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Chemical Composition, Food Safety and Quality Characteristics of Birch Syrup in Comparison to Maple Syrup

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CHEMICAL COMPOSITION, FOOD SAFETY, AND QUALITY CHARACTERISTICS OF BIRCH SYRUP IN COMPARISON TO MAPLE SYRUP

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An Abstract of the Thesis Presented in Partial
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Birch syrup has gained popularity in the market due to its distinctive flavor, natural origin, potential health benefits, and culinary versatility with a wide range of applications. Birch syrup has been attracting attention recently because it has been presented as a unique alternative to one of the most used sweeteners, maple syrup. Apart from the similar production process and artisanal appeal, both syrups have important distinctions such as flavor, chemical composition, and other characteristics that make them unique.

Because of its high consumption and economic impact, maple syrup has been extensively studied to check various factors and aspects such as chemical composition, physical properties, production processes, quality, safety, health benefits, and more. These studies have helped the FDA to establish standards to guarantee the integrity and safety of maple syrup in the market. On the other hand, birch syrup presents regulatory challenges since less research has been conducted on this syrup to establish safe processing methods and Brix level standards to ensure the safety of this product. Therefore, the chemical composition, physical properties, and safety characteristics of birch syrup were investigated and compared to those of maple syrup. Eight batches of blended, heated, and filtered birch and maple syrup samples were collected by the same producer in Temple, ME. Both syrups were analyzed for chemical and nutritive properties (sugars, organic acids, pH, phenolic compounds, minerals), physical properties and quality characteristics (Brix and water activity levels),

and microbial load (total aerobic bacteria, yeast and mold, and fungal inoculation studies). The birch syrup Brix levels (62.2-63.6 degrees Brix) were significantly lower ($p<0.001$) compared to maple syrup (66.4-66.8), although the birch syrup samples also had significantly ($p<0.001$) lower water activity levels (0.800-0.810) compared to the maple syrup samples (~0.850). As expected, sucrose (647.31 ± 15.25 mg/g) was the predominant sugar in maple syrup samples, and birch syrup samples contained mostly fructose (326.53 ± 7.79 mg/g), glucose (241.53 ± 23.63 mg/g), and a much lower sucrose (19.04 ± 14.56 mg/g) content than maple syrup. Because of the differences in sugar ratios, refractometers are most likely not an accurate measure of sugar density in birch syrup since refractometers are built to measure the degrees Brix based on sucrose, not glucose and fructose.

Malic acid was the main organic acid found in both syrups, with birch syrup (5.59 ± 0.68 mg/g) having a higher concentration than maple syrup (2.43 ± 0.03 mg/g). Birch syrup also had more citric and tartaric acid levels, which may contribute to its significantly ($p<0.001$) lower pH (4.62-4.64) levels compared to the maple syrup samples (6.59-6.60) and also contribute to its tangier flavor.

Furthermore, birch syrup had an overall higher mineral content (calcium, potassium, and magnesium) compared to maple syrup, and manganese was also detected in birch syrup (362.2 ppm). These differences in organic acids and mineral content could be due to the tree species, tree physiological differences, and/or production differences since birch sap is boiled for a longer duration than maple sap. Unfortunately, phenolic compounds were not able to be detected in the birch or syrup samples, even though the standards were detected without any issues.

No aerobic bacteria, yeast, or mold colonies were detected among the birch or maple syrup samples, which indicates the hot fill temperatures (85-87.7℃) were adequate to control microbial growth. Based on the fungal inoculation studies, the hot fill temperatures of 85℃-87.7℃ for four minutes appeared to be adequate to also inactivate the inoculated fungal spores of *Eurotium sp* (*Aspergillus* representing the asexual stage), *Penicillium brevicompactum*, and *Rhodotorula mucilaginosa* when the contamination level was 100 spores in 500 µl for both syrups.

Future work could further investigate seasonal variations, producer geography, soil differences, and climate changes to determine if these variables can affect the Brix, pH, water activity levels, sugar, and mineral concentrations in other birch syrup samples. With more data, regulatory agencies can move forward to determine how to best assess an adequate Brix level range and processing parameters to begin creating regulatory definitions to best regulate the growing birch syrup industry.

