine and 2-methyl-6-7-dihydro-5H-cyclopentapyrazine in an arginine-glucose model system, while Reineccius *et al.* (1972) showed a similar pattern for the formation of methylpyrazine, 2,5-dimethylpyrazine, trimethylpyrazine and tetramethylpyrazine during the first 30 min of roasting of cocoa beans at 150°C. The formation of these pyrazines may have been favored by the high reactivity of amino-carbonyl fragment pairs. Shibamoto and Bernhard (1977) reported a large formation of trimethylpyrazine due to the presence of glycine in their model system. This experiment shows a formation of pyrazines influenced by time of heating, pH and water content of the sap system. As a result, no pyrazine was detected before 60 min of heating, when total dissolved solids increased from 3% to 63% with a pH 7.8 (Table 12). No other pyrazines were detected during the single cycle boiling of maple sap.

In the continuous boiling process (Section 3.4.4, Material and Methods), maple sap was heated for a period of 220 min. Unlike the single boiling process, continuous boiling resulted in the formation of several pyrazine compounds. Seven alkylpyrazines previously identified in maple syrup (Alli *et al.,* 1990; Akochi-K. *et al.,* 1994) were detected with the predominance of 2,5-dimethylpyrazine (Table 13). As was the case with the single cycle boiling

Table 13: Pyrazines formed in maple syrup when maple sap is subjected to continuous boiling under laboratory conditions.

Time (min)	Pyrazine compounds (ng/g)							
	2-methyl	2,5-dimethyl	2,6-dimethyl	2-ethyl	2,3-dimethyl	trimethyl	2-eth-3-met	Total
60	ND	1.15(0.03)	ND	ND	ND	2.27(0.15)	ND	3.42
90	ND	1.33(0.01)	ND	ND	ND	3.11(0.22)	ND	4.44
120	1.18(0.05)	6.25(0.32)	1.01(0.00)	2.40(0.11)	1.35(0.01)	5.75(0.73)	1.22(0.08)	19.16
160	5.60(0.12)	9.57(1.01)	7.53(0.09)	3.21(0.15)	3.05(0.00)	7.55(1.05)	5.90(0.00)	42.41
190	7.25(0.19)	14.30(1.21)	11.35(1.00)	7.17(1.02)	3.90(0.05)	8.65(0.16)	7.75(0.93)	60.37
220	9.77(0.65)	15.20(0.00)	15.73(0.55)	7.42(0.00)	5.10(0.37)	10.17(1.23)	8.93(0.82)	72.32
%[1]	*13.51*	*21.02*	*21.75*	*10.26*	*7.05*	*14.06*	*12.35*	

Results are means (standard deviation) of triplicate analysis
0 and 30 min boiling did not generate any detectable pyrazine compound
ND = not detected
[1]% refers to individual pyrazine as a percentage of total pyrazines at 220 min of boiling

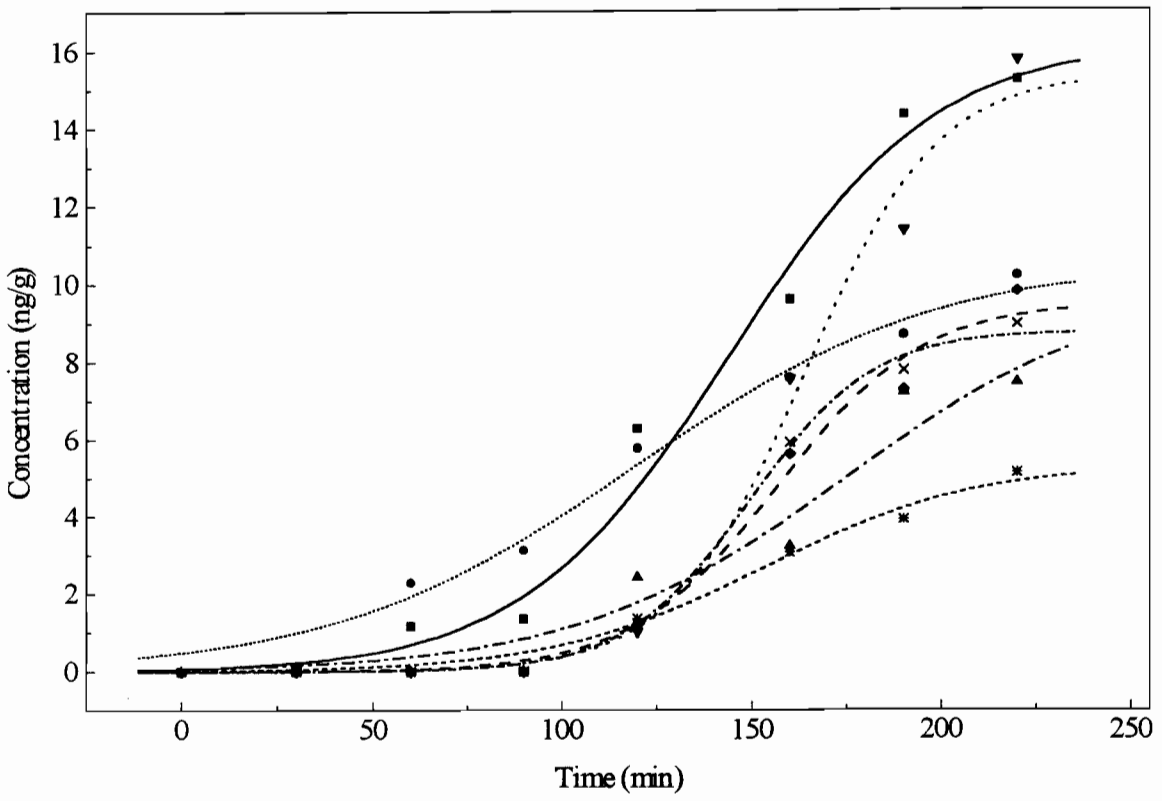


Figure 51: Accumulation of pyrazines during boiling of maple sap.
♦ methylpyrazine, ■ 2,5-dimethylpyrazine, ▼ 2,6-dimethylpyrazine, ▲ ethylpyrazine
✱ 2,3-dimethylpyrazine, ● trimethylpyrazine, × 2-ethyl-3-methylpyrazine

process, no pyrazines was detected after 30 min of heating. Trimethylpyrazine and 2,5-dimethylpyrazine were detected after 60 min of heating. Unlike the single cycle boiling, the concentrations of pyrazines increased as the time of heating increased. Methylpyrazine, 2,6-dimethylpyrazine, ethylpyrazine, 2,3-dimethylpyrazine and 2-ethyl-3-methylpyrazine were detected only after 120 min of heating. Figure 51 shows the accumulation of the pyrazines. Of the two first detected pyrazine, 2,5-dimethylpyrazine showed a steady increase while the increase of trimethylpyrazine slowed down after 150 min of heating; 2,6-dimethylpyrazine which was detected after 120 min of heating showed a rapid increase to reach a level comparable to that of 2,5-dimethylpyrazine at the end of the processing period. The remaining of the "late formed" pyrazines (methylpyrazine, ethylpyrazine, 2,3-dimethylpyrazine and 2-ethyl-3-methylpyrazine) showed a slow increase and 2,3-dimethylpyrazine was the least formed pyrazine compound (5.10 ng/g). Using glucose-glycine model systems, Gwen *et al.* (1993) obtained a greater yield and distribution of pyrazines at alkaline pH (pH 9.0 and 9.64) than at acidic pH. 2,5-Dimethylpyrazine and trimethylpyrazine were predominant predominant pyrazine products.

