INVESTIGATION OF CHEMICAL AND PHYSICAL PROPERTIES OF SOUTHWESTERN WISCONSIN MAPLE SYRUP

by

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ABSTRUCT

Maple syrup is generally produced in the early spring from February through April when maple sap runs from the maple trees. It is traditionally known that maple syrup tends to be darker when the maple syrup is produced from the sap in the later season. According to the maple syrup grading system in the United States, the darker syrup is given lower grades and values than the lighter syrup. Interestingly, some consumers, however, prefer dark syrup to light syrup. Therefore, the seasonal variation of important maple syrup parameters was investigated. The other aspect of this study was to determine whether the filtration process in maple syrup production removes some nutritional value from the product. In this research, five different seasonal filtered maple syrup samples and their unfiltered samples were compared with respect to their physical properties (density, absorption spectrum, solid content, and water activity) and their chemical properties (pH, mineral contents and sugar contents). It was found that the concentrations of calcium, iron, fructose, and glucose tended to be higher, while sucrose tended

to be lower as the season progressed. The filtration process did not seem to be a significant factor in influencing the concentrations of minerals and sugars.

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One hot summer day, in the Chemistry Department at Stout, Dr. Martin Ondrus asked me if I was interested in working on research about maple syrup. It was totally an unexpected and a surprising proposal since I had already had a rough idea of my thesis topic, and my knowledge about maple syrup was limited to its use as a sweetener for pan cake. However, after the meeting with Dr. Ondrus and two persons from maple farms in Wisconsin, my eyes became just brilliant and my heart was pumping really hard. "This will be my thesis!" That was also the first time that I realized that the maple syrup industry is quite popular in Wisconsin. I was quite excited to think that my research may contribute to our local industry. I am really thankful for Dr. Ondrus and two maple syrup farmers who initiated this research and put a fire in my mind. One of the maple farmers, Ruth Rupp, was the person whom I have worked with on my research from the beginning to the end, and always gave me advice with encouragement whenever needed from her home town. When I visited her at Westby to learn the maple syrup production process, she and her husband, Ken Rupp, warmly welcomed me and treated me as if I were one of the family members. The experience I had with them and their wonderful friends I met there is unforgettable.

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Chapter I: Introduction

Maple syrup is one of the most common sweeteners in the United States. The maple syrup industry is quite dominant in Canada and eastern North America including Wisconsin. Maple syrup is produced from maple sap, a slightly sweet, transparent liquid, tapped from maple trees. Maple sap is boiled down to 1/35 to 1/40 in volume when it becomes syrup. Maple syrup is then pasteurized, filtered, graded, and packaged before it is marketed. Compared to table sugar, which consists of only sucrose, maple syrup contains a variety of nutrients such as minerals, organic acids, amino acids, and vitamins as well as sucrose and other sugars such as glucose and fructose. Stuckel and Low (1996) studied the chemical composition of 80 pure maple syrup samples produced in the United States and Canada. The research focused on sugars (glucose, fructose, sucrose), organic acids (malic acid, fumaric acid), and minerals (Ca, Mg, K), and all 80 samples contained those chemicals, although the contents varied according to the production area.

The United States legal definition of maple syrup states that maple syrup is the "syrup made by the evaporation of maple sap or by the solution of maple concrete (maple sugar) and contains not more than 35 percent of water, and weighs not less than 11 pounds to the gallons (231 cubic inches)" (Willits & Hills, 1976, p. 93). As it is seen in this definition, maple syrup production is not only to produce sweet syrup but also to standardize the properties of maple syrup to ensure a certain quality. In fact, it is very important to pay particular attention to the density and the color of the product in order to obtain high quality maple syrup due to its grading system. According to the United States Standards for Grades of Maple Syrup, the grade is determined mainly by the color of the product measured by means of accepted grading kits such as the United States

Department of Agriculture (USDA) permanent glass color standards. Lighter maple syrup receives higher grades, while darker syrup receives lower grades. The standards give three grade levels: United States Grade A, United States Grade B, and Substandard. United States Grade A is the highest grade in the United States (with the exception of the state of Vermont, where "Fancy" grade is the highest) and it is the lightest color among the three grades. United States Grade A is subdivided into three more grades, United States Grade A Light Amber, United States Grade A Medium Amber, and United States Grade A Dark Amber according to the color of product. United States Grade B is darker than United States Grade A Dark Amber, and it has a stronger maple flavor than United States Grade A. It is used for reprocessing such as cooking and manufacturing, and it is considered unsuitable for consumer labeling. Substandard is not usually available to consumers and is used only for commercial cooking and manufacturing. The standards also describe higher grade maple syrup as having better flavor, clearer color and fewer defects.

The color of the maple syrup is mainly a result of the time of season the maple sap is collected from maple trees. It is generally known that the later in the season the sap is collected, the darker the color of the resulting maple syrup. Driscoll (1998) described in her article how the dark color of maple syrup is a function of the size of sugar molecules in maple sap. As the season progresses, the temperature warms and wild yeasts begin to break down the sugar molecules into smaller components which absorb more light than larger molecules. In fact, microorganisms convert sucrose that is in maple sap to glucose and fructose (invert sugar) by enzymatic hydrolysis (King & Morselli, 1983; Morselli & Whalen, 1991). Driscoll (1998) also mentioned that the colors of maple syrup

as well as chemical compositions are quite dependent on the environments where the maple trees are grown. There has been some research done on the relationship between chemical composition and the color of maple syrup. Robinson, Mac Lean, and Mac Connell (1989) researched heavy metal concentrations in maple syrup produced in Canada and the group found that the later season or darker samples contained more copper and zinc than earlier season or lighter samples.

Statement of the Problem

Driscoll (1998) determined from blind taste tests of maple syrups that consumers tended to prefer darker maple syrup since they wanted syrup that tasted like the "real thing." This result is quite interesting since the maple syrup preference given by consumers does not agree with the level of grade, where the lighter maple syrup is given a higher grade. Though the color of maple syrup is dark, there is a possibility that its nutritional value is equal to or better than lighter syrup. In this study, five maple syrup samples which were produced, respectively, during the maple syrup season in 2004 were analyzed for their physical properties (density, absorption spectrum, solid content, and water activity) and their chemical properties (pH, mineral contents and sugar contents). Maple sap used for these samples was collected 5 times from March 17 through April 4 (a four days period for the first four samples, and a three days period for the last sap) in 2004 from Westby, Wisconsin and each collected sap was separately processed by the syrup maker, Ocooch Mountain Acres, LLC (Westby, Wisconsin). This company also provided another five unfiltered samples corresponding to the same samples as the five filtered samples mentioned above, and this research compared the unfiltered samples to filtered samples in terms of physical and chemical properties.

Study Objectives

To analyze both unfiltered samples and filtered samples by the objective measurements described below.

1) Density: density was determined by measuring the weight of 1 mL of each sample using analytical balance.
2) pH: pH was determined using a pH meter with a glass combination electrode.
3) Visible and UV light absorption spectrum: absorbance of wavelength was determined using a UV-visible spectrophotometer by measuring absorbance in the range of 250nm to 750nm.
4) Solid content: solid content was determined by drying samples using a vacuum oven.
5) Water activity: water activity was determined using a digital water activity meter.
6) Mineral contents: Mineral contents (K, Ca, and Fe) were determined using an atomic absorption (AA) spectrophotometer.
7) Sugar contents: sugar contents (glucose, fructose, and sucrose) were determined using high performance liquid chromatography (HPLC).

Definition of Terms

Maple syrup: In this research, maple syrup is recognized as pure maple syrup as defined earlier. By law, syrups which contain at least 2% of pure maple syrup may be called "maple syrup" and allowed to be labeled with the word "maple syrup" on the syrup products (Doner, L. W., 2003); however, no other certified quality is given in these types

of products. The pure maple syrup, on the other hand, is graded according to the color and quality described in this paper.

Season: In this research, season corresponds to the time of the year when maple sap runs from maple trees or it is collected from the trees. The season usually comes around the end of February to the beginning of March, and it will end in April, when the weather warms. *Seasonal effect* on maple syrup is often discussed in this research. The maple syrup samples used in this research were made from the maple sap collected at different times of the season. Therefore, the term indicates how the different times of the season influence the maple syrup properties.

Limitations of This Study

The result of this research is somewhat limited for general application since the maple syrup samples were from a specific year, and it is expected that the results could vary year by year because maple syrup is more or less influenced by the environmental factors such as soil condition, weather, and temperature, etc. The results represent a specific case, and chronologically accumulated data were necessary to evaluate the seasonal variation of maple syrup properties.

Chapter II: Literature Review

Introduction

There is no exact historical evidence found concerning who first discovered the way to make maple syrup. This is a rather controversial and legendary topic among historians and anthropologists. However; "it is probable that the Indians were the first both to tap maple trees and to distill their sap into syrup" (Mower, 2004, p. 44). Hence, it is believed that maple syrup is one of the oldest agricultural products originating from North America (Willits & Hills, 1976). Lawrence and Martin (1993) indicated that the origin of maple syrup was Indians besides maize and tobacco. In fact, the term, "Indian melasses" and "Indian sugar" were frequently used in the diaries by the early settlers in North America. Maple syrup and maple sugar, a condensed form of maple syrup, used to be staple sweeteners in North America though production rate has been varied according to the cost or supply of white sugar and the supply of farm labor (Willits & Hills, 1976). Table 1 shows the comparison of the production of cane sugar and maple syrup in the selected years from 1918 to 1960. The data indicate that the production of maple syrup has been decreasing while the production of sugar cane has been increasing in the later period of time. Though it is not clearly observed from Table 1, it is mentioned that during the World War I and World War II in this period when cane sugar was in short supply, maple syrup and sugar played an important role in order to meet the demand of sweeteners in the United States (Willits & Hills, 1976).

Table 1

Comparison of the Production of Cane Sugar and Maple Syrup in the Selected Years From 1918 to 1960 in the United States

Year	Total production of maple syrup and maple sugar (tons)[a]	Total production of sugar cane for sugar (thousand tons)[b]
1918	20190	4220
1925	11691	2644
1930	14438	2910
1935	13017	13224
1940	9617	12413
1945	3829	13621
1950	7394	14656
1955	5726	16689
1960	4079	15750

Source: [a]Willits & Hills, 1976; [b]USDANational Agricultural Statistics Service

The maple trees are naturally grown only in eastern North America due to the characteristics of the soil, climate and geology in the area though some other regions such as portions of Europe and Asia have also planted them on a smaller scale. The term," Maple trees" is a generic designation of about 13 maple (*Acer*) species which produce maple sap. However, *A. saccharum* (sugar maple) and *A. nigrum* (black maple) are the two main species for producing maple syrup amounting to about 75% of the total production (Doner, 2003), because of the higher concentration of sugars in their

sap compared to other maple species such as *A. rubrum* (red maple) and *A. saccharinum* (silver maple). Due to their high populations of maple trees, Canada and the United States are the two main maple syrup producers in the world. Production involves the area from Maine west to Minnesota and from Quebec south to Indiana and West Virginia. Vermont, New York, Maine, Wisconsin, and New Hampshire are the major maple syrup producers in the United States in recent years. Table 2 shows the top five producer states and their production from 1995 to 2004. Vermont, New York and Main state produced maple syrup about 60 to 70% of total production in the United States. During the term, Wisconsin stayed the fourth and fifth largest producer in the United States except 2001. There was no specific trend in terms of the production in each country and in the United States, rather it continued increasing and decreasing year by year. This indicates how the production is influenced by the environmental condition in each year.

Table 2

Top Five Highest Producer States and Their Production of Maple Syrup From 1995 to 2004[a]

Year	The top five producer states and their production (thousand gallons)					Total production in the United States (thousand gallons)
1995	VT (365)	NY (208)	MA (162)	WI (98)	PA (65)	1096
1996	VT (550)	NY (343)	MA (167)	WI (110)	OH (90)	1567
1997	VT (395)	NY (269)	MA (185)	OH (95)	WI (87)	1298
1998	VT (375)	NY (263)	MA (150)	OH (78)	PA (72)	1194
1999	VT (370)	NY (222)	MA (190)	OH (95)	WI (75)	1216
2000	VT (480)	MA (270)	NY (239)	NH (80)	WI (65)	1307
2001	VT (290)	MA (232)	NY (220)	OH (96)	PA (69)	1137
2002	VT (510)	MA (275)	NY (260)	NH (83)	WI (79)	1457
2003	VT (420)	MA (285)	NY (210)	WI (76)	NH (60)	1260
2004	VT (500)	MA (290)	NY (255)	WI (100)	NH (83)	1507

Note: VT = Vermont, NY = New York, MA = Maine, WI = Wisconsin, OH = Ohio, NH = New Hampshire, PA = Pennsylvania

Source: [a]USDA National Agricultural Statistics Services

Production Process of Maple Syrup

The production process consists of two main parts: maple sap production and maple syrup production. In maple sap production, maple sap is first tapped and collected from maple trees. Even though the season in which sap runs from maple trees is quite short, lots of careful preparations are necessary before the season begins, such as choosing healthy trees in good condition and constructing a maple sap collecting system in the woods (Koelling & Heiligmann, 1996). Traditionally, metal buckets are used to tap the maple sap, and they are attached to the tree by hanging them down from spouts. Tapholes are made on the trees for this purpose under certain regulations such as "two taps per tree is maximum." The standard diameter of a taphole is 7/16 inch and the depth is not more than 2½ inches (Lawrence & Martin, 1993). Instead of using metal buckets, some farmers use transparent plastic bags. Unlike the bucket containers, the plastic bags are easy to carry and are likely to have less contamination from the environment. However, there are some problems including difficulties collecting sap if the sap is frozen in the bags, tearing of bags when the amount of sap exceeds bag capacity, or damaging bags when squirrels or rodents nibble them. Since these methods are quite labor intensive and are not suitable for collecting sap from many trees, another method using plastic tubes has been developed since about 1970 (Doner, 2003). Today, this method is widely used for medium- to large-scale production. Each tube is attached on the tree in the same way as a bucket but all tubes are eventually connected to a main line and the line brings the sap to a main tank with little labor force. There are some problems as squirrels nibble the tubes and make holes; however, this method significantly reduces production cost and sap collecting time. The season of maple sap collection comes anytime after the trees

have lost their leaves in the fall until late spring when the tree buds begin to leaf out. Jasperson (2005) described that the right temperature when sap runs well is approximately 20 degrees Fahrenheit at night and 40 degrees Fahrenheit the next day. How much sap is collected and eventually how much maple syrup is produced is remarkably affected by weather, temperature and other environmental conditions. This is why the production is not consistent from year to year. The storing of sap is quite an important step in the production of quality maple products. Storage tanks are used not only to store sap until it is processed but also to supply a constant flow of sap to the evaporator (Lawrence & Martin, 1993).