DEDICATION

I dedicate this thesis to my adorable, loving parents. Thank you for being the best parents, best friends, and guardian angels ever; I love you with all my heart.

To the most generous and kind people I have ever met, my Uncle Boubacar and Aunties Adiara and Mama, for the support, their pieces of advice, encouragement, and guidance.

Moreover, I dedicate this research paper to my favorite human being, my husband, for giving me a sense of perseverance to continue, and for the love, support, and sacrifices.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1 LITERATURE REVIEW

1.1. Maple Syrup

1.1.1. Introduction

Maple syrup is a popular natural sweetener that many people enjoy during breakfast and/or while cooking. Maple trees from the *Acer* species are tapped and the tree sap is collected in the spring, boiled down, and concentrated to produce syrup (Ball, 2007). The *Aceraceae* or maple family; includes approximately 13 *Acer* species responsible for maple sap production. When it comes to syrup production, *Acer saccharum* (sugar maple) and *Acer nigrum* (black maple) are more commonly tapped and contribute to approximately 75% of the total maple syrup production due to their higher sugar concentration compared to other maple species, such as *Acer rubrum* (red maple) and *Acer saccharinum* (silver maple) (Doner, 2003). Maple syrup is more commonly produced in the northeastern part of North America since the specific soil, climate, and geological conditions of the region support maple trees and sap yield (Ball, 2007). Maple syrup is an important commodity for Maine, other states in New England, and in Canada. The highest-producing states in the United States include Vermont, New York, Maine, Wisconsin, and New Hampshire. A smaller amount of maple syrup is also produced as far south as Ohio and West Virginia. From 1995 to 2004, the states of Vermont, New York, and Maine together contributed to about 60% to 70% of the total maple syrup produced in the United States. However, maple syrup production can fluctuate each year, as it is quite susceptible to environmental conditions (Duchesne and Houle, 2014). However, Vermont, New York, and Maine have remained the primary maple syrup producers in the United States (See Table 1) in recent years. Vermont has consistently held the lead in maple syrup production, which showed an

important increase in production from 2018 to 2022. In 2022, Vermont accounted for 50.7% of the total U.S. maple syrup production. New York, as the second-largest producer, has maintained a consistent production level throughout the years, contributing 16.8% to the total U.S. production in 2022. Although Maine's production has displayed some fluctuations, its overall contribution has remained relatively stable, comprising 13.4% of the total U.S. production in 2022. The overall U.S. maple syrup production increased from 4,199,000 gallons in 2018 to 5,028,000 gallons in 2022, reflecting a combined increase in production trend among the maple-producing states (USDA, 2022). It is important to note that despite being a significant maple syrup producer domestically, the United States is also considered the largest importer of maple products (See Table 2). In 2022, U.S. imports represented about 49.8% of all maple sugar and maple syrup imported globally. This trend could be attributed to the possibility that the demand for maple syrup in the United States has surpassed domestic supply, particularly during peak demand periods like the maple syrup season, or due to increased consumer interest in products derived from maple (Agriculture and Agri-Food Canada, 2023). Because of its distinctive flavor, the limited geographical production, and the associated high production expenses, maple syrup is worth an estimated $126 million every year just in North America alone (Perkins et al., 2009).

Maple syrup contains many nutrients such as phenolic compounds, minerals, pyrazines, phytohormones, organic acids, amino acids, and vitamins, and also sucrose and other sugars, including glucose and fructose. Maple syrup’s bioactive substances have been researched since they are reported to possibly improve health by providing antioxidant properties and hindering cell proliferation and mutations (Ramadan et al., 2021). The

indigenous Americans first recognized the nutritional value and sweetness of pure maple syrup (Mower, 2004). Many researchers have since observed and confirmed that maple syrup possesses a higher nutritional value compared to other commonly used sweeteners. In 1996, Stuckel and Low investigated the chemical and nutritive composition of 80 maple syrup samples. They mostly focused on the sugars (glucose, fructose, sucrose), organic acids (malic and fumaric acid), and minerals (calcium, magnesium, and potassium) in the syrup samples. All eighty samples contained these compounds, but the quantities were different depending on the region of production. During syrup production, a mixture of natural phenolics (found in the sap) and formed compounds (pyrazine, carbonyl, and others) develop in maple syrup during the evaporation process (Ball, 2007). Maple syrup goes through extensive heating to convert sap into syrup, a process that naturally leads to the presence of phenolics from the sap and additional non-natural compounds resulting from chemical reactions during production (Li and Seeram, 2010). For example, Li and Seeram (2011) proposed a new phenolic compound, Quebecol, that appears to be formed through the chemical reactions during maple syrup production, since LC-MS results showed that Quebecol was not found in the initial maple sap.