Laboratory prepared maple syrup contained 15.20 ng/g of 2,5-dimethylpyrazine (21.02% of total pyrazine) and 15.73 ng/g of 2,6-dimethylpyrazine (21.75% of total pyrazine) (Table 13); These relative percentages were lower than those reported for commercial maple syrups (Table 10). The concentration for 2-ethylpyrazine (7.42 ng/g) was higher than

that found in commercial maple syrups (trace amounts, Table 10). The quantities of 2,3-dimethylpyrazine (5.10 ng/g) and 2-ethyl-3-methylpyrazine (8.93) were also higher in laboratory prepared maple syrup, when compared with the commercial maple syrups. These differences could be attributed to extended heating (6 to 8 hours) associated with the preparation of commercial maple syrups; a heating time of 220 min was used for preparation of the laboratory syrups in the present study.

The difference between the commercial syrups and the laboratory prepared syrups (Tables 11 and 14) prompted to investigate the fate of pyrazines when the syrup is subjected to heat for an extended period, as is the case for commercial maples syrups. Heating commercial light maple syrup for 30 min resulted in a decreased of the total pyrazine concentration, but with a large increase of 2-methylpyrazine (Table 14). The levels of 2,5-dimethylpyrazine, 2,6-dimethylpyrazine and trimethylpyrazine decreased dramatically, whereas 2,3-dimethylpyrazine and 2-ethyl-3-methylpyrazine remained unchanged. After 50 min of heating the quantity of 2-methylpyrazine continued to increase and that of 2-ethyl-3-methylpyrazine decreased. The levels of the other pyrazines remained unchanged. Total pyrazine levels of the 30 and 50 min heating were not significantly different ($p<0.05$). The decrease of certain pyrazines may have been the result of volatilization of pyrazines, their involvement in the further reactions (Reineccius *et al.*, 1972) or their degradation.

Table 14: Pyrazines in commercial maple syrup and commercial maple syrup after heating for 30 min and 50 min

	Pyrazine compounds (ng/g)							
Samples	2-methyl	2,5-dimethyl	2,6-dimethyl	2-ethyl	2,3-dimethyl	trimethyl	2-eth-3-met	Total
Maple syrup	7.90(0.25)	16.17(0.11)	21.30(1.21)	tr	1.32(0.02)	6.75(1.00)	3.20(0.00)	56.64a
%[1]	*13.95*	*28.55*	*37.61*		*2.33*	*11.92*	*5.65*	
30 min heating	21.53(1.55)	6.86(0.91)	6.24(0.02)	tr	2.01(0.01)	3.76(0.07)	3.15(0.10)	43.55b
%	*49.44*	*15.75*	*14.33*		*4.62*	*8.63*	*7.23*	
50 min heating	25.82(0.88)	5.03(0.03)	7.18(0.31)	tr	2.55(0.00)	3.47(0.12)	1.26(0.01)	45.31b
%	*56.99*	*11.10*	*15.85*		*5.63*	*7.66*	*2.78*	

Results are means (standard deviation) of triplicate analysis
[1]% refers to individual pyrazine as a percentage of total pyrazines in the same treatment
Mean scores with the same letter within the same column are not significantly different at 0.05 level

4.13. Kinetics of formation of pyrazines during heat processing of maple sap to syrup.

Figures 52 to 58 show the plots of the quantities of pyrazine formed versus time of continuous boiling of maple sap for methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, ethylpyrazine, 2,3-dimethylpyrazine, trimethylpyrazine and 2-ethyl-3-methylpyrazine, respectively. An induction period was associated with the formation of all pyrazines. The absence of pyrazines or lack of formation during the induction period may be due in part to low initial concentrations of sugar and amino acid fragments. It is known that for the Maillard reaction to proceed, the reducing sugar and amino acid must be converted to Amadori rearrangement products; this rearrangement is dependent on water activity (a_W). Leahy (1985) reported a direct relationship between a_W and the accumulation of pyrazine compounds.

After the induction period however, a linear relationship between the formation of pyrazines and time of reaction was observed which permitted the determination of rate constant (k) for the formation of the pyrazines (Table 15). The rate constant for the seven pyrazines investigated ranged from 0.04 to 0.13 ng/min for a heating time of 220 min at 105°C. The slope of the linear portion of the reaction curve, the region where pyrazines were detected, was consistent with a *pseudo*-zero order reaction (Stamp and Labuza (1983). This indicates that the rate of accumulation of products from this system may not be dependent on reactants. The lack of a definite mathematical relationship

Table 15: Rate constants for pyrazine formation in maple syrup

Pyrazines compounds	rate constant *k* (ng/min)	r2
methylpyrazine	0.08	0.98
2,5-dimethylpyrazine	0.09	0.95
2,6-dimethylpyrazine	0.13	0.98
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

between reactants and products, which was also observed for pigment formation in the Maillard reaction (Labuza and Satlmarch, 1981), is explained by *1)* a high reactant/product ratio (Leahy and Reineccius, 1989), *2)* the multiple steps involved and *3)* the influences of competitive and consecutive reactions that occur along with the formation of pyrazines. Leahy and Reineccius (1989) found that *k* was dependent of temperature and the model system. Huang *et al.* (1989) reported a *pseudo*-zero order rate for the formation of pyrazine, 2-methylpyrazine and 2,6-dimethylpyrazine in model systems, with activation energy of 19.5, 24.8 and 20.8 Kcal/mole, respectively. Rate constants for methylpyrazine and 2,5-dimethylpyrazine at 120^{o}C were 0.38 and 0.08 ng/min, respectively. In comparison to rates shown in Table 15, the substantial difference between rate constants for methylpyrazine may be attributed to the heating temperature. Huang *et al.* (1989) reported the formation rate of pyrazine, 2-methylpyrazine and 2,6-dimethylpyrazine in a model system follows a *pseudo*-zero order reaction with activation energies for pyrazine, 2-methylpyrazine and 2,6-dimethylpyrazine as 19.5, 24.8 and 20.8 Kcal/mole, respectively. Leahy and Reineccius (1989) reported a *pseudo*-zero order reaction for the formation of pyrazine, methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine and 2,3-dimethylpyrazine. The same pyrazine compound had different activation energies depending on the model system.