When enough sap is collected for the maple syrup production, the sap is heated to a boil. There are two major types of boiling pans which are currently used for maple syrup production. The first type is a flat bottom open pan. This is a more traditional type and uses enclosed fire box. It is typically heated exclusively by wood fires. It usually takes more time to obtain syrup than with a modern type of pan. The pan is detachable, and syrup is collected by scoping it out. Eventually the pan is slanted to collect the rest. The modern type of pan consists of two parts: a sap pan and a syrup pan. The sap pan is deeper than the syrup pan, and it is more heat-efficient with a heat exchanger. A large amount of water evaporation is expected from this pan but the sap is not yet viscous enough to be syrup in this step. The concentrated sap is then brought to the syrup pan, which is usually less deep and has chambers which are connected to each other to create a one way flow. The higher the syrup density becomes, the further the syrup moves and eventually the syrup is collected at the end when the syrup reaches the right density. A variety of heat sources are available for this pan, and they include wood, fuel oil, gas, and

steam. In either way, the sap is boiled down to 1/35 to 1/40 in volume when it becomes syrup. This value will vary according to the sugar content of sap: sap from *A. saccharum* (sugar maple) has higher sugar content; therefore, it takes less time for evaporation than the sap from other maple trees.

To obtain the right density of maple syrup is crucially important in order to meet the acceptable quality and color of product. Traditionally, the density is determined from the shape the syrup makes when dropped from a scoop, which is called "brow test" or "apron test." This method; however, depends on long experience and skill. Today, there is some equipment used to measure the density of the finished product such as a refractometer and a hydrometer. Using a refractometer is simple and accurate; however, this equipment is quite expensive, and although this method gives a precise value when maple syrup is at room temperature, it will be inaccurate at higher temperatures (Willits & Hills, 1976; Koelling & Heiligmann, 1996). For this reason, a hydrometer is more commonly used for measuring density of maple syrup. The density of maple syrup is determined by displacing a volume of the syrup with the floating body of the hydrometer and is based on the Archimedes principal (Willits & Hills, 1976). A thermometer is always used along with a hydrometer, and taking account the temperature, the density is used to determine the sugar concentration on the Brix scale or Baumė scale. Most states and provinces set the legal minimum concentration of maple syrup at 66 degrees Brix (66 grams of solid content per 100 grams) at 68 degrees Fahrenheit (Koelling & Heiligmann, 1996). According to Vermont law, the concentration should be "ranging from the equivalent of 36 degrees Baumė (66.9 degrees Brix) to 37 degrees Baumė (68.9 degrees Brix) at 60 degrees Fahrenheit" (Lockhart, n. d.). If the density is below 66.9 degrees

Brix, maple syrup tends to ferment and easily get spoiled, and if it is above 68.9 degrees Brix, the syrup tends to crystallize. In order to obtain effective boiling process, some pre-treatment of sap might be used. Reverse osmosis is one of those ways, and the objective of this method is to remove a substantial portion (approximately 75%) of the water from the sap, concentrating it to between 7 degrees and 10 degrees Brix before it enters the evaporator, thereby reducing evaporator fuel costs and boiling time (Koelling & Heiligmann, 1996).

After maple syrup is collected from a boiling pan, the syrup might be pasteurized before the filtration process. Pasteurization eliminates microbial hazards and reheats the stored maple syrup. R. Rupp at Occoch Mountain Acres, LLC. (Westby, Wisconsin) utilizes vat pasteurization and she brings it up to 200 degrees Fahrenheit and holds there for 5 minutes or more before filtration is carried out. She adds a small amount of filter aid (diatomaceous earth) for the next filtering process.

The syrup after evaporation will usually be suspended with sugar sand, which is calcium and magnesium salts of malic acid (Koelling & Heiligmann, 1996). Sugar sand causes maple syrup to have a darker or cloudier color than actual maple syrup. Therefore, it is quite important to filter those impurities to obtain sufficient clarity of the product. There are mainly two types of filtration methods currently used. One is called gravity systems. In this method, hot syrup is poured through a wool or Orlon felt bag prepared in the collection tank, and it is usually packaged directly from the tank. The other method is called pressure filterization. Pressure filters consist of a mechanical pump which forces the syrup through a series of filter plates and disposable filter pads. This process enables rapid processing and the highest clarity.

Before the syrup is packaged, it should be graded. Grading is described later in this paper. Finally, maple syrup is packaged and ready for sale. The packaging method is called hot packing, and the temperature will be kept at 180 degrees Fahrenheit or higher in order to prevent mold and yeast growth. The temperature should not be greater than 200 degrees Fahrenheit, however, because of the possibilities of darkening of the color. Maple syrup is filled in air-tight containers as top as possible and the air space in the container is sterilized by inverting the containers as soon as they are sealed (Perrin, 1980). For the containers used for the packaging, there are three major types: glass, plastic and metal containers. Each type of container has both benefits and weak points; therefore, which type of container is used should be considered according to the needs. Glass containers are most common for 1-quart and smaller size (Koelling & Heiligmann, 1996). Transparent glass permits the natural color to be seen and this stimulates customers to buy products. On the other hand, glass container tends to be heavy and fragile so containers will be specially packaged or put in secondary containments. Plastic containers have a variety of sizes. They are inexpensive, recyclable and have a high resistance to breakage. The plastic used for these containers is often porous to air. The color of the syrup will be changed when kept for a prolonged period. In order to prevent the oxidation reaction, coated plastic containers have been developed, and they have achieved a much longer shelf life. Metal containers are suited for packaging in the 1 quart to 1 gallon size. They are often preferred because artistic graphics on the metal containers gives nostalgic impression to customers and those are often popular among collectors. There is, however, a possibility of loosing metal fragments or rust; therefore, a careful check of the container prior to use is important. After packaging, proper labels are placed

on the package before marketing. Those labels contains product and producer identification, description of the product, suggested uses for the product, grade of product (if available), volume (liquid or weight) of product, and nutritional value.

Grading System of Maple Syrup

It is traditionally known that the color of maple syrup produced in the later season becomes darker and the flavor of the maple syrup becomes stronger than the maple syrup produced in the early season. Some research has been done for the darkening of maple syrup, and the relationship between the color and the quality of the syrup had been discussed (Morselli & Whalen, 1980; Morselli, 1988; Morselli & Whalen, 1989; Morselli & Whalen, 1991). The density of maple syrup is also important for ensuring the quality of product. As mentioned before, if the density is too low, the product easily undergoes fermentation, and if the density is too high, it causes excess darkening and the sugars in the syrup are easily crystallized. In order to obtain consistent and acceptable quality of maple syrup for consumer sale, certain standards for grades of maple syrup have been issued (Willits & Hills, 1976).

The first issue of the United States Standards for Table Maple Syrup became effective February 15, 1940. It graded the maple syrup packed in containers for table use. For the maple syrup in larger containers, for reprocessing purpose, the United States Standards for Maple Syrup for Reprocessing was issued at the same time. The second issue of the United States Standards of Grades of Maple Syrup became effective January 14, 1980 with some modifications. The 1940 standards for table syrup consist of four grade levels: United States Grade AA (Fancy) Table Syrup, United States Grade A Table Maple Syrup, United States Grade B Table Maple Syrup, and Unclassified Table Maple

Syrup. In the 1980 standards, instead of having United States Grade AA, Grade A was subdivided into three levels: Grade A Light Amber, Grade A Medium Amber, and Grade A Dark Amber. The grading systems of maple syrup between the United States and Canada and from state to state have also changed over time (Lawrence & Martin 1993). Table 3 shows the various approaches to maple syrup grading in the United States, Vermont, and Canada.

Even though the grades shown in Table 3 differ, the color of maple syrup is the most important standard for grading. For the purpose of measuring the color, accepted grading kits such as the United States Department of Agriculture (USDA) permanent glass color standards and colored glycerine solution grading kits are used (Koelling & Heiligmann, 1996). The colored glycerine solution grading kits are created just for temporary use. The color tends to be dull; hence, they should be used while they are new. The color value of maple syrup may be indirectly indicated by transmittance (%T). Transmittance is determined by a spectrophotometer. It indicates the color intensity of maple syrup defined as percent transmission of light through the syrup as compared to analytical regent glycerol fixed at one hundred percent transmission. For example, the United States Grade A Light Amber maple syrup should have a light transmittance of higher than 75%T at 560nm. This transmittance value corresponds to 0.125 in absorbance. Although transmittance is one indicator of the color of maple syrup, this is not essential for grading syrup, and the grading kits for color standards should be used along with transmittance. As well as using color as a standard of grading maple syrup, some other factors are considered. Vermont law requires not only color, density, clarity, and purity but also flavor as one of the essential standards for grading maple syrup,

described as "a delicately sweet, original maple flavor characteristic of fancy grade" (p. 58) in the law (Lockhart, n. d.). Lockhart (n. d.) mentioned that flavor is even more important than color in the grading of syrup. A syrup that would be classified as Grade A Medium Amber maple syrup in color will be graded down to Grade A Dark Amber maple syrup if the flavor does not meet the requirement for Grade A Medium Amber.

Table 3

Various Approaches to Maple Syrup Grading in the United States, Vermont, and Canada

Color	The United States	Vermont	Canada
Light	Grade A Light Amber	Fancy	No. 1 Extra Light
↑	Grade A Medium Amber	Grade A Medium Amber	No. 1 Light Grade A
	Grade A Dark Amber	Grade A Dark Amber	No. 1 Medium Grade A
	Grade B	Grade B	No. 2 Amber
↓	-	Commercial	No. 3 Dark
Dark	Substandard	Substandard	-

Source: Koelling & Heiligmann, 1996

Influential Factors of the Darkening of Maple Syrup

Since the color of maple syrup is significant when grading the syrup, factors influencing of the darkening of maple syrup have been a primary concern over the years. Since most of precursors of color and flavor of maple syrup come from maple sap, it is thought that the change of chemical compositions in the sap makes a difference of the color of the syrup (King & Morselli, 1983). The main precursors concerning flavor and color of maple syrup include sucrose, small amounts of amino acids, phenolic substances, organic acids, and irons. King and Morselli (1983) pointed out that those chemical compositions are changed due to the change of metabolic system of maple trees mainly by temperature and also due to the genetic differences among those trees. When the temperature warms, microorganisms such as wild yeast and bacteria are more likely to metabolize maple sap, and they break down sucrose in the sap into monosaccharides, glucose and fructose. Driscoll (1998) mentioned in her article that smaller molecules such as glucose and fructose absorb more light than sucrose, resulting in darker color when sap is concentrated as maple syrup.

Morselli (1988) discussed the influences of a variety of environmental stresses on maple syrup. Those stresses were classified into two groups: biotic causes and abiotic causes. Biotic causes include drought, insect defoliation, pathological conditions, and extreme temperature change. Abiotic causes include overtapping, the use of the paraformaldehyde (PFA, (CH_2O) n) pellet in the taphole, and root damage by cattle grazing and by snowmobiles and heavy equipment. One note about PFA should be added here. In the past, PFA was placed in tapholes in order to retard the growth of microorganisms and obtain a higher quality and quantity of maple sap; however, PFA is

no longer allowed to be used and is not even produced or distributed in the United States or Canada due to its influence on the compartmentalization of maple trees. Though the use of PFA has been abandoned, the old manual still recommends its use, and the influence from the past may still remain (Morselli, 1988; Koelling & Heiligmann, 1996). Morselli (1988) also added abiotic causes which were indirectly or directly produced by human technological advances such as the pollutants from acid rain, greenhouse effects, and increased ozone level. Those biotic and abiotic stresses influence the chemical compositions in the sap and eventually the grades of maple syrup.

The darkening of maple syrup also happens during storage. Morselli and Whalen (1989) summarized a case observed by food inspectors of the Vermont Department of Agriculture. The inspectors found that maple syrup was color-layering and finally darkening in bulk and retail storage. Morselli and Whalen (1989) warned that "syrup darkening may become a major economic issue if syrup in bulk/retail storage would change color grade from that originally identified by the producer/packer/consumer" (p. 33). They cited three possible causes as a factor of a color-layering/darkening in their article. One is blending of syrup. If dark grade syrup is mixed with light grade syrup, invert sugars in the dark syrup may begin the color-layering/darkening during storage in uncontrolled temperature. The next is syrup oxidation. The oxygen in the head space of containers may interact with the chemicals in the syrup resulting in the color-layering/darkening. The last is reverse osmosis concentrated sap. Since the sap concentrated by reverse osmosis contains a higher concentration of invert sugars, it may affect the color-layering/darkening.

Morselli and Whalen (1991) studied aseptic tapping of sugar maple in order to obtain light color grade syrup. Since the fermentation of sucrose into monosaccharides by microbial activity is one of the main factors for the darkening of the syrup, aseptic tapping was expected to prevent darkening of the syrup. In this research, all equipment was sterilized prior to use, and the bark of maple trees was also sterilized by alcohol. They found that the sap obtained by the aseptic tapping technique produced a greater amount of lighter amber syrup (92.8%) than the sap obtained by the traditional tapping technique (61.8%), and no syrup obtained by the aseptic way was darker than medium amber syrup. They also found that if the aseptic tapping technique was used for collecting sap, the color of the syrup stayed light amber or medium amber color even as the season progressed.

pH of Maple Syrup

The value of pH indicates the degree of the acidity or basicity of the sample. pH is defined as

$$pH = - \log [H^+]$$

or

$$pH = X, \text{ when } [H^+] = 1.0 \times 10^{-X}$$

which corresponds to that the higher the concentration of hydrogen ion in solution, the higher the degree of acidity and the lower the value of pH. Since chemical compositions in maple syrup can be influenced by changes in environmental or production conditions as a season progresses, there is a great possibility that the pH of the maple syrup may differ. Table 4 shows the pH of maple syrup samples produced from the eight different areas in the United States and Canada. The mean value of total samples was 6.63 with the

rage from 4.73 to 8.70, and the mean value of any area was also slightly below neutral (pH = 7.0). The average pH of maple syrup produced from some areas such as Quebec, Vermont, Massachusetts, and Nova Scotia gave slightly lower value than other areas.

Table 4

pH of Maple Syrup Produced From the 8 Different Areas in the United States and Canada

Area	pH	Range
Ontario[a]	6.96[b] ± 0.46[c]	5.70-7.85
Quebec [a]	6.50 ± 0.49	5.64-7.74
Quebec[d]	6.82	4.73-8.17
Nova Scotia[d]	6.32	5.54-8.26
New Brunswick[d]	6.64	5.61-8.70
Vermont[a]	6.58 ± 0.26	6.11-7.04
Massachusetts[a]	6.51 ± 0.21	6.27-6.98
Wisconsin[a]	6.85 ± 0.56	6.20-7.90
New Hampshire[a]	6.88 ± 0.09	6.79-6.97

Note: [b]Mean, [c]Standard deviation

Source: [a]Stuckel & Low, 1996; [d]Robinson, MacLean, and MacConnell, 1989

Table 5 shows the pH of maple sap and maple syrup over the course of the season. The pH of maple sap was significantly lower than the pH of maple syrup. This result indicates that the process of production from sap to syrup is also a main influential factor of pH or a chemical composition. Robinson et al. (1989) mentioned that the pH increase from sap to syrup was probably because of the removal or conversion of the organic acids during the evaporation process. Table 2 also shows the pH decrease from early season to late season in both the sap and syrup. This is probably because of the change of chemical compositions caused by microbial activity or metabolic changes of maple trees.