Phenolics have drawn favorable attention due to their possible contributions in preventing diseases and promoting human health (Shahidi and Ho, 2005). It was reported that extracts of maple syrup enriched with phenolic compounds displayed inhibitory effects on the proliferation of human lung, colorectal, prostate, brain, and breast tumor cell lines (Legault et al., 2010).

Table 1: United States maple syrup production by state (gallons)

	2018	2019	2020	2021	2022	2022 % Share
Vermont	1,940,000	2,070,000	1,950,000	1,750,000	2,550,000	**50.70%**
New York	806,000	820,000	804,000	647,000	845,000	**16.80%**
Maine	539,000	520,000	590,000	514,000	672,000	**13.40%**
Wisconsin	225,000	270,000	265,000	365,000	440,000	8.80%
Michigan	165,000	195,000	170,000	150,000	190,000	3.80%
Pennsylvania	142,000	157,000	178,000	168,000	164,000	3.30%
New Hampshire	163,000	148,000	154,000	127,000	167,000	3.30%
Others	219,000	0	0	0	0	0.00%
United States	4,199,000	4,180,000	4,111,000	3,721,000	5,028,000	**100.00%**

Source: Adapted from Statistical overview of the Canadian maple industry (2022).

Table 2: Top importers of maple sugar and maple syrup — by value (thousands of Canadian dollars)

	2018	2019	2020	2021	2022	2022 % Share
United States	**250,537**	**266,617**	**304,500**	**348,758**	**381,260**	**49.8%**
Netherlands	19,659	11,574	22,114	25,482	63,165	8.3%
Germany	36,683	37,605	44,019	50,590	41,132	5.4%
France	16,349	23,512	26,608	31,182	38,087	5.0%
United Kingdom	25,264	31,417	37,347	38,645	37,506	4.9%
Japan	27,428	28,783	28,825	37,013	35,266	4.6%
Australia	19,985	22,021	27,291	25,708	28,349	3.7%
Canada [1]	14,780	14,709	15,495	11,011	16,937	2.2%
Denmark	8,529	8,469	10,422	11,322	11,606	1.5%
South Korea	6,137	5,276	9,139	11,913	11,293	1.5%
Italy	5,772	8,274	10,112	13,663	10,268	1.3%

Table 2 Cont. Top importers of maple sugar and maple syrup — by value (thousands of Canadian dollars)

Poland	**6,787**	**7,316**	**7,063**	**8,218**	**8,225**	**1.1%**
Switzerland	5,305	5,409	7,494	7,434	7,858	1.0%
Ireland	3,259	3,521	6,115	5,921	6,464	0.8%
Israel	2,796	2,773	4,686	6,396	5,970	0.8%
Others	44,865	48,439	61,120	69,727	62,040	8.1%
Total	494,135	525,715	622,350	702,983	765,426	100.0%

Source: Adapted from Statistical overview of the Canadian maple industry (2022).

1.1.2. History

The late winter maple season (early February to late April) was eagerly awaited because it marked the arrival of spring (Lagace et al., 2015). This season was referred to as the "sugaring season" because most of the collected maple sap was condensed into maple sugar rather than syrup. Although the exact timing and specific method used for the initial production of maple syrup is still unknown, by the mid-1600s, European newcomers and the indigenous people of North America both were collecting maple sap and transforming it into maple syrup through boiling. This tradition led to the establishment of a new spring industry (Perkins et al., 2022). The materials used for the boiling and evaporation process of sap have evolved and become more advanced over time. However, the necessity of condensing sap by applying heat to obtain the desired color and caramelization has remained consistent. One innovation that led to a better quality of the final product was the introduction of multiple kettles. This technique included the transfer of partially concentrated sap from one kettle to another as the sugar content progressively increased. As a result of this process, the boiling time needed for sugar concentration was lessened and a lighter-colored syrup was developed (Perkins et al.,2022). During the 1900s, the