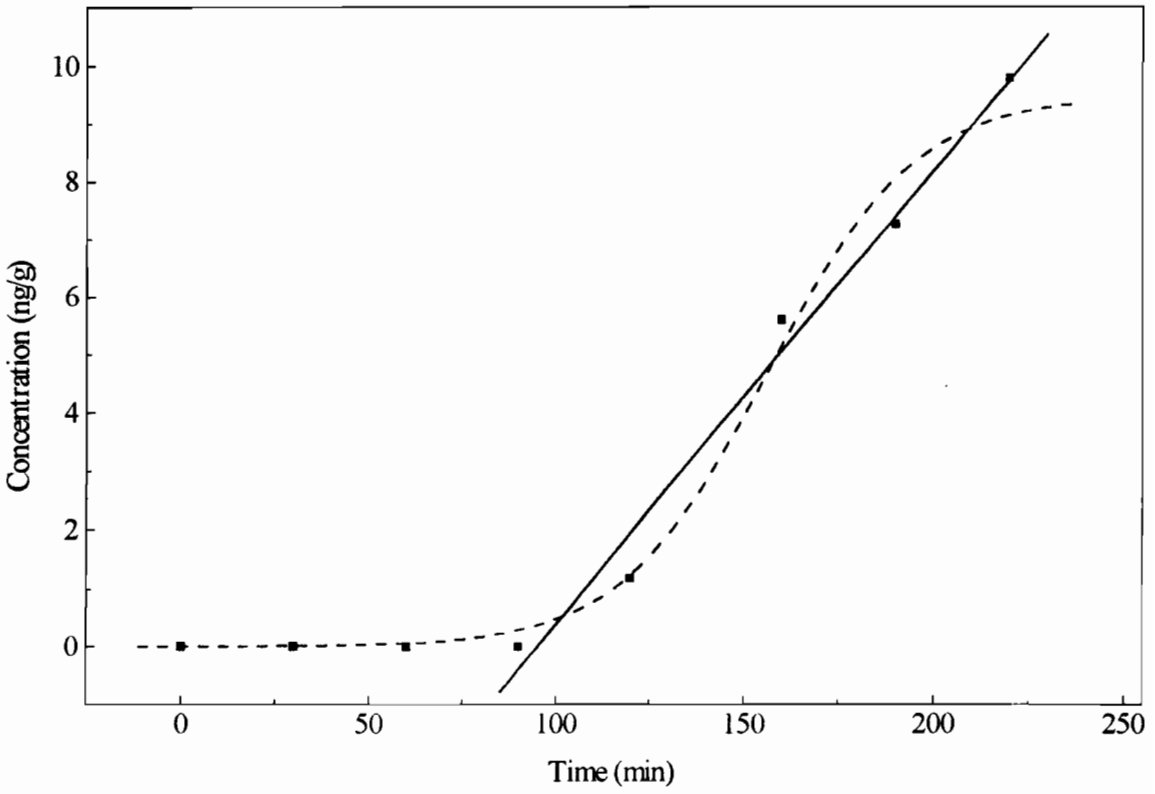


Figure 52: Plot of methylpyrazine concentration *vs* time of heating of maple sap.

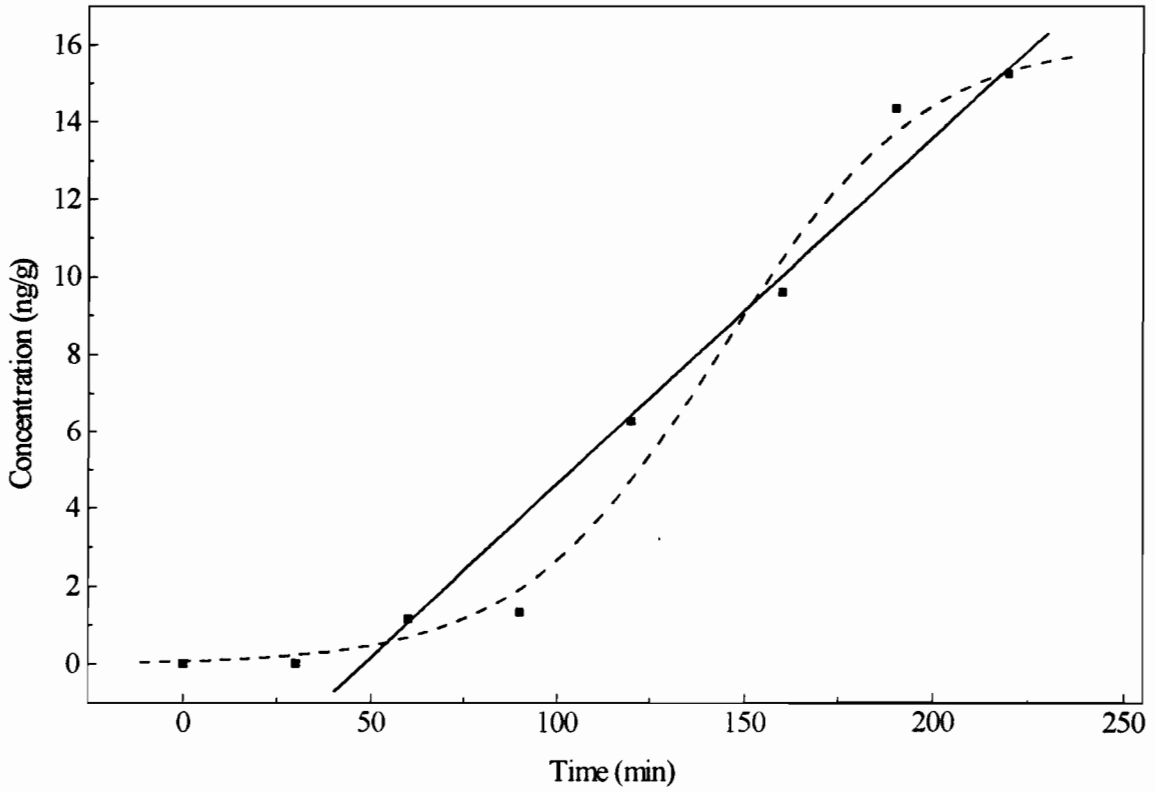


Figure 53: Plot of 2,5-dimethylpyrazine concentration *vs* time of heating of maple sap.

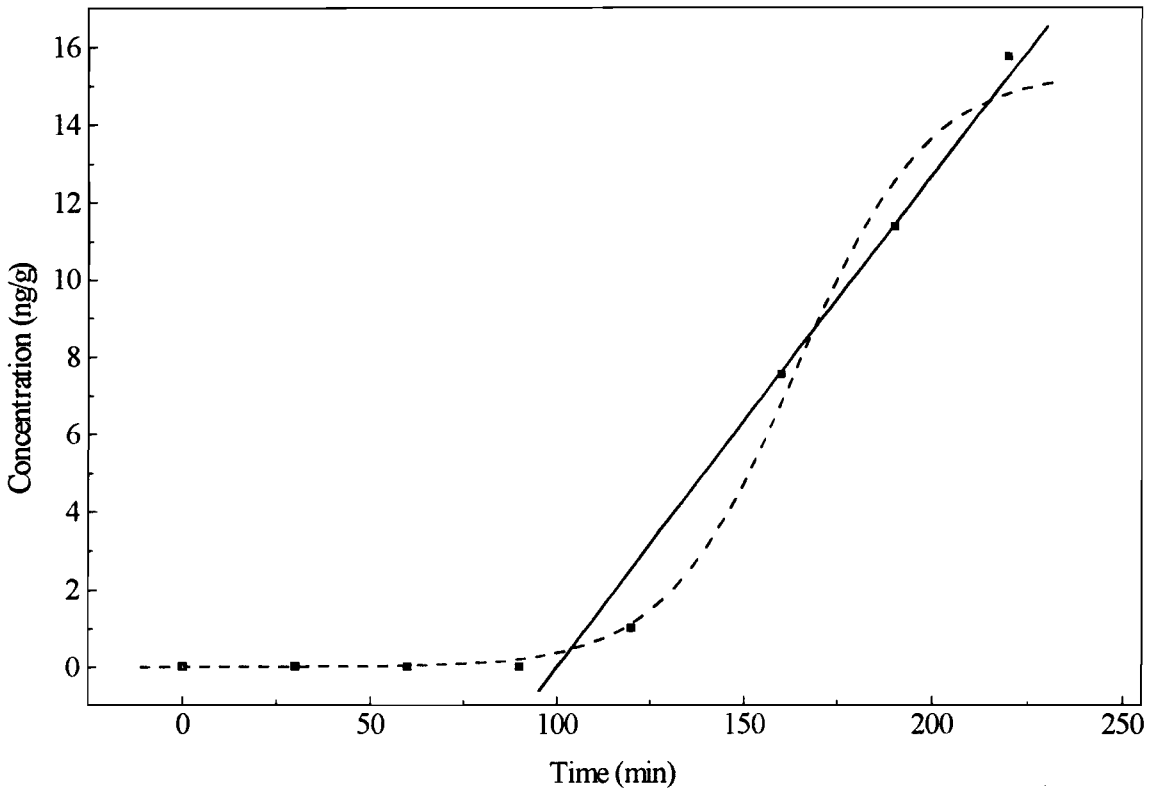


Figure 54: Plot of 2,6-dimethylpyrazine concentration *vs* time of heating of maple sap.