Table 5

pH of Maple Sap and Syrup Compared by the Sampling Time in a Season

	Early Season		Middle Season		Late Season	
	Sap	Syrup	Sap	Syrup	Sap	Syrup
pH[a]	4.85	6.71	4.50	6.54	4.47	6.48

Source: [a]Robinson, MacLean, and MacConnell,1989

Density and Solid Content of Maple Syrup

Density of maple syrup may be determined by refractometry or hydrometry in order to meet the standard of maple syrup. From the density of syrup, the concentration of sugar can be calculated. Two scales are usually used: Baumė scale and Brix scale. In the syrup industry, the Baumė scale is a measure of the density. The Brix scale is a measure of concentration. The Brix scale technically shows the total percentage of dissolved solids in maple syrup; however, it represents "what the percentage of sugar would be if the density of the solution were due only to dissolved sugar" (Willits & Hills,

1976, p. 83). This is because almost 98% of dissolved solids in maple syrup are sugars. Therefore, the Brix scale is considered to be equal to the sugar content of maple syrup. For instance, 67 degrees Brix means 67 pounds of sugars contained in 100 pounds of maple syrup. On the other hand, Baumė scale does not directly indicate the solid content of maple syrup. It is a measurement of the density and is usually converted to the Brix scale by using a conversion table, or by using a hydrometer, which has both a Brix scale and a Baumė scale. Density may also be measured by weight/volume method only if volume and weight of maple syrup can be accurately measured.

According to the data obtained by Stuckel and Low (1996), a mean value of 80 maple syrup samples was 67.0 ± 1.6 degrees Brix with the range from 62.2 to 72.4 degrees Brix at 22 degrees Celsius (72 degrees Fahrenheit). Table 6 shows the degrees Brix values of maple syrup produced from six different states in the United States and Canada. According to the legal solid content of maple syrup, the minimum concentration of solid content is defined as 66 degrees Brix at 68 degrees Fahrenheit (Koelling & Heiligmann, 1996). When the mean value of solid content obtained by Stuckel and Low (1996) is re-calculated by adding 1.8 in order to convert it to the solid content at 68 degrees Fahrenheit (Willits & Hills, 1976), it becomes 67.2 degrees Brix with the range from 62.4 to 72.6. Though the mean value meets the standard of maple syrup, some maple syrup samples do not seem to be proper for sale.

Table 6

Solid Content of Maple Syrup Produced From the six Different Areas in the United States and Canada

Area	Solid content (Degrees Brix)	Range
Ontario[a]	67.2[b] ± 1.3[c]	63.6-69.1
Quebec [a]	66.5 ± 1.4	63.2-69.5
Vermont[a]	66.7 ± 0.9	65.2-68.4
Massachusetts[a]	67.6 ± 1.1	65.5-68.7
Wisconsin[a]	67.5 ± 0.9	65.8-68.7
New Hampshire[a]	67.6 ± 4.9	62.2-74.0

Note: [b]Mean, [c]Standard deviation

Source: [a]Stuckel & Low, 1996

Robinson et al. (1989) measured the solid content of maple sap and syrup produced from three different provinces in Canada at three different sampling times. Solid content of maple sap and syrup was measured by weight/volume measurement. In this method, moisture in the maple syrup samples was removed by drying, and the dry residues were weighed out. According to the data shown in Table 7, the solid content of maple sap and syrup tended to decrease as the season progressed though solid content of maple syrup was not as clear as the solid content of maple sap. This is probably because the solid content in the syrup depends on how long the sap is boiled, and each sample used in the experiment was processed in the different way.

Table 7

Solid Content of Maple Sap and Syrup Compared by the Sampling Time in a Season

	Early Season		Middle Season		Late Season	
	Sap	Syrup	Sap	Syrup	Sap	Syrup
Solids (%)[a]	2.69	65.01	2.61	64.16	2.40	64.55

Source: [a]Robinson, MacLean, and MacConnell, 1989

Water Activity of Maple Syrup

In order to prevent microbial growth in food, it is important to lower the moisture content of food. In fact, drying or desiccation is one of the major ways for food preservation. However, moisture content does not always reflect the food perishability. According to Fennema (1996), it has been reported that the food which has lower moisture content can be more perishable than the food which has higher moisture content. Today, water activity is the accepted indicator of possible microbial growth in food. It is also related to a quality factor for organoleptic properties such as hard/soft and crunchy/chewy (Robert, & Bradley, Jr., 1998). Water activity for food is defined by the ratio of water vapor pressure of food substrate to the vapor pressure of pure water at the same temperature (Jay, 1996). Water activity is defined as follows:

$$a_w = P/P_0$$

or

$$a_w = ERH/100$$

where:

a_w = water activity

P = partial pressure of water above the sample

P_0 = vapor pressure of pure water at the same temperature

ERH = equilibrium relative humidity surrounding the product

In the equation, a higher partial pressure from the food sample means more water is available for microorganisms. Generally, the smaller the water activity, the greater the prevention of microbial growth.

In general, bacteria require higher water activity for their growth than molds and yeasts. Most spoilage bacteria do not grow below $a_w = 0.91$, whereas, most spoilage yeasts and molds can grow with water activities as low as 0.88 and 0.80, respectively (Jay, 1996). However, it has been reported that food poisoning bacteria, *Stapylococcus aureus*, has been found to grow as low as $a_w = 0.86$ and growth at $a_w = 0.75$ has been reported for some halophilic (salt-loving) bacteria. Some xerophilic (dry-loving) molds and osmophilic (high osmosis pressure-loving) yeasts have even been found at water activities of 0.65 and 0.61, respectively.

Some research has been done regarding the influence of water activity on growth of fungi and bacteria. Beuchat (1983) mentioned that fungal spoilage of foods tends to be more serious than bacterial spoilage at low water activity (0.61 – 0.85) not because fungi can grow faster at low water activity but because competitors (bacteria) are absent and fungi can adapt themselves in the limited environment. It has been reported that pH and water activity have a synergic influence on preventing fungal growth of bakery products with the concentration of carbon dioxide in the package (Guynot et al., 2003), and the influence on preventing the growth of *Escherichia coli* with nisin (Cerrutti et al, 2001).

There is little research found regarding the relation between water activity and maple syrup spoilage; however, it has been reported that some species of fungi such as

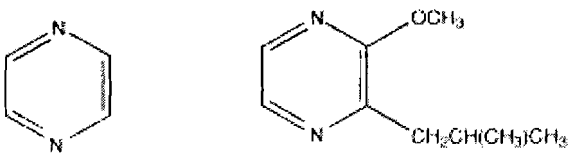
the genera *Aspergillus* and *Penicillium* were observed in the maple syrup, which was reheated at 82 degrees Celsius before packing and was stored at 24 and 30 degrees Celsius (Whalen & Morselli, 1984). There is a general belief among maple syrup makers that microbial contamination in syrup generally occurs only if syrup is packaged at a density below 66.0 degrees Brix. However, microbial contamination may occur when process and storage conditions are inadequate regardless of the degrees Brix in the syrup product. It is quite important to note that some parameters such as temperature, pH or a nutritional condition in syrup more or less affect microbial growth. In fact, abundant nutrients in the media tend to increase the range of water activity and temperature over which microorganism can survive or at which germination and growth of fungal spores will occur (Jay, 1996; Beuchat, 1983).

Flavor Compounds of Maple Syrup

The characteristic flavor of maple syrup known as maple flavor is developed when sap is cooked to syrup during the boiling process. The maple flavor comes from not just one type of compound but rather from a variety of flavor compounds. Researches have detected up to twenty-five flavor compounds including several unknown compounds in a chloroform extract of maple syrup (Filipic, Underwood, & Dooley, 1969; Underwood, 1971). Kermasha, Goetghebeur, and Dumont (1995) identified ten kinds of phenolic compounds and one furfural compound from maple sap and syrup. Pyrazine compounds in maple syrup have also been reported as flavor compounds of maple flavor (Alli et al., 1990; Akochi-K, Alli & Kermasha, 1997).

Pyrazine (1,4-Diazabenzene) compounds are some of the dominant flavor compounds of maple flavor as well as other types of food. The flavor of pyrazine

compounds is often described as roasted or cooked (Maga & Sizer, 1973; Mabrouk, 1976). Maga and Sizer (1973) reviewed past research that alkylpyrazines were identified in roasted peanuts, cocoa products, cooked meat, coffee, and potato chips. Pyrazine compounds are also responsible for the characteristic odors of some vegetables (Vollhardt & Schore, 1998). In fact, some pyrazine compounds are found in raw potatoes, green bell peppers, and green peas (Maga & Sizer, 1973). The structures of pyrazine and 2-methoxy-3-isobutylepyrazine (green bell pepper flavor) are shown in Figure 1.



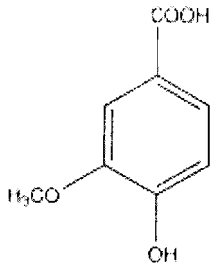
Pyrazine 2-methoxy-3-isobutylepyrazine

Figure 1. The structures of pyridine and a pyridine derivative.

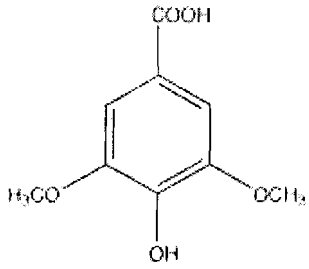
The formation of pyrazine compounds has been researched and several pathways were described (Koehler & Odell, 1970; Shibamoto et al., 1979; Hwang et al., 1994; Akochi-K et al., 1997). The formation of pyrazine compounds is involved in the chemical pathways of the Maillard reaction. The Maillard reaction is also known as non-enzymatic browning, which is responsible for the darkening and the favorable flavor of cooked food initiated by reducing sugars and amino acids. The most common route for the formation of pyrazine compounds in food systems is by the interaction of α-decarbonyls derived from sugars and amines derived from α-amino groups of amino acids through Strecker degradation (Hwang et al.,1994; Akochi-K et al, 1997). The condensation of α-

aminoketones, which are the products from Strecker degradation, produces pyrazine compounds (Nagodawithana, 1994). In the composition of maple sap, sucrose, glucose, fructose and trace amount of amino acids are a great source for the formation of pyrazine compounds. Some pyrazine compounds identified include: methylpyrazine, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, ethylpyrazine, 2-ethyl-6-methylpyrazine, and 2-ethyl-3-methylpyrazine (Alli et al., 1990; Alli & Kermasha, 1997). Alli and Kermasha (1997) found that the formation and concentration of pyrazine compounds was influenced by temperature, heating time, and pH of boiling sap.

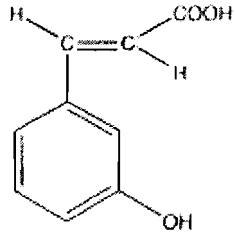
Another family of flavor compound is phenolic compounds. They are volatile substances responsible for the characteristic aromas for many kinds of raw and processed foods such as vegetables, fruits, coffee, chocolate, and wine (Bennion & Scheule, 2000; Mathai, 2000). It is also known that phenolic compounds are associated with browning color of certain vegetables and fruits such as potatoes, apples, bananas, and peaches. Phenolic compounds are easily oxidized when they are exposed in the air with the oxidizing enzymes, which are existed in vegetables and fruits, called oxidases. The oxidative reaction ends with the brown color products (Bennion & Scheule, 2000). Recently, phenolic compounds have been focused as main source of aromatic compounds in maple sap and syrup, and some of them have been identified. The structures of some of phenolic compounds are shown in Figure 2. Among those phenolic compounds, vanillin is one of the most important flavor compounds derived from ligneous material with respect to flavor contribution (Filipic et al., 1969). It is well known that vanillin, syringaldehyde, and dehydroconiferyl alcohol are produced by the degradation of lignin (Filipic & Underwood, 1964).Underwood and Filipic (1964) found out that vanillin in



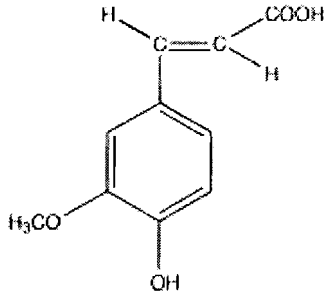
vanillic acid[a]



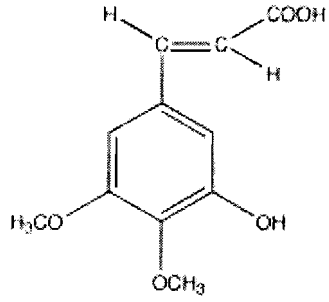
syringic acid[a]



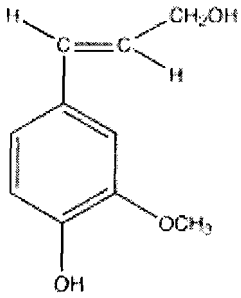
p-coumaric acid[b]



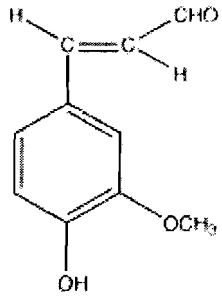
ferulic acid[b]



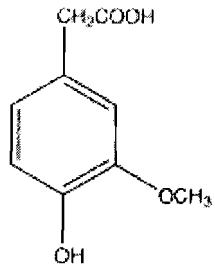
sinapic acid[b]



coniferyl alcohol



coniferyl aldehyde



homovanillic acid

Figure 2. Some structures of phenolic compounds found in maple syrup.

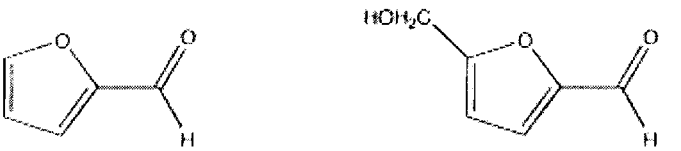
Note: [a]benzoic acid derivatives, [b]cinnamic acid derivatives

Source: Kermasha et al., 1995

syrup can be derived from the trace amount of vanillin contained in the sap, by oxidation of the coniferyl aldehyde and ether-insoluble ligneous material, and/or the dihydroxyconiferyl alcohol produced while the sap is being boiled in the early stage when pH is alkaline. Kermasha et al. (1995) identified ten kinds of phenolic compounds: vanillic acid, syringic acid, homovanillic acid, coniferyl alcohol, vanillin, *p*-coumaric acid, syringaldehyde, sinapic acid, ferulic acid and coniferylaldehyde, from maple sap, concentrates of the sap by reverse osmosis, and syrup. According to the research, sinapic acid is the major phenolic compound identified in maple sap, whereas ferulic acid, syringal, and vanillin are the major compounds identified in maple syrup. The research also shows the significant effect of harvesting time of sap on those phenolic compounds. There is a tendency that the later the sap is taken from the maple trees, the more the total phenolic compounds in the maple products. It has also been observed that when the producers of maple products are different, the concentrations of those phenolic compounds are greatly different. This final result indicates that a variety of maple flavor is created according to the harvesting and the processing of maple sap as well as the conditions of climate and soil.

Other compounds such as furfural compounds are also identified as an important source of maple flavor. Furfural compounds in maple syrup are considered as products of probable carbohydrate origin (Filipic et al., 1969). Underwood (1971) identified two furfural compounds, furfural and hydroxymethylefurfural (HMF) from maple sap and syrup (Figure 3). Although maple sap contains furfural compounds in low concentration, the drastic increase of the concentration will occur by thermal processing. It is also mentioned that these compounds are related to the heavy, acrid caramel flavor of maple

syrup. Kermasha et al. (1995) found that HMF concentration in maple sap, concentrates, and syrup increased as seasons progressed, and HMF concentration in the syrup was much higher than the concentration in the sap. Underwood (1971) also identified several other flavor compounds of carbohydrate origin such as acetol, pineapple furanone (2,5-dimethyl-4-hydroxy-3(2H)furanone), acetic and propionic acids, isomaltol and cyclotene.



furfural hydroxymethylefurfural (HMF)

Figure 3. Structures of furfural and a furfural derivative.

Mineral Contents of Maple Syrup

The amount of minerals contained in maple syrup is quite small; however, they play an important role in forming some characteristics of maple syrup.

It has been reported that the total mineral content in maple syrup is 0.66% or 1.00% of the dry solids (Willits & Hills, 1976). According to White and Underwood (1974), the total mineral content in maple syrup was in the range between 0.38 to 1.5% (as cited in Alexander, 1998). The major mineral composition found in pure filtered maple syrup includes: potassium, calcium, magnesium, manganese, phosphorus, and iron (Morselli, 1975). Some trace cations such as sodium, zinc, copper, tin, and lead may be contained in maple syrup (Koelling & Heiligmann, 1996).

There are some benefits found in maple syrup regarding minerals. Willits and Hills (1976) mentioned that minerals have been "useful in establishing the purity of maple syrup, and they contribute an astringency to the syrup that many find desirable" (p. 66). Nutritional benefits of minerals in maple syrup have also been reported. Byrnes (2000) mentioned that unlike refined sugars such as white and brown sugars, natural sugars and carbohydrates such as honey, maple syrup, fruits, and cane sugar are naturally combined with the vitamins and minerals, which are needed for enzymatic digestion and assimilation in the body. He also added that all natural sugars are healthful in a limited amount.

Although the minerals in maple syrup are considered to be acceptable for the human body, trace amounts of heavy metals have been reported. Robinson et al. (1989) researched the concentration of copper, iron, lead, and zinc in maple sap and syrup produced in Canada. Among these heavy metals, lead is one of the major contaminants found in foods. Food can be contaminated by lead though air pollution, leaded gasoline, water pipes, improperly manufactured ceramic ware, and lead crystal (Boyle, 2001). Accumulated lead is hazardous, and it causes health problems such as diminished intelligence, impaired development, anemia, and inhibited immune response. The rest of these three heavy metals, copper, iron, and zinc are classified as trace minerals, which the human body requires in small amounts from the daily diet. These three metals are deeply related to the formation of hemoglobin and enzymes, immunity, and wound healing. Although minerals are necessary nutrients, they can be hazardous if more than necessary amounts are consumed. Robinson et al. (1989) compared the concentration of these four heavy metals in maple sap and syrup to the concentrations found in the Canadian water

supply and Canadian diet. They found that most of the concentrations found in maple sap and syrup samples were within the range of the concentrations of water and diet. The exception was the lead concentration in maple syrup, which was a little higher than the range in Canadian diets. This was probably due to the contamination from equipment. They also researched the change in the concentrations of heavy metals in maple sap and syrup according to the different sampling times. Copper and lead concentrations in maple sap decreased as the season progressed, while zinc increased. Iron concentration showed little change. Though lead concentration in maple syrup decreased slightly, specific trends were not observed for copper, iron, and zinc in the maple syrup samples.

Finally, minerals also react with organic acids to form sugar sand (Willits & Hills, 1976). Sugar sand is undesirable sediment formed at the bottom of the boiling pan, and accumulated sugar sand eventually decreases the boiling efficiency. Calcium found in maple sap is the major mineral to form sugar sand. It reacts with malic acid and forms calcium malate. Other minerals such as potassium, magnesium, manganese, iron, copper, and molybdenum were also found in sugar sand.

Sugar Contents of Maple Syrup

Sugar is the main constituent of maple syrup, and the typical sugar content in the dry matter of maple syrup is about 98.0% (Willits & Hills, 1976). The main sugar found in maple syrup is sucrose, but small amounts of glucose and fructose are also found. Sucrose is also the main sugar of cane sugar, sugar beets, molasses, and sorghum, while fructose is the main sugar in honey and fruits. Stuckel and Low (1996) reported the sugar concentrations of 80 pure maple syrup products produced in the United States and Canada. According to the data, the concentration of sucrose, glucose, and fructose found

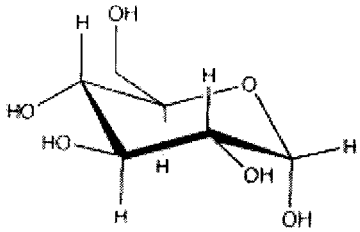
in these maple syrup products ranged from 51.7 to 75.6%, 0.00 to 9.60%, and 0.00 to 4.00%, respectively. Compared to maple syrup, Willits and Hills (1976) mentioned that maple sap typically contains 97.0% sugar in dry weight or 2.0% sugar in wet weight, most of which is sucrose (96.0% of dry matter). Other sugars include raffinose, glycosyle sucrose, and oligosaccharides, yet no glucose and fructose are reported. Although glucose and fructose are quite minor sugars found in maple sap, they are important factors related to color and flavor of maple syrup. As previously mentioned, glucose and fructose tend to be produced in maple sap as the season progresses, mainly due to the fermentation of sucrose by microorganisms and the metabolisms of the trees under warmer weather (King & Moreselli, 1983).

The conversion of sucrose into glucose and fructose occurs by hydrolysis in an acidic condition or in the condition with an enzyme called invertase (Pancoast & Junk, 1980). Another name of sucrose is α-D-glucopyranosyl-β-D-fructofuranoside, which is named from the structure of the combination of two monosaccharides: α-D-glucopyranose (α-D-glucose) and β-D-fructofuranose (β-D-fructose). The structures of α-D-glucose, β-D-fructose, and sucrose are described in Figure 4. This conversion is also called inversion because the specific rotation $(\alpha)_D$ is changed from positive (sucrose: +66.5°) to negative (α-D-glucose: +52.5°, β-D-fructose: -92°, average: -20°). Glucose and fructose that result from the inversion of sucrose are called invert sugars. It is thought that invertase from microorganisms is one of the possible causes of enzymatic conversion of sucrose into the invert sugars in maple sap. It is generally known that the primary sources of invertase are certain yeasts such as *Saccharomyces cervisiae*, *S. carlsbergensis*, and certain fungi such as *Aspergillus oryzae* and *A. niger* (Pancoast &

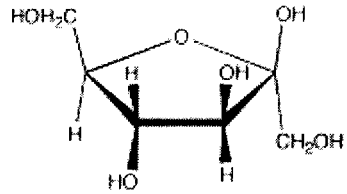
Junk, 1980). King and Morselli (1983) isolated the types of microorganisms from the plastic tubing walls used for maple sap collection, such as yeasts, aerobes, fluorescent pseudomonads, pseudomonads, gram positives, enterics, and fermenters and non-fermenters. Labbe, Kinsley, and Wu (2001) also reported gram negative bacteria, *Pseudomonas fluorescent*, and yeasts, *Candida* spp. as main microorganisms isolated from maple sap. A variety of microorganisms found in maple sap and its collecting tools could be responsible for producing invertase and convert sucrose into glucose and fructose. Though some organic acids such as citric acid are contained in maple sap, acid inversion of sucrose seems to be less likely to occur due to the increase in pH during the boiling process. Akochi-K et all. (1997) reported that the pH changed from neutral (pH = 7.2) to alkaline (pH = 9.2) during the first 40 min of boiling process of maple sap. The increase in pH is probably due to the loss of organic acids and increase of amines. Regarding the influence of glucose and fructose on the color and the flavor of maple syrup, they are both important sugars due to the contribution to the non-enzymatic browning reaction or Maillard reaction, and their potential to produce flavor compounds such as pyrazine compounds and furfural compounds.

The technology for measuring the types of sugars and their concentrations is particularly important in order to detect adulterated sugar products. It is mentioned that additional cane sugar or corn syrup added into maple syrup does not noticeably change the taste of maple syrup, but it greatly increases the syrup production (Benton, 2005).In fact, there is an actual case that happened in the past in which pure Vermont maple syrup distributed to consumers was actually only flavored Iowa corn syrup (Lewis, 2002). Benton (2005) mentioned the importance of confirming the concentration of sucrose,

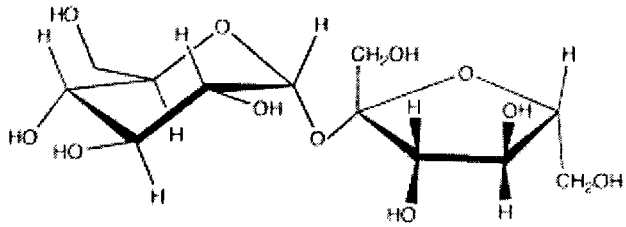
glucose, and fructose in order to ensure the purity of maple syrup. Currently, a complex carbon-isotope ratio test or pulsed-amperometric detection (PAD) method as an alternative is widely used as a reliable technique among sugar makers. It has also been reported that Fourier Transform infrared (FTIR) spectroscopy, near-infrared (NIR) spectroscopy, and Fourier Transform-Raman (FT-Raman) spectroscopy were all successfully used in order to identify the adulterated maple syrup with corn syrup (Paradkar, Sakhamuri, & Irudayaraj, 2002).



α-D-glucopyranose (α-D-glucose)



β-D-fructofuranose (β-D-fructose)



α-D-glucopyranosyl-β-D-fructofuranoside (sucrose)

Figure 6. Structures of α-D-glucose, β-D-fructose, and sucrose.

Chapter III: Methodology

Materials

Ten glass bottles of maple syrup including five unfiltered and five filtered samples were supplied by the syrup maker, Ocooch Mountain Acres, LLC, Westby, Wisconsin. Maple sap used for these samples was collected five times from March 17 through April 4 in 2004 from Westby, Wisconsin, and each collected sap was separately processed by this syrup maker. Since the first four samples were made from the sap collected for a four days period, and the last sample was made from the sap collected for a three days period, the samples are designated, 4D-N, 8D-N, 12D-N, 16D-N, and 19D-N for unfiltered samples, and 4D-F, 8D-F, 12D-F, 16D-F, and 19D-F for filtered samples in this paper. The photos of ten maple syrup samples are shown in Figure 5. The earliest seasonal samples, 4D-N and 4D-F, had the lightest color, while the latest seasonal samples, 19D-N and 19D-F, had the darkest color. Filtered samples were produced by going through all processes including boiling, pasteurization, filtration, and packaging. Unfiltered samples were collected in glass bottles right after the boiling process, but no pasteurization and filtration were applied. Hence, small amount of sediments were observed at the bottom of glass bottles of the unfiltered samples.

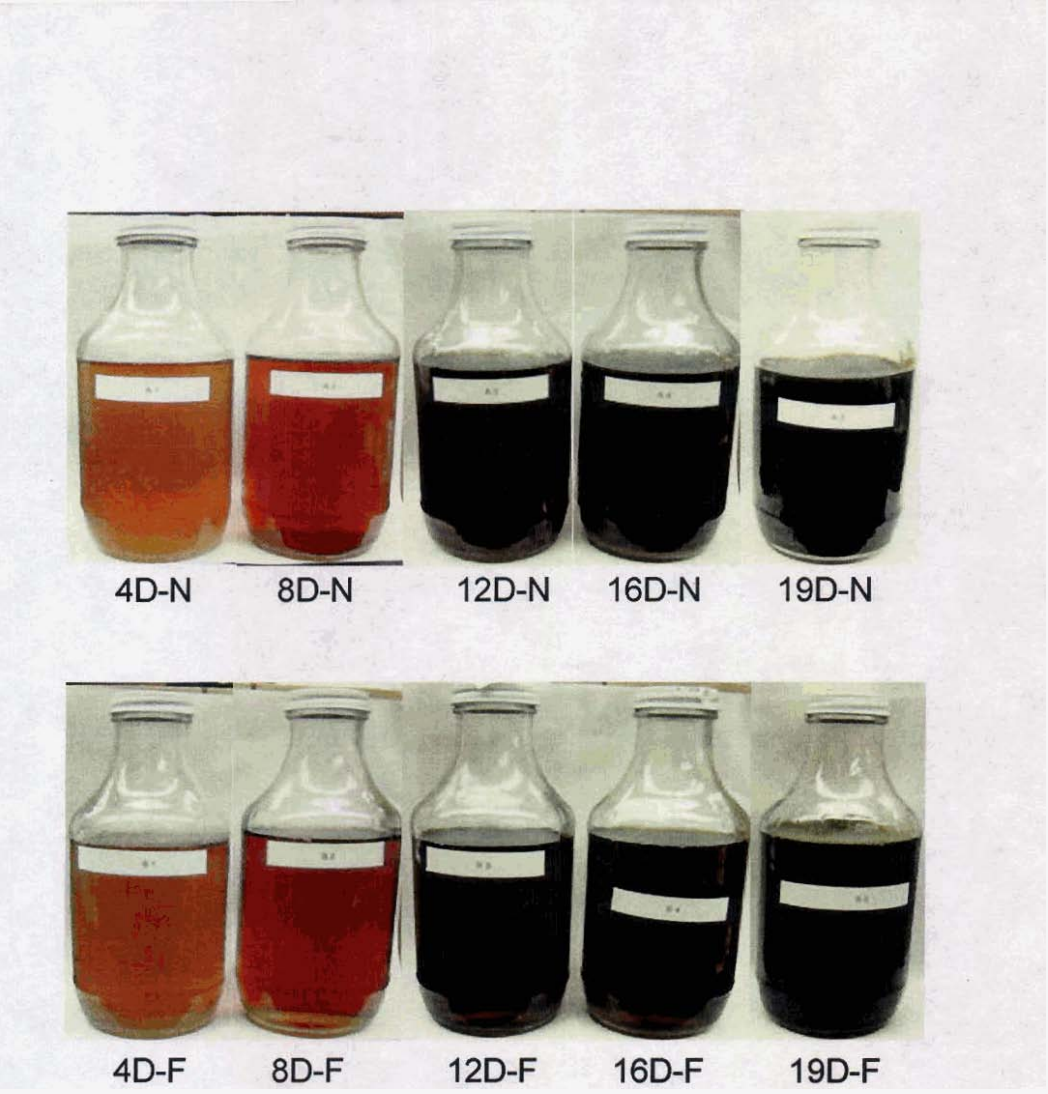


Figure 5. Photos of unfiltered maple syrup samples (4D-N, 8D-N, 12D-N, 16D-N, and 19D-N) and filtered maple syrup samples (4D-F, 8D-F, 12D-F, 16D-F, and 19D-F) supplied by the syrup maker, Ocooch Mountain Acres, LLC, Westby, Wisconsin.

Methods

Prior to experiments, small but sufficient portions of maple syrup samples for the planned analyses were separated from the glass bottles into small plastic bottles in order to prevent microbial contamination in the main bottles. All glass bottled samples and separated plastic bottled samples were kept at 4 degrees Celsius until the beginning of each experiment. Maple syrup samples separated in plastic bottles were used for all experiments except sugar analysis, where samples were used from glass bottles. The maple syrup samples taken from the plastic bottles were kept at room temperature prior to each experiment.

Density and pH Analysis

Prior to the measurement of density and pH, about 15 mL of each maple syrup sample was prepared in a 25 mL-flask. Ten samples prepared in the flasks were then put in a Fisher Isotemp Water Bath (FisherScientific) with the water temperature set at 25 degrees Celsius. All flasks were covered with Parafilm (Pechiney Plastic Packaging) in order to prevent water from coming into the flasks. Samples were kept in the water bath long enough for the temperature of the samples to equilibrate at 25 degrees Celsius.