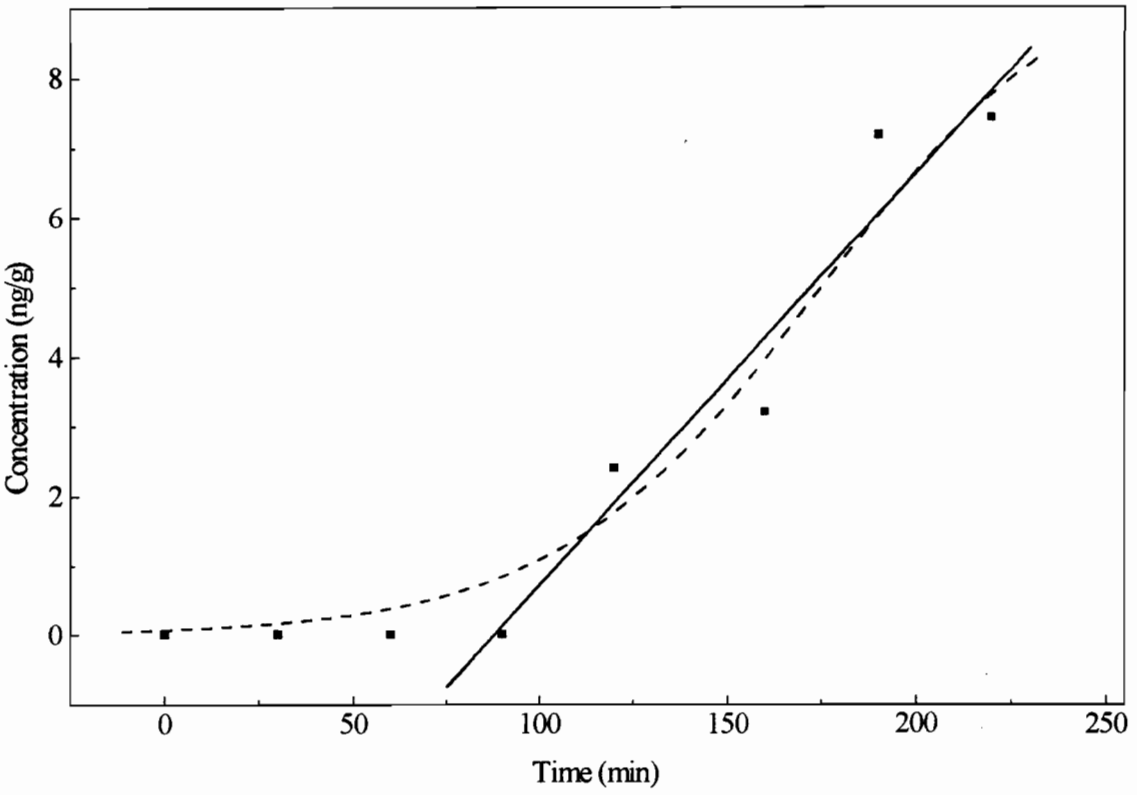


Figure 55: Plot of ethylpyrazine content *vs* time of heating of maple sap.

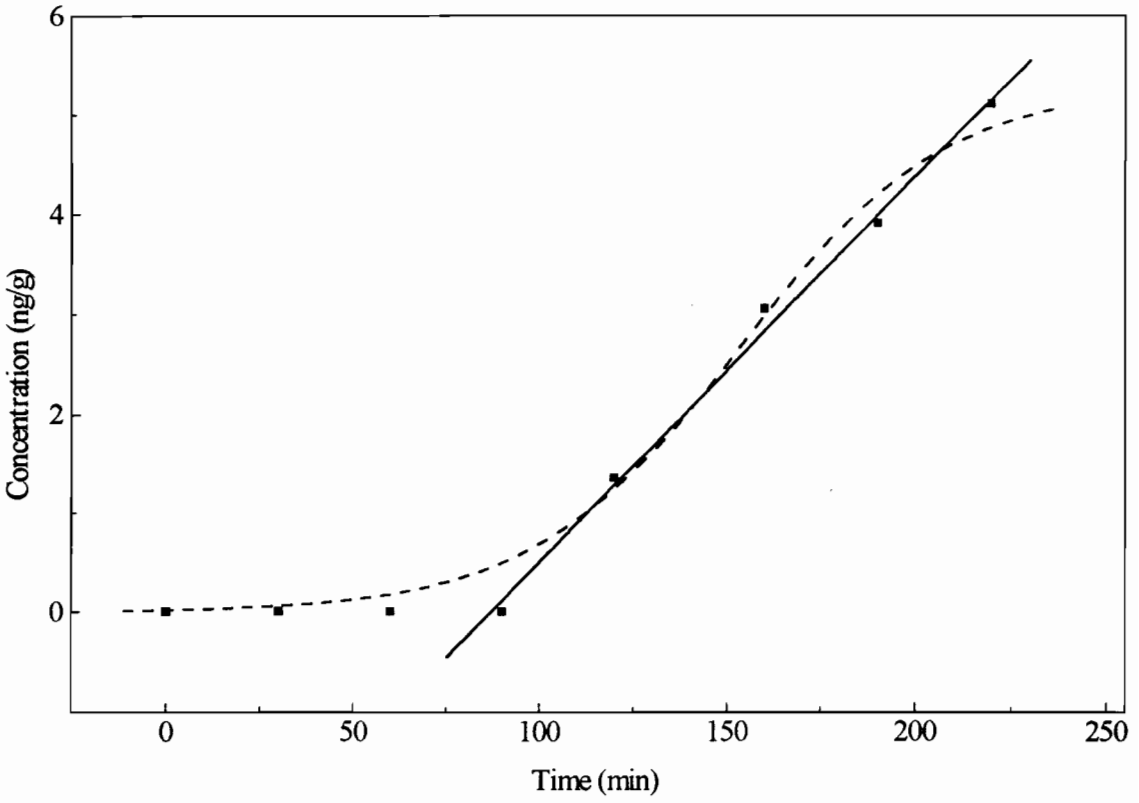


Figure 56: Plot of 2,3-dimethylpyrazine concentration *vs* time of heating of maple sap.

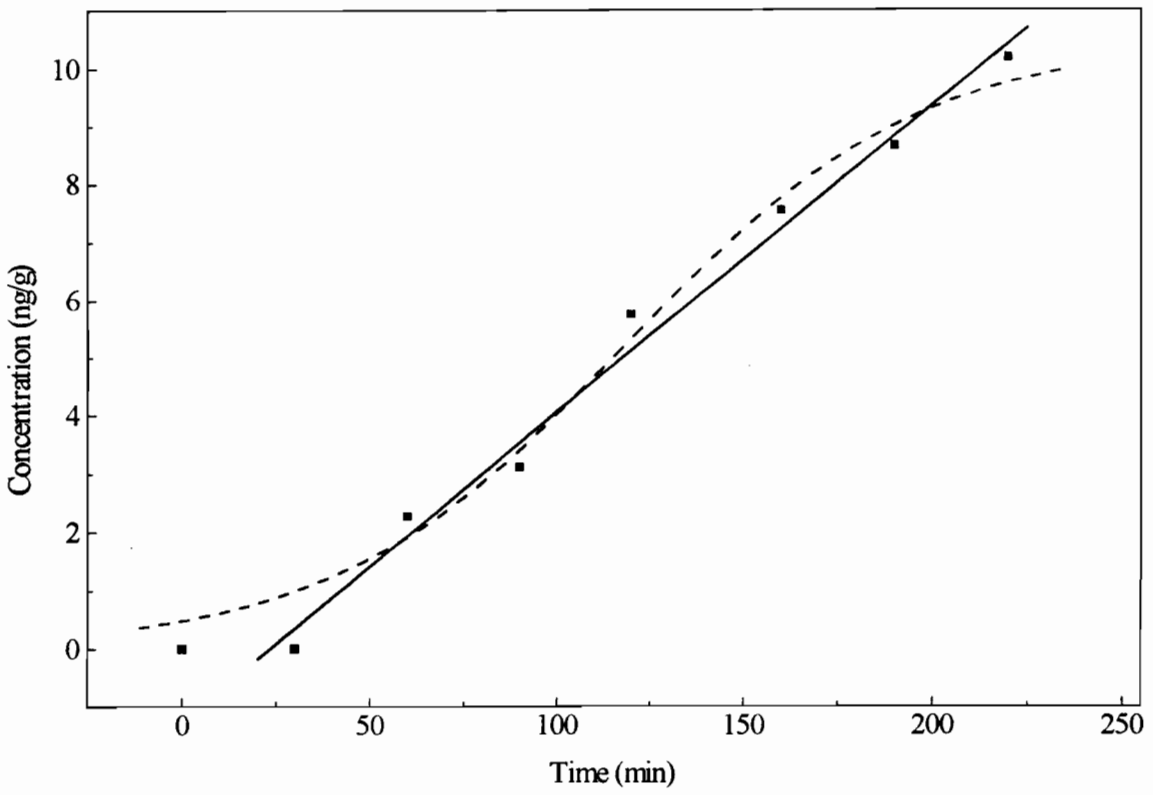


Figure 57: Plot of trimethylpyrazine concentration *vs* time of heating of maple sap.

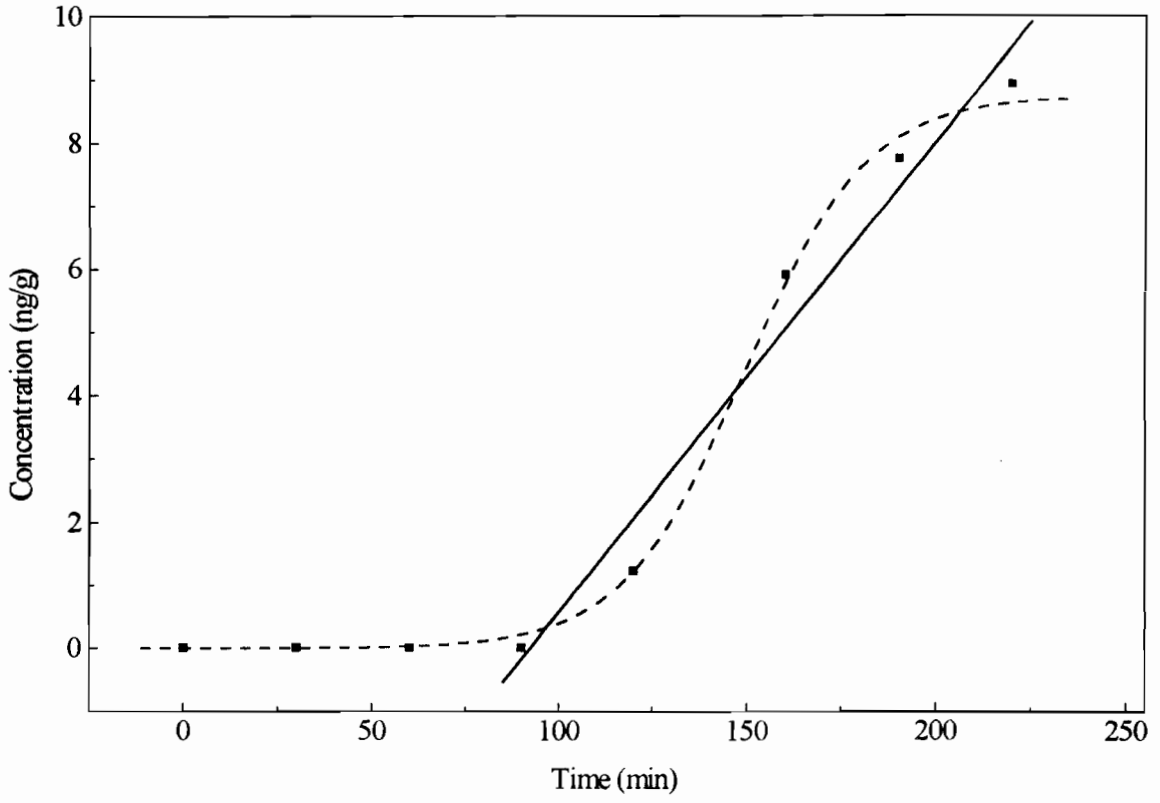


Figure 58: Plot of 2-ethyl-3-methylpyrazine concentration *vs* time of heating of maple sap.

4.14. Sensory evaluation of maple syrup

Table 16 shows "maple flavor" quality and taste performance scores for light, medium and amber maple syrup grades. The medium and amber grade syrups were judged by panelists to have the highest "maple flavor" quality of the three grades investigated. Mean score for the amber syrup (6.74 on a scale of 0 to 10) was significantly different ($p<0.05$, Appendix 1) from that of light syrups.

Taste preference scores for the medium amber grade syrups were also higher and significantly different ($p<0.05$, Appendix 2) from that the light grade syrup.

These results show that the three grades of maple syrup investigated have different levels of the "maple flavor" quality, and also confirm that the "maple flavor attribute is an important factor that determine consumer preference judgments for maple syrups. Effects of syrup colors did not contribute to the outcome of the tests, since in the experimental design, color differences between syrup grades were blocked. Sensory evaluation of maple syrup conducted with color discrimination have shown a preference for darker syrups (Belford *et al.* 1991; Sendak, 1982; Sendak, 1978).

On the basis of evidence presented and the practice of higher market values for lighter maple syrups, there is inconsistency between consumer preference and maple syrup pricing. Similar to maple syrup, birch syrup derived from birch trees of Finland (Kallio *et al.*, 1985 and Kallio 1988) was reported to have stronger, caramel and burnt aromas and weaker vanillin

Table 16: Flavor quality and preference rating of maple syrups

Syrup sample	Total* (ng/g)	Attributes rated	
		Maple flavor	Preference
light	57.29^{b}	5.98^{b}	4.53^{a}
medium	70.68^{c}	5.78^{a}	4.92^{b}
amber	48.89^{a}	6.74^{c}	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
** Total concentration of pyrazines in each syrup grade*

flavor than maple syrup. The report does not indicate if the syrup was prepared from early season flow sap and aseptic conditions of tapping, collecting and processing of the sap.