In order to determine the density of maple syrup, hydrometry and refractometery are the methods commonly used among maple syrup makers. The direct weighing method was not recommended due to its requirement of high accuracy of weight measurement (Willits & Hills, 1976). However, analytical balances have been improved in recent years, and it has become possible to determine the weight of samples more accurately. Since the quantities of maple syrup samples were quite limited in this experiment, direct weighing method was chosen for the measurement of the density.

Density was determined by measuring the weight of the maple syrup sample and the specific volume (1.00 mL). To measure the exact 1.00 mL of sample, a Repeater 4780 (Eppendorf) was used with the 12.5 mL combitip. The combitip was immersed into the maple syrup sample kept at 25 degrees Celsius in the water bath, and then the filling lever was slowly slid upwards. The sample was then delivered with the dial set at 4 into a small beaker prepared on an analytical balance (AG balance-AG 135, Mettler-Toledo GmbH), and the weight was recorded to four decimal places. Three measurements were done per sample (triplicate), and the average for each sample was calculated.

pH was determined using a model 6050 (Sargent-Welch Scientific Company) pH meter with a glass combination electrode. Calibration of the meter was accomplished with pH 7.0 and 4.0 buffers. After density was determined, the glass electrode was directly immersed into the rest of maple syrup sample in the flask. pH was measured once per each sample.

Visible and UV Light Absorption Spectrum

In order to carry out the qualitative or quantitative analysis of an analyte in a given solution, spectroscopy such as UV-Vis spectrophotometry, mass spectroscopy, atomic absorption (AA) spectrophotometry, and infrared spectrophotometry are used (Penner, 1998). Among those methods, UV-Vis spectrophotometry includes both UV (Ultraviolet) range, which has a spectrum that ranges in wavelength from approximately 200 to 350 nm, and Vis (visible) range, from approximately 350 to 700 nm, as electromagnetic radiation. When the radiation beam passes though the solution in a cell, radiant power is decreased, since analyte in the solution absorbs the photons of the radiation. The instrument detects the degree of absorption in a certain wavelength;

therefore, the concentration of analyte is determined by the degree of absorption by comparing to references, and the type of analyte or absorption spices are estimated from the wavelength where the maximum absorption occurs.

The power of the incident beam from the energy source and the power of the exit beam remaining after passing though a cell are expressed as transmittance (T), as described in the following equation. Another term, absorbance (A) is also used as convenience since absorbance is proportionally related to the concentration of the absorption species in analyte.

$$T = P/P_0,$$

$$A = \log (P_0/P) = -\log T$$

Where:

T = transmittance

P_0 = radiant power of beam incident on absoption cell

P = radiant power of beam exiting the absorption cell

A = absorbance

Penner (1998) points out the possible indiscriminant error associated with the absorbance/transmittance measurement. In order to minimize the error, it is important to measure the absorbance in the optimum range from 0.2 to 0.8 absorbance units, or from 15% to 65% transmittance with less expensive spectrophotometers. Though the absorbance reading could be extended up to 1.5 or greater with high quality spectrophotometers, it is recommended that the reading be always less than 1.0 for accuracy. If the concentration of analyte in the solution is too high to measure the

absorbance in the optimum range, the sample should be diluted properly prior to the measurement.

The purpose of this experiment was to estimate the type of substance associated with the color of the maple syrup products by applying the qualitative analysis with a UV-Vis spectrophotometer. Prior to the measurement of absorbance, maple syrup samples were diluted appropriately with Milli-Q water (Millipore) according to the color of the products. By using a Repeater 4780, 1.0 mL of a sample was poured into a volumetric flask. Samples 4D-N, 8D-N, 12D-N and 4D-F, 8D-F, 12D-F were diluted by the factor of 50 in a 50 mL volumetric flask, and samples 16D-N, 19D-N and 16D-F, 19D-F were diluted by the factor of 100 in a 100 mL volumetric flask. The sample was poured directly into the flask, and each flask was filled to volume with Milli-Q water.

Absorbance of each sample was measured by using a Hewlett Packard 8452A Diode Array UV-Vis spectrophotometer with the wavelength range from 250 nm to 750 nm. For a sample-holding cell, there are several choices of composition; however, there is a limitation of the usage of the cell that the cell should not absorb radiation in the spectral region being used (Penner, 1998). Due to the measurements in UV range, a 1-cm quartz cell was chosen in this experiment. As a reference, Milli-Q water was used. The relationship between absorbance and wavelength was plotted in the computer as graphics, and the possible compounds were estimated according to the wavelength at which the maximum absorbance was observed.

Solid Content

About 1g of each maple syrup sample was put in a 30-mL beaker by using a Repeater 4780. The weight of the empty beaker and the sample taken in the beaker were

separately measured by using an analytical balance. The sample was dried by using a Fisher Isotemp® Model 282A Vacuum Oven (FisherScientific) until all water was dried out. The degree of the dryness of samples was occasionally checked by visual observation. If the sample did not appear to have completely dried, it was kept in the oven for additional time. The temperature of the vacuum oven was set at 75 degrees Celsius, and the pressure was set at 5.0 to 8.0 in Hg. After the samples were dried, they were kept in a desiccator until their temperature equilibrated with room temperature. The total weight of the beaker including the sample was measured, and then solid content was calculated with the following equation. Duplicate measurements were done per sample.

$$\text{Solid content (\%)} = (Wt-Wc)/Wb\times 100$$

$$= Wa/Wb\times 100$$

Where:

Wt (g) = weight of a beaker including a dried sample after dried

Wc (g) = weight of a empty beaker

Wb (g) = weight of a sample before dried

Wa (g) = weight of a sample after dried

Water Activity

Water activities of maple syrup samples were measured by using an AquaLab Series 3 Model TE (Decagon Devices, Inc.). The instrument was kept with a set temperature at 25 degrees Celsius prior to measurements. The small portion of maple syrup sample, temperature equilibrated with room temperature, was poured in a plastic sample dish until the bottom of the dish was covered by the sample, and then the dish was

loaded in the instrument. Water activity was automatically measured in approximately 5 minutes. The measurement was done once per each sample.

Mineral Analysis

The concentrations of three kinds of minerals, potassium (K), calcium (Ca), and iron (Fe) in maple syrup samples were determined by using a flame atomic absorption (AA) spectrophotometer. Atomic spectroscopy including atomic absorption spectrophotometry and atomic emission spectrophotometry has been used for measuring the mineral nutrients and toxicants in foods (Miller, 1998). The atomic absorption spectrophotometer measures the quantities of the absorption of electromagnetic radiation by the completely separated atoms in the gaseous state. Atomic absorption spectrophotometers consist of five main components: radiation source, atomizer, monochromator, detector, and readout device. When an analyte in a solution was separated well into atoms or ions in the atomizer (a nebulizer-burner system is used in flame AA systems), ground state atoms or ions are shifted to an excited state, and they absorb energy from a radiation source. A hollow cathode lamp specified for each element is used for the radiation source, and it produces the radiation including a specific wavelength which can be absorbed by the atoms. The amount of radiation absorbed by the atoms is described by Beer's law.

$$A = \log (I_0/I) = abc$$

Where:

A = absorbance

I_0 = intensity of radiation incident on the flame

I = intensity of radiation exiting the flame

a = molar absorptivity

b = path length through the flame

c = concentration of atoms in the flame

The attenuated beam from the source then reaches at monochromator (typically an ultraviolet-visible grating monochromator), where a specific wavelength is isolated for the final readout. A photomultiplier tube (PMT) is used as a detector, and it converts the radiant energy into an electric signal, which eventually comes out as analog or digital readout at a readout device.

In order to carry out the quantitative analysis, standard solutions of these three elements were prepared. For the potassium standard solution, 1.907 g of dried KCl was put into 1-L volumetric flask, and it was filled to volume with Milli-Q water (1000 mg/L K^+). Then, 10 mL of this solution was put into a 100-mL volumetric flasks for preparing 100 mg/L K^+ solution. From this solution, 0.5, 1.0, 2.0, and 4.0 mg/L K^+ standard solutions were prepared in each 100 mL-volumetric flask with Milli-Q water. For the calcium standard solution, 2.497 g of dried $CaCO_3$ was dispersed in 50 mL of Milli-Q water. Approximately 10 mL of HCl (0.1N) was added drop-by-drop for the complete dissolution of $CaCO_3$. The solution was diluted in a 1-L volumetric flask with Milli-Q water (1000 mg/L Ca^{2+}), and then a 100 mg/L Ca^{2+} solution was prepared in a 100-mL volumetric flask as well as K^+ solution. From this solution, 0.5, 1.0, 2.0, and 4.0 mg/L Ca^{2+} standard solutions were prepared in each 100-mL volumetric flask with Milli-Q water. For the iron standard solution, 1.000 g of metallic iron (99.5% purity), which was cut into small pieces, was put in 50 mL of 6N HCl, and it was kept for approximately 10 days when all iron was dissolved as Fe^{2+} ion. The solution was diluted in a 1-L

volumetric flask with Milli-Q water (1000 mg/L Fe^{2+}), and 100 mg/L Fe^{2+} solution was prepared in a 100- mL volumetric flask. From this solution, 0.1, 0.3, 0.5, and 0.8 mg/L Fe^{2+} standard solutions were prepared in each 100-mL volumetric flask with Milli-Q water.

According to Stuckel and Low (1996), the average concentrations of K^+ and Ca^{2+} of the eight maple syrup samples produced in Wisconsin were 2091 ± 385 mg/L and 775 ± 279 mg/L, respectively. Morselli (1975) reported that the concentration of Fe^{2+} in maple syrup ranged from 0 to 36 ppm, which is much smaller than the concentration of K^+ or Ca^{2+} (less than 1/100). Since the range of mineral concentrations of maple syrup samples needs to be in the range of the mineral concentrations of standard solutions, the samples were diluted prior to the measurements with the appropriate ratio determined by those mineral concentrations in maple syrup in the past research of Stuckel and Low (1996), and Morselli (1975). For the potassium and calcium analysis, maple syrup samples were diluted by the factor of 1000, and for iron analysis, the samples were diluted by the factor of 100. For the potassium and calcium analysis, 0.50 mL of each maple syrup sample was delivered into a 50-mL volumetric flask by using a Repeater 4780 (Eppendorf) with the dial of 2, and the weight of the sample was measured by an analytical balance. After the volumetric flask was filled to volume with Milli-Q water (100 times dilution), 10 mL of the solution was delivered into a 100-mL volumetric flask in order to prepare a 1/1000 diluted maple syrup sample. For the iron analysis, 0.50 mL of each maple syrup sample and 1.0 mL of 6N HCl were delivered into a 50-mL volumetric flask in order to prepare a 1/100 diluted maple syrup sample. As a blank,

Milli-Q water was used for the potassium and calcium analysis, and Milli-Q water with 1 mL of 6N HCl prepared in a 50-mL volumetric flask was used for the iron analysis.

Mineral contents were measured by using a Thermo Elemental SOLAAR S4 atomic absorption spectrophotometer with appropriate hollow cathode lamps as light sources. The wavelengths for the measurements of minerals were set at 766.5 nm for potassium, 422.7 nm for Calcium, and 248.3 nm for iron. Air-acetylene was used as oxidant-fuel combinations. Standard solutions and diluted maple syrup samples were measured three times per each (triplicate). By using standard curves, mineral contents in the maple syrup samples were determined.

Sugar Analysis

The concentrations of three mono- and disaccharides, glucose, fructose, and sucrose, in maple syrup samples were determined by using high performance liquid chromatography (HPLC). HPLC is used for the analysis of mono-, di-, and oligosaccharides, or it can be used for polysaccharides after hydrolysis. Main components of an HPLC system include pump, injector, column, detector, and recorder/integrator/data system.

In order to carry out the quantitative analysis, standard solutions of these three sugars were prepared. Five different concentrations: 0.1, 0.25, 0.5, 1.0, and 2.0% were used for the standard solutions of glucose, fructose, and sucrose. In order to prepare a 0.1% standard solution, about 0.1 g of each sugar measured by an analytical balance, was dissolved together in Milli-Q water in a small beaker. The solution was then transferred into a 100-mL volumetric flask, and it was filled to volume with Milli-Q water. 0.25, 0.5, 1.0, and 2.0% of standard solutions were prepared in the same way by measuring 0.25,

0.5, 1.0, and 2.0 g of each sugar followed by diluting the solution with Milli-Q water in each 100-mL volumetric flask.

Stuckel and Low (1996) reported that the average concentrations of glucose, fructose and sucrose of the 8 maple syrup samples produced in Wisconsin were 0.27 ± 0.22%, 0.22 ± 0.51%, and 69.9 ± 1.2%, respectively. According to these data, the concentrations of glucose and fructose were in the range of standard solutions (0.1 to 2.0%); however, because the samples were too thick due to the high concentration of total sugars, they were not suited to be injected into HPLC without any dilution. For the glucose and fructose, the maple syrup samples were diluted by the factor of 20, and for the sucrose, the samples were diluted by the factor of 40 in the following procedure. Since the sugar precipitation in the plastic bottles had been observed from a certain time during the experiment, unlike mineral analysis, it was though that the precipitation would greatly affect the sugar content in the maple syrup samples. Since there was no sugar precipitation in the glass bottles (main stocks) when portion of samples were separated into the plastic bottles, the maple syrup samples for sugar analysis were taken from the glass bottles. Since 4D-N, 16D-N, 4D-F, and 16D-F have obtained small amount of sugar precipitation at the bottom of glass bottles during the storage, these bottles were kept in the hot water until all precipitation was dissolved prior to the measurement. For the glucose and fructose, 2.5 mL of each maple syrup sample was delivered into a 50-mL volumetric flask using a Repeater 4780 (Eppendorf), and it was filled to volume with Milli-Q water in order to obtain 20 times dilution. For the sucrose, 1.25 mL of each maple syrup sample was delivered into a 50-mL volumetric flask in order to obtain 40 times dilution. Accurate sample weights were measured by using an analytical balance.

All standard solutions and diluted maple syrup samples were passed through a 0.45 μm syringe filter (Arbor Technologies, Inc.) and collected in vials in order to remove particulates prior to HPLC analysis. Samples were analyzed on a Waters HPLC (system) equipped with a manual UGK injector, and a Waters R401 differential refractometer detector using a Supelcosil^{TM} LC-NH_2 column (250 mm × 4.6 mm, 5 μm) (Supelco). Chromatograms were plotted employing Dynamax MacIntegrator II software (Rainin Instrument Co., Inc). As mobile phase, the solution of 85% acetonitrile and 15% Milli-Q water was filtered and degassed in a vacuum filtration system. Flow rate and injection volume were set at 2.0 mL/min and 20 μm, respectively. From the chromatograms, standard curves were constructed according to the peak areas and sugar concentrations of the standard solutions.

Chapter IV: Results and Discussion

Density and pH Analysis

The density and pH results are shown in Table 8. The very low standard deviation for the densities shows that the reproducibility of the three measurements was quite close and reliable. The means of the pH of five unfiltered maple syrup samples and five filtered maple syrup samples were 6.18 ± 0.251 and 6.49 ± 0.002 , and the means of the densities of five unfiltered maple syrup samples and five filtered maple syrup samples were 1.324 ± 0.005 g/mL and 1.326 ± 0.007 g/mL, respectively. According to the pH measurements, all samples were slightly acidic. This indicates that maple syrup is not a pure solution of sugars, but also contains minerals and organic acids. The unfiltered maple syrup samples showed slightly lower pH than the filtered maple syrup samples. This is probably because of the removal of sugar sand by filtration process. The sugar sand is generally the calcium and magnesium salts of malic acid. It is thought that the removal of the acidic compounds by the filtration process may have affected the pH and increased it in filtered samples compared to unfiltered samples. Besides the removal of sugar sand, filtered samples had longer storage time and additional cooking time in the pasteurization and the packaging process; it could have influenced the compositions of maple syrup, which resulted in the pH change.

Table 8

pH, Density, Solid Content, and Water Activity of Unfiltered and Filtered Maple Syrup Samples

Sample Number	pH	Density (g/mL)	Solid content (%)	Water activity
4D-N	6.28	1.320[a] ± 0.003[b]	67.3	0.842
8D-N	5.96	1.322 ± 0.003	67.7	0.844
12D-N	5.87	1.324 ± 0.002	67.1	0.843
16D-N	6.46	1.329 ± 0.008	69.2	0.841
19D-N	6.32	1.323 ± 0.003	68.3	0.847
4D-F	6.64	1.333 ± 0.004	68.8	0.854
8D-F	6.53	1.322 ± 0.002	67.2	0.851
12D-F	6.36	1.325 ± 0.002	67.1	0.848
16D-F	6.60	1.333 ± 0.006	69.1	0.854
19D-F	6.33	1.318 ± 0.002	65.9	0.855

Note: [a]Mean, [b]Standard deviation

According to the density measurements, all samples were quite similar. The means values of unfiltered and filtered maples were 1.324 ± 0.005 g/mL and 1.326 ± 0.007 g/mL, respectively, and significant difference was not observed between them. The density is generally determined by using a hydrometer and checking the elevation of boiling point. The result indicates the accuracy of the measurement regardless of the sugar concentration in the original maple sap. Although no significant difference was observed among the samples, sample 16D-N, 4D-F, and 16D-F showed a slightly higher pH and density than the other samples. This is probably because the compositions of maple syrup, such as minerals and organic acids, and their amounts will change according to the season when the maple sap was taken. Other than the seasonal effect, process control could also influence the properties of maple syrup since certain types of factors such as weather and temperature are uncontrollable and will be different in each process.

By comparing the data obtained by Stuckel and Low (1996), the pH of maple syrup produced in Wisconsin ranged from 6.20 to 7.90. The mean of the pH of filtered sample obtained from this experiment was in this range, though one of the unfiltered samples was slightly lower than the range. It was reported that the lowest pH was found in the maple syrup produced in Quebec in Canada, which was 5.64 (Stuckel & Low, 1996). From this report, quite a wide range of pH is observed for the maple syrup product.

Solid Content

The result of solid content by weight ranged from 65.9% (19D-F) to 69.2% (16D-N) (Table 8). The major contributor of the solid content is the sugar content, which will

be approximately 98.0% of the total solid (Willits & Hills, 1976). For this reason, sugar content of maple syrup is approximated by the solid content, or vise versa. Sample 16D-N, 4D-F, and 16D-F gave slightly higher solid content than other samples. As discussed in the previous section, 16D-N, 4D-F, and 16D-F also gave slightly higher density than others. This result indicates the exact correspondence between solid content and density. The mean of solid content of unfiltered samples and filtered samples was 67.92 ± 0.850% and 67.63 ± 1.322%, respectively. This data shows that there is quite small difference in solid content between unfiltered and filtered samples.

Robinson et al. (1989) measured the solid content of maple syrup produced from three different provinces in Canada at three different sampling times. The mean solid content of the maple syrup samples was 64.41% (ranging from 43.81% to 68.34%), and there was no clear tendency of the change of solid content observed as the season progressed. The data obtained in this experiment did not show the clear tendency between the different seasonal samples, either. The mean of moisture content calculated by subtracting the mean of solid content from 100 for unfiltered and filtered samples was 32.08% and 32.37%, respectively. According to the data obtained by Stuckel and Low (1996), the moisture content of maple syrup produced in Wisconsin ranged from 28.1% to 33.0%. From this data, the moisture content and the solid content of the ten maple syrup samples used in this experiment are well within the range observed for Wisconsin maple syrup.

Water Activity

The result of water activity ranged from 0.841 (16D-N) to 0.855 (19D-F) (Table 8). The maximum water activity was obtained from the maple syrup which had the

minimum solid content or maximum moisture content, and the minimum water activity was obtained from the maple syrup which had the maximum solid content or minimum moisture content. This result indicates that the higher the moisture content the maple syrup sample is, the higher the water activity. Moisture content is, however, not the same as water activity; moisture content simply indicates how much water is in the sample, and water activity indicates how much free water is available for microorganisms. In general, the food which has higher moisture content tends to have higher water activity though some exceptions are found (Fennema, 1996).

It is desirable that the water activity is low enough to prevent any pathogenic bacteria growing in maple syrup. According to Jay (1996), most spoilage bacteria do not grow below $a_w = 0.91$, although some specific bacteria such as *Stapylococcus aureus* and certain halophilic type of bacteria have been found at lower water activity in food products. The microorganisms which could be found in the highly sugared food are generally molds and yeasts. This is because they are much more tolerant in dry condition, and they can grow at $a_w = 0.61$ in lowest case. Most spoilage molds and yeasts; however, do not grow below $a_w = 0.80$ and 0.88, respectively (Jay, 1996). According to the data obtained, all water activities were below 0.86, which indicates that pathogenic bacteria are not likely to grow in the maple syrup. Although most spoilage molds and yeasts are hardly seen in the condition, there is still possibility that some types of molds and yeasts could be found in syrup if once contaminated.

Another characteristic of the experimental data is that the average water activity of filtered samples (0.852 ± 0.003) was slightly higher than unfiltered samples (0.843 ± 0.002). This is probably because the filtered samples contain much fewer impurities than

unfiltered samples, and the free water in the filtered samples vaporized with less interaction with impurities compared to unfiltered samples.

Visible and UV Light Absorption Spectrum

Figure 6 and 7 shows the relation between absorbance and wavelength of each maple syrup samples. Since 16D-N, 19D-N, 16D-F, and 19D-F were diluted by the factor of 100, while other samples were diluted by the factor of 50, the order of the height of the peaks are not correctly corresponded to the original concentration of maple syrup samples. The relation between the season when maple sap was taken and the maximum absorbance modified by dilution factors are shown in Figure 8 and 9. In Figure 8 and 9, the x axis represents the average sampling time; for instance, in case of the sample 4D-N, since the sample was collected from day 0 through day 4, the average, day 2, was chosen. Similarly, day 6 was chosen for 8D-N since the sample was collected from day 4 through day 8. Interestingly, all maximum absorbance were observed in the same wavelength in ultraviolet region (271 nm to 275 nm). This result indicates that the maximum absorbance was obtained by the same or similar type of compounds existed in the maple syrup. One possibility for the source of these compounds is a series of phenolic compounds. Kermasha et al. (1995) used HPLC equipped with an ultraviolet diode array to determine the detection limit of ten kinds of phenolic compounds. Those phenolic compounds were detected with the wavelength at 280 nm. Phenolic compounds are basically a type of substituted benzenes such as benzoic acid derivatives, cinnaic acid derivatives, and coniferyl alcohol. It has been reported that the highest absorption for benzene was obtained at 261 nm, 268 nm for 1,3,5-hexatriene, and between 250 and 290 nm for simple substituted benzenes (Vollhardt & Schore, 1998). It is thought that there

are a variety of phenolic compounds and possibly other variety of substituted benzenes contributed this result.

Another finding was that the later the season when the maple sap was taken, in other words, the darker the color of maple syrup is, the greater the maximum absorbance is. In Figure 8 and 9, the average sampling time seemed to be exponentially proportional to the maximum absorbance. This result indicates that the darkening of the color of the maple syrup samples may be caused by the certain group of compounds found in higher concentration in the later season.

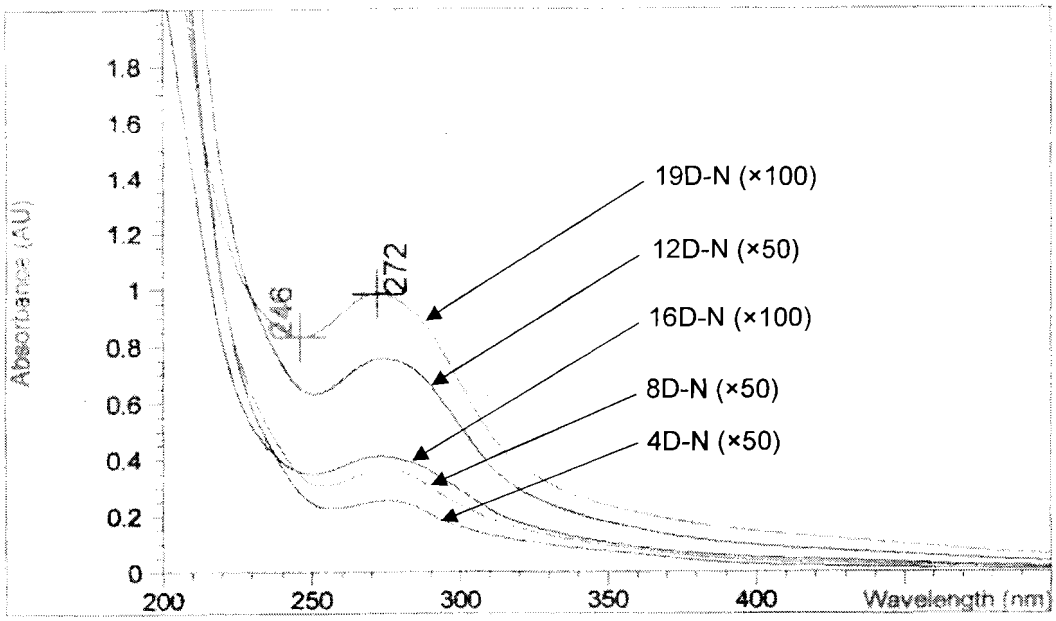


Figure 6. Visible and UV light absorption spectrum of unfiltered maple syrup samples.

Note. The numbers in parentheses are the dilution ratios.

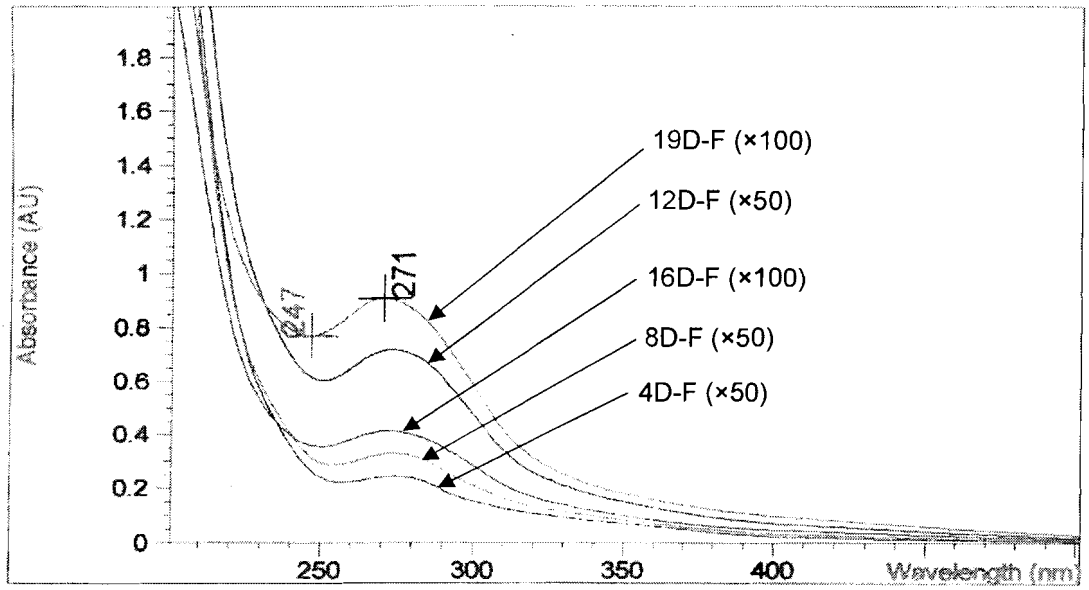


Figure 7. Visible and UV light absorption spectrum of filtered maple syrup samples.

Note. The numbers in parentheses are the dilution ratios.

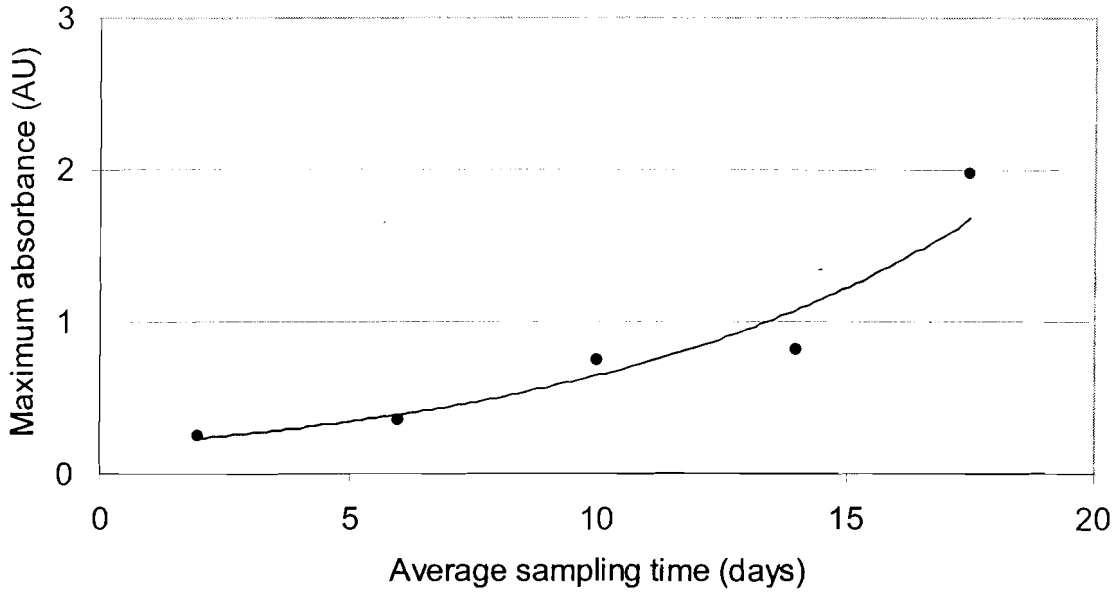


Figure 8. Chronological change of maximum absorbance of unfiltered maple syrup samples.