ANOVA for "maple flavor quality" (Appendix 1) shows significant treatments (syrup samples) and panelists effects at the 0.05 levels of probabilities and non-significant replications and panelists by treatments (syrup samples) effects. This indicates that the panelists are in agreement in their scoring inspite of their differences. For maple syrup preference test, treatment (syrup samples) effects were not significant, however, panelists by treatments had a significant effect at the 0.05 levels of probabilities (Appendix 2).

4.14.1. Relationship between taste performance and pyrazines

The total pyrazine content (Table 10) of the amber (darker colored) syrup (49 ng/g) which was significantly different from the total pyrazine contents of the lighter syrups (light, medium) and 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-dimethylpyrazine, 2,5-dimethylpyrazine and 2-ethyl-3-methylpyrazine (Table 16), and *(2)* were preferred over the light syrup which had a lower quantities (Table 10) of 2,3-dimethylpyrazine (1.70 ng/g) and 2-ethyl-3-methylpyrazine (3.53 ng/g) but higher quantity of 2,5-dimethylpyrazine (16.93 ng/g). This suggests that of the pyrazines identified, 2,3-

dimethylpyrazine, 2,5-dimethylpyrazine and 2-ethyl-3-methypyrazine might 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 (4.23 ng/g; Table 10) quantity of trimethylpyrazine, suggesting a possible inverse relation of trimethylpyrazine to maple flavor.

V. SUMMARY

The characteristic flavor of maple syrup, like that of many processed foods, is derived during processing. In the case of maple syrup, this occurs during heat processing of maple sap to syrup. Thermally-induced flavor compounds formed during boiling of maple sap are important contributors to the flavor and aroma of the final syrup. For example, thermal degradation of sugars and polyphenolic compounds in the sap results in the formation of aliphatic, carbonyl and phenolic compounds; heat induced reaction between amino acids and reducing sugars results in the formation of pyrazines in maple syrup.

A modified version of an existing solvent extraction method was used to isolate pyrazines. Extraction efficiencies were evaluated using a different internal standard for the three critical steps of the methods. As a result of the modification, recoveries of pyrazines increased to above 90%. In this study, thirteen pyrazines were isolated from maple syrup and characterized by gas chromatography / mass spectrometry analysis. These include methylpyrazine, 2,3-dimethylpyrazyne, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, ethylpyrazine, trimethylpyrazine, 2-ethyl-6-methylpyrazine, 2-ethyl-3-methylpyrazine, tetramethypyrazine, 2-ethyl-5-methylpyrazine, 2ethyl-6-methylpyrazine, 2-ethyl-3,5-dimethylpyrazine and 3-ethyl-2,5-dimethylpyrazine. These pyrazine compounds have been identified as flavor and aroma compounds in various processed foods.

Maple syrup was prepared in laboratory conditions that simulate conventional industrial processing. The processing conditions were established

by preliminary experiments following which a six-compartment stainless steel pan was designed for use. The pH of boiling sap increased from 7.2 to 9.2 followed by a decrease to a final pH 7.3. The decrease of pH coincided with an increase of total dissolved solids. The number and total concentration of pyrazines increased with time of processing. A period of induction preceded their formation and accumulation. The rate constant for the formation of pyrazines in maple syrup was consistent with a *pseudo*-zero order.

Heating of commercial maple syrup resulted in the decrease of pyrazine concentrations. This suggest that the distribution and content of certain pyrazines are affected by processing.

The study of pyrazines in various samples have shown that no pyrazine was present in maple sap from which the syrup is prepared. 2,6-dimethylpyrazine was predominant in commercial maple syrup samples, and represented as much as 46% of the total pyrazine concentration in the medium syrup sample. Amber and medium grade syrups contained comparable concentrations of trimethylpyrazine and 2-ethyl-3-methylpyrazine. Different pyrazine distribution pattern and concentrations was also observed for non-maple syrups and artificial maple flavorings.

Sensory evaluation for maple flavor quality and taste preference of maple syrup have shown that medium and amber grades had the highest degree of maple flavor attribute and scored the highest taste preference. A study of the relationship between total pyrazine content and taste preference of maple syrup have shown an inverse relationship between maple syrup flavor and total

pyrazine concentration. Comparison of individual pyrazine concentration have shown that the medium and amber syrups contained comparable quantities of 2,3-dimethylpyrazine, 2,5-dimethylpyrazine and 2-ethyl-3-methylpyrazine, but contained differents quantities of 2-methylpyrazine, 2,6-dimethylpyrazine and trimethylpyrazine. The light syrup however, contained lower quantities of 2,3-dimethylpyrazine and 2-ethyl-3-methylpyrazine but a higher quantity of 2,5-dimethylpyrazine. This suggests that of the pyrazines identified, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine and 2-ethyl-3-methylpyrazine in combination with the other pyrazines be important in the taste preference of maple syrup. Individual pyrazine comparision have also suggested that their may be a possible inverse relation of trimethylpyrazine to maple syrup flavor.

APPENDIX

1: Analysis of varance (ANOVA) for maple syrup flavor

Source	df	Some of squares	Mean square	F-value
treatments	2	79.11	39.56	12.00**
judges	16	401.92	25.12	7.62**
replicats	4	3.62	1.81	0.55ns
judges * treatments	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*

2: Analysis of variance (ANOVA) for maple syrup taste prefenrene

Source	df	Some of squaress	Mean square	F-value
treatments	2	10.13	5.06	2.48ns
judges	18	152.23	8.46	4.15**
experiments	4	3.07	0.77	0.38ns
judges * treatments	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*

APPENDIX 3

Quantitation of Alkylpyrazines in Maple Syrup, Maple flavors and Non-maple syrups

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ABSTRACT

A modified solvent extraction method was developed and evaluated for the quantitation of pyrazine compounds in maple syrup by capillary gas liquid chromatography. The content of total identified pyrazines in the amber, medium and light maple syrups were 48.89 ng/g, 70.68 ng/g and 57.29 ng/g respectively. Commercial non-maple syrups showed total identified pyrazine content ranging from 14.25 ng/g to 20.56 ng/g. Artificial maple flavorings showed values ranging from 1.17 µg/g to 4.93 µg/g. The most abundant pyrazine in the maple syrup samples was 2,6-dimethylpyrazine representing 34% to 43% of the total pyrazines in these samples; 2,5-dimethyl and 2-methyl-pyrazines were also present in considerable amount. Trimethylpyrazine was present in maple syrups and artificial maple flavorings but present only in trace quantities in non-maple syrups; this suggest that trimethylpyrazine could play a role in imparting the characteristic maple flavor. No pyrazine was found in maple sap from which maple syrup is obtained. Gas chromatography/mass spectrometry analysis led to the identification of the following pyrazines which have not been previously identified in maple syrup: 2-ethyl-3-methyl-, 2-ethyl-6-methyl-, 2-ethyl-5-methyl-, 2-ethyl-3,5-dimethyl, 3-ethyl-2,5-dimethyl- and 2,3,5,6-tetramethyl- pyrazine.