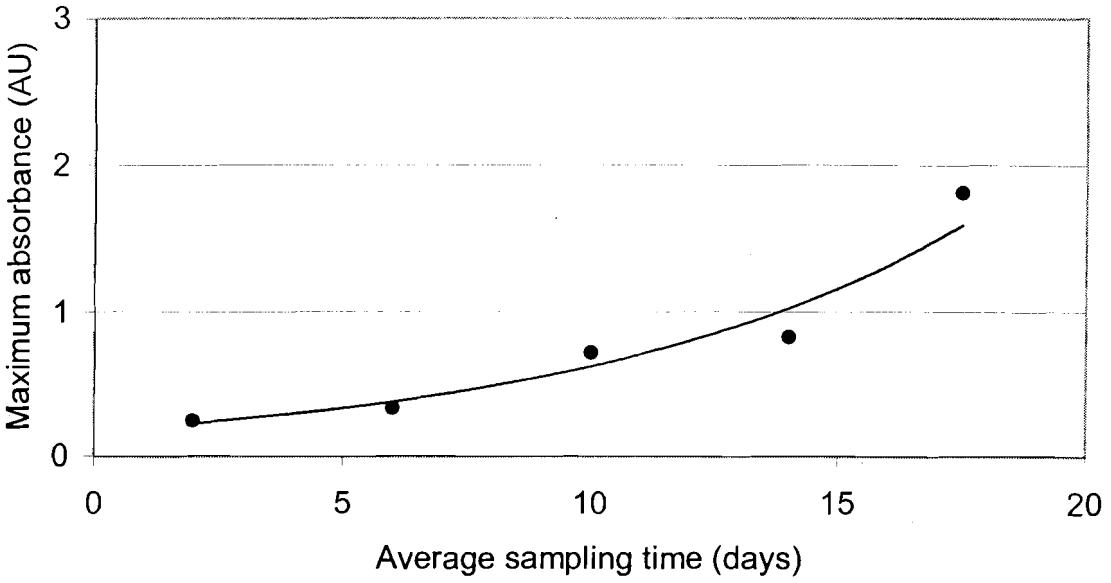


Figure 9. Chronological change of maximum absorbance of filtered maple syrup samples.

Mineral Analysis

From the standard curve of each mineral analysis, the correlation (R-squared value) for potassium (K^+), calcium (Ca^{2+}), and Iron (Fe^{2+}) was obtained as 0.9976, 0.9992, and 0.9692, respectively. These values represent the accurate data analysis. The standard curve of K^+ is shown in Figure 10 as an example. The bar charts in Figure 11, 12, and 13 shows the concentration of K^+, Ca^{2+}, and Fe^{2+} of filtered and unfiltered maple syrup samples.

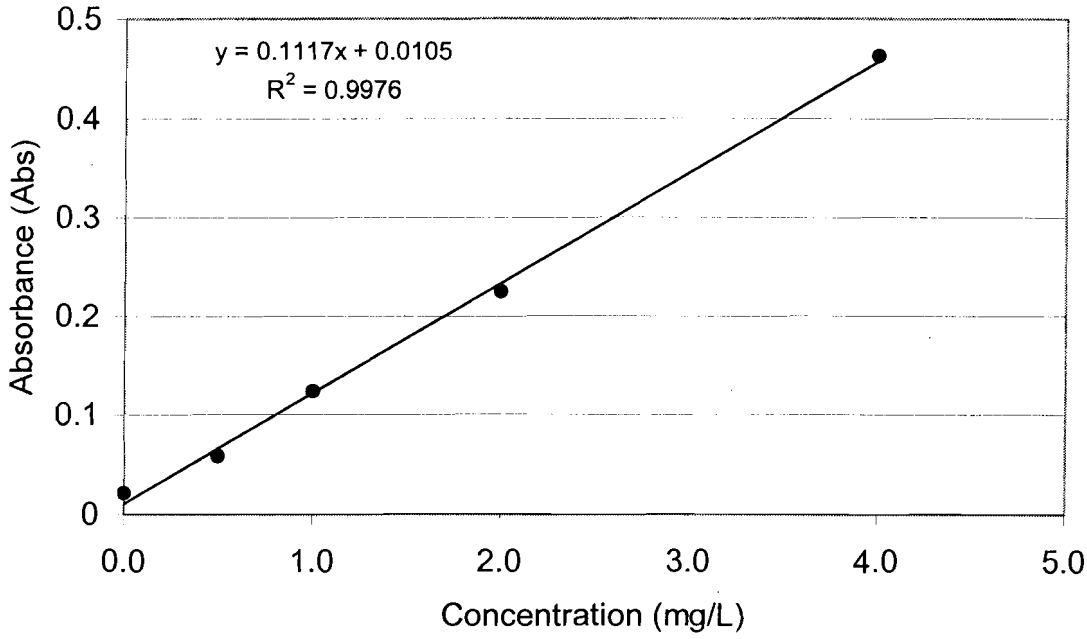


Figure 10. Standard curve of potassium.

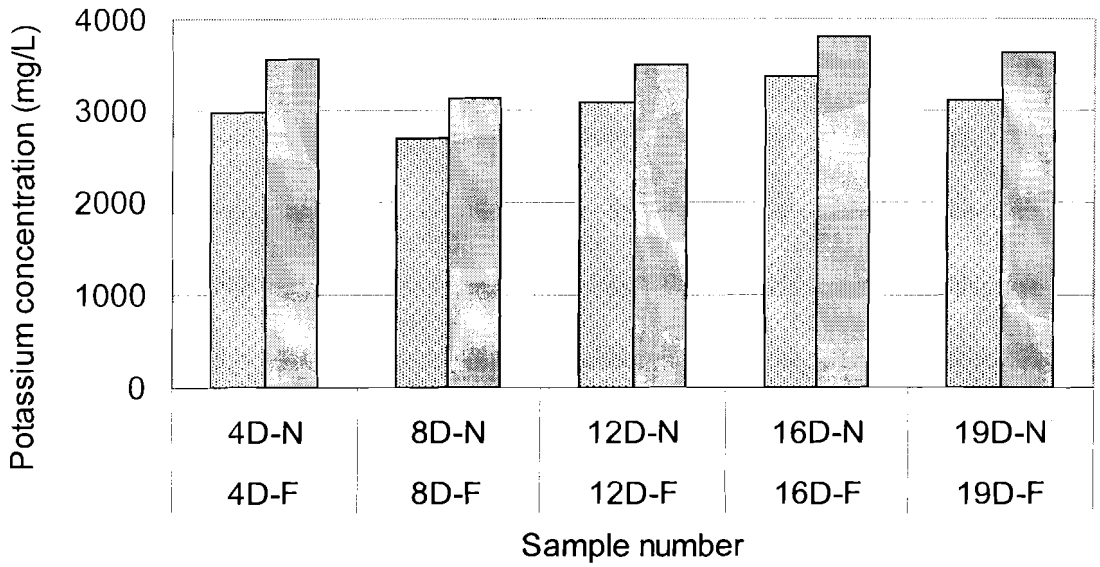


Figure 11. Potassium concentration of unfiltered and filtered maple syrup samples.

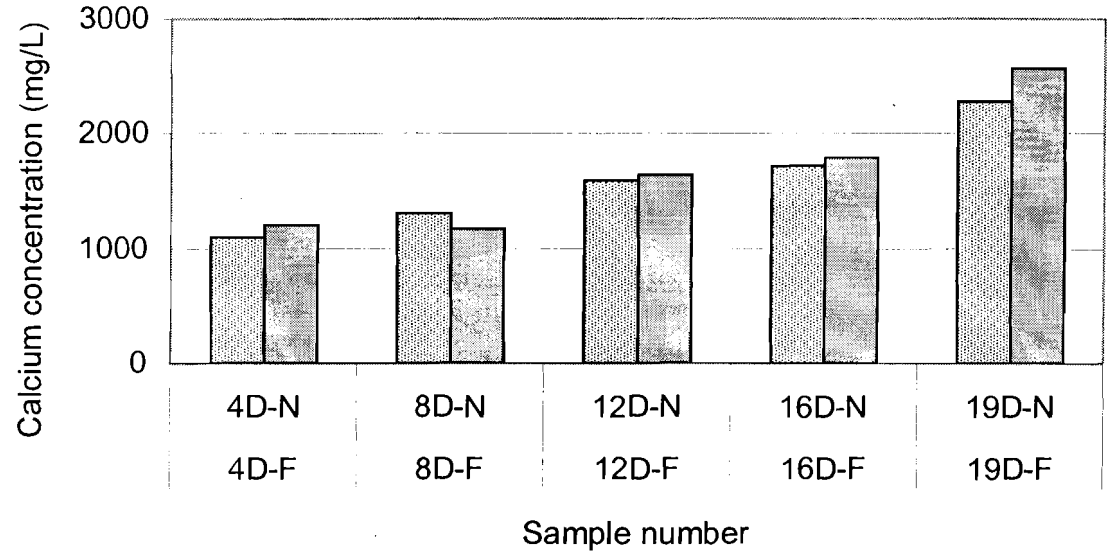


Figure 12. Calcium concentration of unfiltered and filtered maple syrup samples.

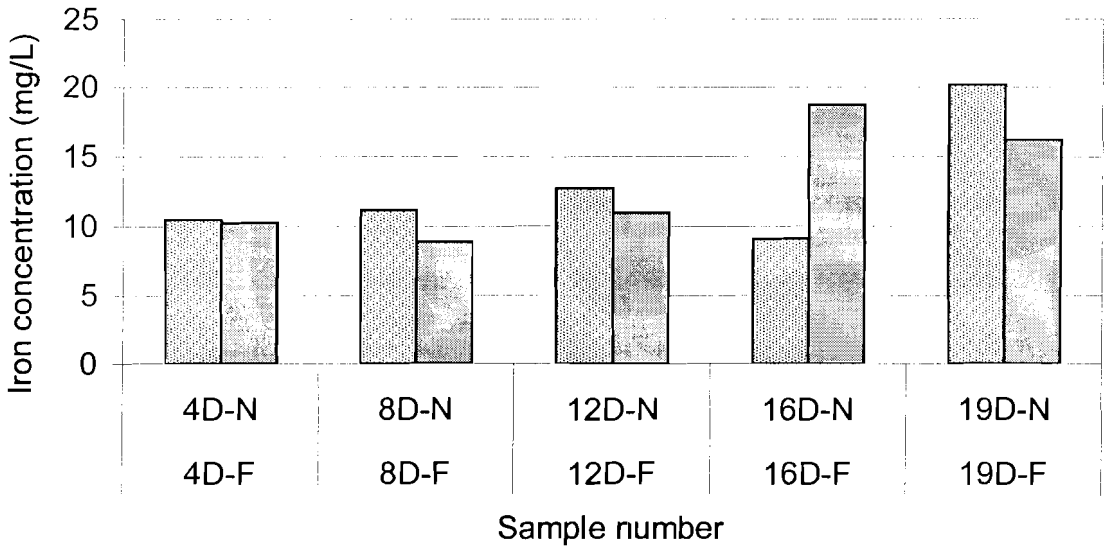


Figure 13. Iron concentration of unfiltered and filtered maple syrup samples.

By comparing the filtered samples, the K^+ concentration did not change significantly, and it seemed to keep the same level through the whole season. The Ca^{2+} concentration, on the other hand, showed clear elevation as the season progressed. The Fe^{2+} concentration did not show any clear tendency in the change of concentration through the season although a slight tendency of elevation was observed. This result indicates that the seasonal effects such as temperature, weather, and other environmental influences affected the metabolisms of maple trees, and more Ca^{2+} and possibly more Fe^{2+} were contained in the maple sap; however, potassium concentration would not be strongly affected by seasonal effects. The average concentrations of K^+, Ca^{2+}, and Fe^{2+} in filtered samples from this investigation were 3526 ± 17 mg/L, 1676 ± 23 mg/L, and 13 ± 2 mg/L, respectively. According to the data obtained by Stuckel and Low (1996), the average concentrations of K^+ and Ca^{2+} in the maple syrup produced in Wisconsin are 2091 ± 385 mg/L and 811 ± 310 mg/L, respectively. Robinson et al. (1989) measured the Fe^{2+} concentration of maple syrup produced in eastern Canada. The average Fe^{2+}

concentration was 3.71 mg/L (ranging from 0.41 to 44.01 mg/L). By comparing the data obtained in this experiment to the data in the literature, the concentration of all minerals measured in this experiment was relatively higher than the literature values. One reason to explain this difference is that since the average values included the latest seasonal maple syrup samples (19D-N and 19D-F), the values became potentially higher than the literature values. The lowest values among samples in filtered samples of K^+, Ca^{2+}, and Fe^{2+} were 3141 ± 17 mg/L, 1177 ± 17 mg/L, and 9 ± 1 mg/L, respectively. They were reasonably within the range of concentrations of maple syrup products found in the literature, except K^+ concentration, which was, however, quite close to the range.

The average concentrations of K^+, Ca^{2+}, and Fe^{2+} in unfiltered samples from this study were 3047 ± 146 mg/L, 1599 ± 12 mg/L, and 13 ± 1 mg/L, respectively. By comparing the unfiltered samples to the filtered samples, the concentration of K^+ in unfiltered samples was visibly lower than those in filtered samples. This tendency was slightly observed in Ca^{2+} concentration. The concentration of Fe^{2+} in unfiltered samples was, however, slightly higher than those in filtered samples. It was expected that the mineral concentration of filtered samples would be the same or lower than unfiltered samples since some minerals such as calcium are removed as sugar sand by filtration process. Some types of additional minerals; however, could have been introduced into maple syrup from the pasteurization, filtration, and packaging process.

From the result, there is a possibility that Ca^{2+} or other minerals which increase in concentration when the season progresses are also related to the darkening of maple syrup as well as phenolic compounds. On the nutrition side, it will be necessary to conduct other research to make sure those minerals are available for the human body as

nutrients; however, it is a quite interesting fact that some types of minerals are found in higher concentration in the darker (or lower graded) maple syrup.

Sugar Analysis

One of the chromatograms of standard solution (2.0% fructose, glucose, and sucrose) obtained from HPLC is shown in Figure 14. The first peak represented the primary injected sample (2.0% standard sugar solution), which is called a solvent front, and the second, third, and fourth peaks represented fructose, glucose, and sucrose, respectively, which were separated from the injected original solution. This order was the same in all other standard solutions, though the peak area varied according to the sugar concentration. The retention time for fructose, glucose, and sucrose in the standard solutions were recorded and used for distinguishing the type of sugar from the peak concentration in the chromatogram obtained from the maple syrup samples. From the standard curve of each sugar analysis, the correlation (R-squared value) for fructose, glucose, and sucrose was obtained as 0.9986, 0.9987, and 0.9992, respectively. These values represent the accurate data analysis. The standard curve of sucrose is shown in Figure 15 as an example. The chromatogram of 20 times diluted maple syrup sample (4D-N) is shown in Figure 16 as the example of the chromatogram used for determining the concentration of fructose and glucose, and the chromatogram of 40 times diluted maple syrup sample (4D-N) is shown in Figure 17 as the example for determining the concentration of sucrose. The concentration of fructose, glucose, and sucrose is shown in Table 9. The average concentration of fructose, glucose, and sucrose of filtered maple syrup samples was 0.66 ± 0.37%, 2.09 ± 0.71%, and 65.67 ± 3.93%, respectively. The average concentration of fructose, glucose, and sucrose in unfiltered maple syrup samples

was 0.62 ± 0.49%, 2.04 ± 0.81%, and 65.23 ± 3.61%, respectively. There was no important difference observed between filtered and unfiltered samples in each sugar concentration. According to the data obtained by Stuckel and Low (1996), the concentration of fructose, glucose, and sucrose in maple syrup samples produced in Wisconsin was 0.22 ± 0.51%, 0.27 ± 0.22%, and 69.9 ± 1.2%, respectively. By comparing the data obtained from this experiment to the literature data, fructose and glucose concentration was slightly higher but sucrose concentration was slightly lower than the literature data of the Wisconsin maple syrup. This data indicates the possibility that the more inversion of sucrose may have occurred in the trees before sap was taken. Stuckel and Low (1996) also reported that the concentration of fructose, glucose, and sucrose in the maple syrup produced in four states in the United States and two provinces in Canada ranged from 0.00 to 4.00%, 0.00 to 9.60%, and 51.7 to 75.6%. These data indicate that the compositions of sugars in maple syrup are varied significantly according to the producing area.