Keywords: *maple, sap, syrup, flavor, artificial flavorings, pyrazine.*

INTRODUCTION

The presence of alkylpyrazines (substituted alkyl derivatives of 1,4-diazine) in both fresh and processed foods and the contribution of these compounds to characteristic flavors have been reported extensively (Maga, 1982). Despite their recognized contribution to food flavors, many reports only deal with the presence of pyrazines compounds and their associated flavor with little quantitative information. Among the foodstuffs for which quantitative data have been reported are baked potatoes (Butter *et al.*, 1973), potato chips (Deck *et al.*, 1973), cocoa beans (Reineccius *et al.*, 1972; Chaveron *et al.*, 1989), soy sauce (Nunomura *et al.*, 1978), extruded malt (Fors and Eriksson, 1986) and birch syrup (Kallio, 1987). Quantitative data also have been reported for pyrazines formed in model systems (Koehler and Odell, 1970; Shibamoto and Bernhard, 1977; Huang *et al.*, 1989). This relative lack of quantitative information on pyrazine in foodstuffs may be attributed to the relatively complex nature of the heat induced flavors in processed foods. Chemical compounds, including pyrazines, which impart characteristic flavors to foods and foodstuffs are generally present as part of very complex mixtures. For instance, compounds having large differences of polarities and molecular weights as well as different functional groups can contribute to a particular flavor (Perkins, 1989); these components can be soluble in water and/or oil within the food or can be bound to macromolecules (Dumont, 1987). These factors contribute to difficulties in characterization, as well as quantitation of specific flavor compounds. Pyrazine compounds have low thresholds, are present in trace amounts (Koehler *et al.*, 1971; Shibamoto, 1986) and very frequently are in combination with other compounds to produce characteristic flavor. For example, although some nutty and roasted notes can be attributed to the presence of alkylpyrazines, the characteristic flavor of this heated foodstuff

involves a complex blend of pyrazines and other classes of volatiles such as carbonyls, pyrroles, furans (Bondarovich, 1967); these non pyrazine compounds are formed under similar conditions that result in the formation of pyrazines.

The identification of pyrazines in certain specialty foodstuffs such as syrups and the possible contribution of the pyrazine to the characteristic flavor of these foodstuffs present a need for more quantitative information on the types and amounts of pyrazines. Although these compounds are present in sub microgram/Kg quantities, gas liquid chromatographic analysis has been shown to be satisfactory for their quantitation. Nevertheless, other techniques for analyzing heterocyclic flavor compounds at trace levels (150 ppb to 1 ppm) have emerged; an example is the simultaneous polarometric/chemometric technique with application of a differential pulse at the surface of a static mercury drop electrode (Niyongnian *et al.*, 1992). In the quantitative analysis of pyrazines, the extraction of those compounds in the presence of numerous other volatiles present a challenge. Generally, volatile flavor compounds from foodstuffs are extracted for quantitation by solvent extraction, distillation, head space and trapping techniques with cryofocussing for very early eluting volatiles, or a combination of these techniques (Reineccius, 1989). Selective separation of volatiles based on functional group characteristics is often used. Pyrazine compounds and other nitrogen containing organic bases can be selectively separated by an aqueous hydrochloric acid extraction resulting in the formation of hydrochloride salts. The N containing compounds are

subsequently recovered by adjusting the pH. Chaveron *et al.* (1989) compared different methods for the extraction of pyrazine compounds, these included column extraction (Reineccius *et al.*, 1972), extraction by shaking, steam distillation, micro-distillation and various combinations of these techniques.

The present work was carried out to quantitate the alkylpyrazines present in commercial maple syrups and to determine the possible contribution of these pyrazines to certain maple syrup characteristics by comparison of the type and quantities of alkylpyrazines of maple syrups with those of commercial non-maple syrups and artificial maple flavors.

MATERIALS and METHODS

Materials

Maple sap was obtained from the Morgan Arboretum, Macdonald Campus, McGill University (Ste Anne de Bellevue, Québec). The sap was collected during the 1992 season and kept frozen (-30^{o}C) until processed. Commercial pure maple syrup 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). Three commercial non-maple syrups: Aunt Jamima™ , Crown Corn Syrup™ and Old Tyme™ were purchased from a local supermarket. Artificial maple flavor: LD595, LD596, LD597 and LD598 were obtained from Quest International (Montréal, Canada). Pyrazine standards were purchased from Aldrich Chemical Co. (Milwaukee, WI.). Solutions of the pyrazines standards were prepared as described by Akochi-K. *et al.* (1993). Double distilled chromatographic grades of diethylether and dichloromethane were purchased from BDH (Montréal, Canada).

Extraction of Pyrazine Compounds

A method used previously for the extraction and identification of pyrazines in maple syrup (Alli *et al.*, 1990), (Fig. 1a) was evaluated for extraction efficiency; a series of three internal standards was used. A quantity (approximately 0.1 g; exact quantity determined) of each internal standard was added to the sample to be extracted, as indicated in Figure 1a and 1b. The recovery of each internal standard was determined at the end of the completed extraction procedure. Pyrazine (1,4-diazine) which was not previously identified in maple syrup was used as internal standard (IS) #1 to determine the recovery of pyrazine during the entire extraction procedure (Fig. 1, steps 1 to 4); Reineccius *et al.* (1972) used the parent pyrazine as IS for the quantitation of pyrazine substituted compounds in cocoa beans. Undecanone was used as IS #2 to determine the recovery of step 3 of the extraction procedure; Huang *et al.* (1989) used undecanone as internal standard in the quantitation of pyrazines formed from amino acid-glucose model systems. Dodecane was used as IS #3 to determine the extraction efficiencies of step 4 of the procedure; Hunziker (1989) used dodecane as IS for measurement of recovery of a group of flavor components. The results of these recovery experiments (Table 1) indicate that improvement in the extraction efficiencies is required in the procedure except for the final step (step 4, Fig. 1a). To improve the recoveries of the internal standards, the extraction procedure was modified (Fig. 1b) as follows: syrup sample (100 g) was diluted with distilled water (100 mL); the mixture was adjusted to pH 3 by dropwise addition of HCl (11% v/v) and stirred at medium speed with a magnetic stirrer (10 min). Sodium chloride (30 g) was added and the mixture was extracted with diethylether (150 mL) with stirring for a further 30 min. The aqueous phase was separated and adjusted to pH 11 and extracted with dichloromethane (5x20 mL). The dichloromethane extract was concentrated to 5 mL with a rotary evaporator and then to a final volume of 0.5

mL using a stream of nitrogen. Commercial non-maple syrups, artificial maple flavor as well as lyophilized maple sap were analyzed for pyrazines using the method described above, the quantities of these samples used in the extraction procedure were as follows: non-maple syrup, 100 g; artificial maple flavor, 5 g; freeze dried maple sap, 30 g.

Gas Chromatography Analyses

A Varian gas chromatograph (model 3700) equipped with a flame ionization detector was used. On-column injection was used for samples of non-maple syrup which contained low quantities of pyrazines, while splitless injection mode was used when the quantities were high as in the case of the artificial maple flavors. The following conditions were used for analysis: Supelcowax 10™ fused sillica capillary column (30 m length x 0.32 mm i.d. with a 0.1 μ film thickness; Supelco, Canada); injector port temperature, 200°C; detector temperature, 250°C; nitrogen carrier gas flow rate, 1 mL/min. Oven temperature was programmed from 40°C to 150°C at a rate of 1°C/min with a 6 min initial temperature hold. All chromatograms were recorded and integration was done using a Hewlett-Packard model HP-3390A integrator which was programmed to calculate response factors for each pyrazine used in the standard mixture. Samples were analyzed in triplicate.

Gas Chromatography/Mass Spectrometry Analyses

Gas chromatography/mass spectrometry (GC/MS) analyses were performed using a gas chromatograph (Hewlett Packard HP Model 5890 series II) coupled to a mass spectrometer (HP 5971B series). The ionization energy was 70 eV. Helium was used as carrier gas. The analytical conditions were similar to those used for gas chromatography. Pyrazines were tentatively

identified based on their EI fragmentation data compared to data in the system library.

RESULTS and DISCUSSION

Table 1 shows the recoveries of the three internal standards which were used. The recoveries of internal standard pyrazine, undecanone and dodecane were 77%, 87% and 96%, respectively, when the extraction procedure described by Alli *et al.* (1990) for the identification of pyrazine was used. Modification to the extraction procedure described in the Materials and Methods section resulted in recoveries of 97%, 95% and 97% for pyrazine, undecanone and dodecane, respectively. The improvement of recovery of IS pyrazine was from 77% to 97% and that of undecanone was from 87% to 95%; this suggests that the extraction efficiency was improved between step 1 and step 4 of the method by the modification. A likely explanation for the observed improved recovery of pyrazine is that addition of hydrochloric acid results in the conversion of pyrazines to their hydrochloride salts; the salts are non-volatile and therefore the likelihood of loss by volatilization from the aqueous phase is reduced. The subsequent "salting out" by addition of NaCl serves to enhance the extraction of volatile, non-nitrogenous compounds by diethylether in the next step of the extraction procedure. The improved recovery of standard #2 (undecanone) suggests that adjustment to pH 11 instead of pH 8 resulted in more efficient release of nitrogen containing flavor compounds from the aqueous to the subsequent dichloromethane phase. No significant improvement

was achieved in the recovery of standard #3 dodecane, suggesting the transfer of nitrogenous flavor compounds from the aqueous to the dichloromethane phase was not a critical factor in the initial extraction procedure. Table 2 shows the recoveries of the standard pyrazines using the original procedure (Fig. 1a) and the modified procedure (Fig. 1b). With the exception of 2-ethylpyrazine, the recoveries of pyrazines were increased to above 90%; these observed increased recoveries confirmed the enhanced extraction of pyrazines for quantitation by the modified method.

Figure 2 shows a typical chromatogram obtained from the analysis of pyrazines in commercial maple syrup. In addition to the six previously identified pyrazines (2-methyl-, 2,5-dimethyl-, 2,6-dimethyl-, 2-ethyl-, 2,3-dimethyl- and trimethyl- pyrazine) in maple syrup (Alli *et al.*, 1990), six new pyrazines were tentatively identified in this work by GC-MS analysis. Table 3 shows the EI fragmentation data for these pyrazines (2-ethyl-3-methyl-, 2-ethyl-6-methyl-, 2-ethyl-5-methyl-, 2-ethyl-3,5-dimethyl, 3-ethyl-2,5-dimethyl- and tetramethyl-pyrazine (peaks #8, #9, #10 #11, #12 and #13, respectively, Fig. 2) that have not been previously identified in maple syrup. 2-ethyl-3-methyl- and tetramethylpyrazine were positively identified by their retention times in comparison to those of standards.

The total concentration of the seven identified pyrazines in the three samples of maple syrup ranged from 49 ng/g to 71 ng/g (Table 4). The total pyrazine concentration among the three maple syrup samples of different grades were significantly different ($p<0.05$) with the syrup grade "medium" having the highest quantity of total pyrazines and the "amber" grade having the lowest quantity. Commercial non-maple syrups contained substantially lower

quantities of total pyrazines than maple syrups; the total pyrazine concentration of the six identified pyrazines in the three samples of non-maple syrups ranged from 14.2 ng/g to 20.6 ng/g (Table 4). The commercial samples of artificial maple flavor contained substantially higher total quantities of the seven identified pyrazine concentration ranging from 1.17 µg/g to 4.93 µg/g. Total concentration for a group of nine pyrazines identified in roasted cocoa bean ranged from 1.36 µg/g to 8.5 µg/g (Reineccius *at al.*, 1972). The principal pyrazine in the maple syrup was 2,6-dimethyl pyrazine representing 34% to 43% of the total identified pyrazines in these syrups. This was also the principal pyrazine in two of the four artificial maple flavor samples and one of the three non-maple syrups. Other pyrazines which were present in relatively high concentration in all samples were 2,5-dimethyl- and 2-methyl- pyrazine. Reineccuis *et al.* (1972) who did not succeed in separating 2-methyl-, 2,5-dimethyl- and 2,6-dimethyl- pyrazines, found that, in general, a mixture of these pyrazines represented a substantial proportion of identified pyrazines in roasted cocoa beans; tetramethyl- and trimethyl- pyrazine were also found to be present in large quantities in roasted cocoa beans. Trimethylpyrazine was present in the maple syrups (9% to 14% of total pyrazines) and artificial maple flavors (6% to 9% of total pyrazines) but present in only trace quantities in the non-maple syrups; this leads to the speculation that trimethylpyrazine could play a role in imparting the characteristic maple flavor. On the other hand, 2-ethyl-3-methylpyrazine represented a major component (21% to 37% of the total pyrazines) in the non-maple syrups but a relatively minor component in the maple syrups (6% to 10% of total pyrazines) and the artificial maple flavor (8% to 11% of total pyrazines). 2,3-Dimethylpyrazine was a relatively minor component in all samples, representing 3% to 7% of the total pyrazines in the

maple syrups, 2% to 5% in the non-maple syrups and 6% to 7% in the artificial maple flavor.

CONCLUSION

The efficiency of a solvent extraction method for pyrazines was evaluated with a series of 3 internal standards. The monitoring of these internal standards, through their recovery rate, revealed that *(1),* pyrazines compounds can be efficiently extracted (recovered over 95%) by converting these nitrogen compounds to their salts in acidic medium prior to "salting out" the volatiles and *(2),* pH 11 proved to be adequate for an efficient release of nitrogen containing flavors. Using these modifications, 2-ethyl-3-methylpyrazine and tetramethylpyrazine were positively identified by gas chromatography/mass spectrometry, along with six new tentatively identified pyrazines in maple syrup. These pyrazines were not previously reported in maple syrup and brings the number of pyrazine compounds in maple syrup to thirteen.

Listing of Tables and Figures

Table 1= Table 9, page 92.

Table 2 = Table 9, page 92.

Table 3 = Table 11, page 106.

Table 4 = Table 10, page 102.

Figure 1 = Figures 26 and 27, page 74 and 75, respectively.

Figure 2 = Figure 41, page 107.

APPENDIX 4

Contribution of alkylpyrazines to thd 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 medium and amber syrups was significantly higher ($p < 0.05$) 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 QuÚbec 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; 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) have been reported. The presence of these carbonyl and amino compounds lead to the formation of pyrazines and other flavor compounds during maple sap processing. Several pyrazine formation pathways and mechanisms have been reported (Walradt, 1971; 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 10TM (Supelco, Canada); injector port temperature, 200 oC; detector temperature, 250 oC; nitrogen carrier gas flow rate, 1mL/min. Oven temperature was programmed from 40 oC to 150 oC at a rate of 1 oC/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 22oC 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.

Listing of Tables and Figures

VI. REFERENCES

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