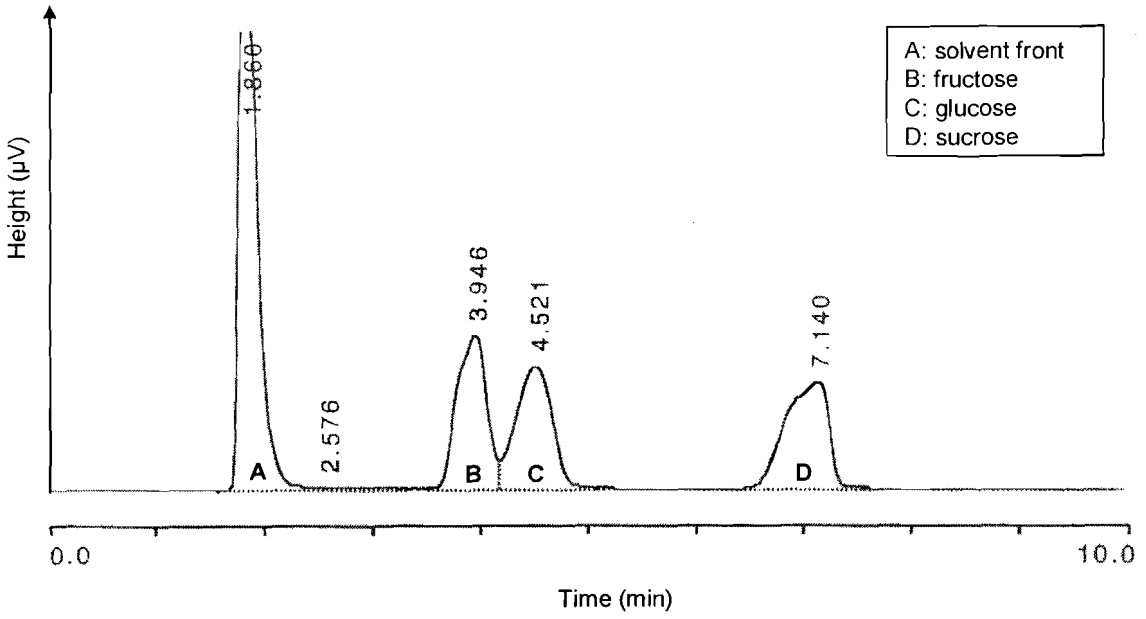


Figure 14. Chromatogram of peak retention time and area for solvent front, fructose, glucose, and sucrose, for 2.0% standard solution.

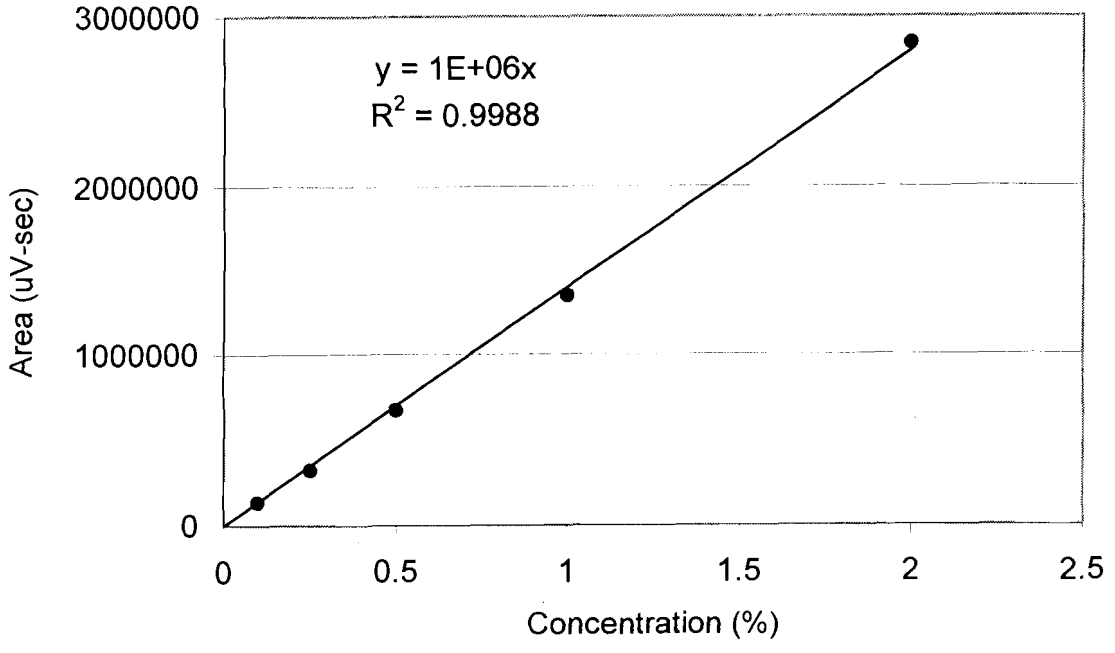


Figure 15. Standard curve of sucrose.

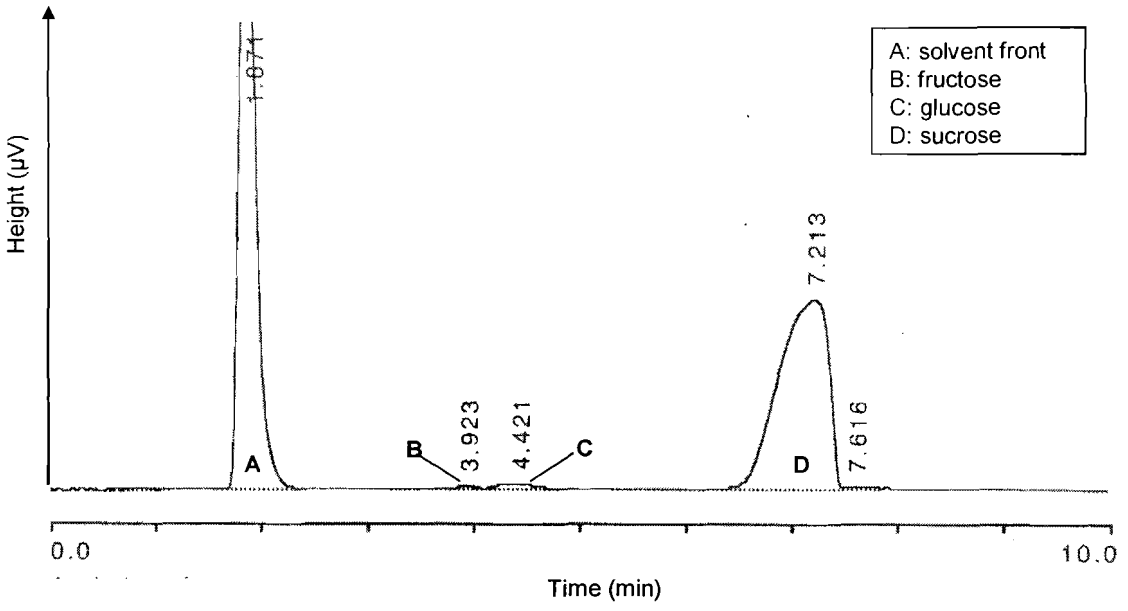


Figure 16. Chromatogram of peak retention time and area for solvent front, fructose, glucose, and sucrose, for the 20 times diluted maple syrup sample (4D-N).

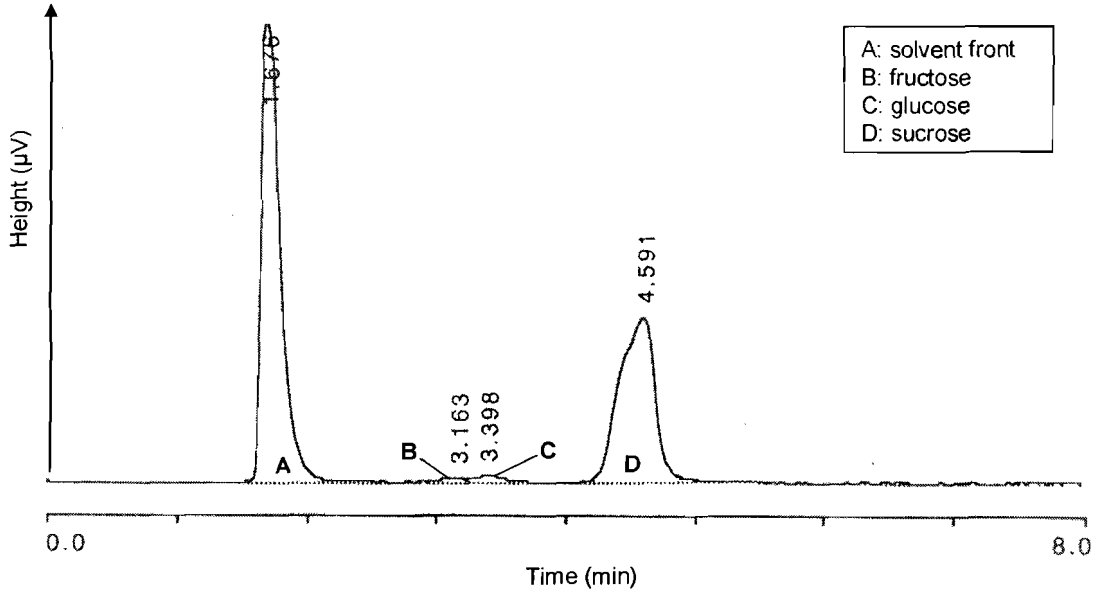


Figure 17. Chromatogram of peak retention time and area for solvent front, fructose, glucose, and sucrose, for the 40 times diluted maple syrup sample (4D-N).

Table 9

Ratio of Fructose, Glucose, and Sucrose Concentration in Unfiltered and Filtered Maple Syrup Samples

Sample Number	Fructose (%)	Glucose (%)	Sucrose (%)
4D-N	0.31	1.24	65.94
8D-N	0.32	1.49	61.98
12D-N	0.55	1.99	68.81
16D-N	0.44	2.14	68.43
19D-N	1.47	3.33	61.00
4D-F	0.42	1.49	70.29
8D-F	0.42	1.48	62.26
12D-F	0.87	2.27	64.78
16D-F	0.38	1.97	69.27
19D-F	1.20	3.21	61.77

In terms of the seasonal effect, there was a slight tendency for the concentration of fructose and glucose to increase as the season progressed, while concentration of sucrose decreased. This change was especially significant in the latest seasonal samples (19D-N and 19D-F). This tendency corresponds to the fact that when the season progresses and the weather becomes warm, bacterial activity proceeds the decomposition of sucrose in the maple sap in maple trees, and the sucrose is converted into the invert sugars (glucose and fructose) (Driscoll, 1998). Driscoll (1998) also noted that more concentration of invert sugars in maple sap would result in a darker maple syrup product. This data supports why early seasonal maple sap is suited to be used to produce maple syrup in order to obtain lighter colored, or in other words, higher graded maple syrup.

Chapter V: Summary and Conclusion

Summarizing this research, five unfiltered maple syrup samples and five filtered maple syrup samples, which were separately produced from the maple sap collected five times during the nineteen day-period season (from March 17 to April 4 in 2004) by the syrup maker, Ocooch Mountain Acres, LLC. (Westby, Wisconsin), were used in order to analyze their physical properties (density, absorption spectrum, solid content, and water activity) and their chemical properties (pH, mineral contents and sugar contents).

The later the maple syrup production season is, the darker the color of the maple syrup products. According to the maple syrup grading system in the United States and Canada, the darker syrup produced in the later season is given lower grades than the lighter syrup produced in the earlier season. Interestingly, some consumers, however, preferred darker syrup to lighter syrup due to the perceived properties of darker maple syrup. There is also a possibility considered that if the syrup is filtered, some nutritional values are decreased.

Due to the previous reasons, it was expected that there were some differences in the physical and chemical properties among the different seasonal maple syrup products and between filtered and unfiltered maple syrup products. The primary findings of this research were summarized as follows:

1. The physical properties (density, absorption spectrum, solid content, and water activity) and the chemical properties (pH, mineral contents and sugar contents) of ten maple syrup samples were successfully measured by using the previously described methods. By comparing the values of pH, solid content, mineral contents, and sugar contents to the literature values, all of the values

were reasonable and found in the literature except K^+ concentration, which was, however, quite close to the range. All values of pH and solid content were in the range found in maple syrup produced in Wisconsin, except one pH value, which was still found in the range of Quebec maple syrup.

2. Seasonal effects: The result of visible and UV light absorption spectrum showed that the later the season the maple syrup sample was produced, the greater the maximum absorbance observed at a wavelength of 271nm to 275 nm. Although specific chemical compounds were not identified in this research, according to the literature, phenolic compounds were some of the possible compounds which are known as the influential factors of the color and flavor of maple syrup. The result of the mineral analysis showed that calcium concentration in maple syrup increased as the season progressed, and a slight increase in iron concentration was also observed. This result indicates that the warmer weather could affect the metabolisms of maple trees, and more calcium and iron exist in the sap later in the season. The result of the sugar analysis showed that fructose and glucose concentrations increased and sucrose concentration tended to decrease as the season progressed. This result corresponded to the fact that the amount of invert sugars affects the darkening of maple syrup. The pH, density, solid content, and water activity did not show any clear tendency in terms of seasonal effect, although 16D-N, 4D-F, and 16D-F showed higher pH, density, and solid content than the other samples.

3. Filtration effects: It was expected that the concentrations of minerals and sugars in filtered samples were lower than the concentrations in unfiltered samples due to the removal of those components by filtration; however, the numbers of the measurements of filtered samples which showed lower concentration of minerals and sugars than unfiltered samples were only 10 out of 30 measurements (33.3%). The details regarding the influence of filtration can not be discussed in this research; however, this data indicates that the filtration process used for producing the maple syrup samples did not seem to be a significant factor in influencing the concentrations of minerals and sugars.

Recommendations for Further Study

There are several suggestions which will provide more applicable data and will explore the properties of maple syrup.

1. Identify the types and concentrations of phenolic compounds contained in maple syrup by using known phenolic compounds as standard solutions for the analysis of visible and UV light absorption spectrum. It is expected that the specific types of phenolic compounds will provide the further approach to study the flavor and the browning of maple syrup.
2. Measure the other nutrients such as organic acids, vitamins, and amino acids in maple syrup in order to evaluate the seasonal effects and the effect of the filtration process on these nutrients.
3. Evaluate sensory characteristics such as aroma and flavor of different seasonal maple syrup samples of different seasons by conducting sensory evaluation with sensory panelists. It was found that maple syrup produced in different seasons showed different chemical properties including mineral, sugar contents, and possibly flavor compounds as well as color. These differences will in some degree affect the sensory characteristics of maple syrup. The comparison to commercial pancake syrup, which includes less than 2 % of pure maple syrup, will provide the preference of the taste of syrup among consumers.
4. Conduct the same or similar type of analysis by using the maple syrup produced from the same place but in different years in order to explore the seasonal effect of maple syrup in a certain period of time. It is desirable to record the environmental factors such as temperature, weather, and the compositions of air

and soil in order to discuss the influences of environmental factors on maple syrup properties.

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