adulteration of pure maple syrup was an issue caused by syrup blenders who were selling heavy quantities of cane and corn syrup mixed with small amounts of maple syrup, which was a concern for maple producers. These blenders falsely labeled and sold this cheaply produced mixture as pure maple syrup, which caused a competitive handicap. In 1906, these concerns were addressed by the introduction of the Pure Food and Drug Act, which paved the way for the creation of the Food and Drug Administration. This legislation assigned rigorous regulations about the accurate labeling of ingredients, forcing syrup blenders to truthfully declare that their syrups contained both cane or corn syrup and maple syrup. Although the act did prevent some blenders from false advertising, it did not entirely put a stop to the blending of maple syrup with other syrups (Perkins et al., 2022).

1.1.3. Production Process

Maple Sap is a dilute mixture containing water (95-99%) and sugar (1-5%), with small quantities of additional elements such as organic acids, proteins, phenolic compounds, amino acids, and phenolic compounds (Perkins et al., 2009). The production of maple syrup mainly consists of evaporating the excess water in the sap, resulting in the concentration of the sap solids, primarily composed of sugars. The production process can be divided into two processing steps: 1) sap collection and 2) syrup production.

In maple sap production, maple producers collect the sap by inserting a tap (spile) into the tree to minimize tap hole damage and prevent sap microbial contamination. Microorganisms are the primary factor in both tree health issues and poor sap quality. In the early 1900s, and even today, some small syrup producers still use spiles and buckets for sap collection. Nowadays, larger syrup producers prefer gravity-fed plastic tubing and pipelines, which tremendously help in reducing sap collecting time and production costs (Figure 1). In certain cases, vacuum pumps are incorporated to facilitate sap flow by

reducing the pressure within the pipeline system (Doner, 2003). The season of maple sap collection typically starts from mid-February and continues until mid-April. As described by Japerson (2005), the ideal temperature for sap flow is around 4℃ during the day and roughly -6℃ during nighttime (Takano, 2006). Therefore, the amount of sap collected, and subsequent maple syrup production are both highly influenced by temperature, weather, and other environmental factors. This explains the inconsistency in production every year. Another crucial step in the production of high-quality maple products involves proper sap storage until it is ready for processing and maintaining a constant sap supply for the evaporator (Lawrence and Martin, 1993).

Once the sap is collected, it goes through the boiling process to evaporate water and achieve syrup concentration. It does take a fair amount of sap to produce syrup, as 40 gallons of sap is typically needed to produce 1 gallon of maple syrup. During the evaporation step, the sap reaches the desired density and develops its characteristic flavor and color due to the chemical reactions that occur during the heating process. The boiling duration plays a part in determining the final syrup color and flavor. Producing high-quality, light-colored syrup depends on early season sap and/or requires minimizing the evaporation time, whereas lengthened boiling results in a darker/lower-grade syrup (Koelling and Heiligrnann, 1996). Furthermore, while glucose and fructose are present in relatively small amounts in maple sap, they significantly influence the color and flavor of maple syrup. These sugars increase in concentration as the sugaring season progresses, primarily due to sucrose fermentation by microorganisms and tree metabolism in warmer weather. Consequently, maple syrup produced later in the season tends to have a darker color and a stronger flavor compared to syrup produced earlier in the season (Takano, 2006). The syrup is filtered, and the most effective filtering occurs while it is still hot,

generally between 85℃ to 87.7℃, which helps to remove any sediment. To prevent the risk of yeast or mold growth, and maintain the purity of the final product, the syrup should be hot-packed. Regardless of the type of container used, it is important to ensure that containers are clean and sanitized (Perrin, 1980). For instance, sanitation for smaller containers can be achieved by boiling them for at least 10 minutes in water. Additionally, it is suggested to quickly seal and invert hot-filled containers because filling syrup into any container, whether sterilized or not, may introduce contamination (Perrin, 1980). Several studies have explored the influence of bottle material on the quality of the final syrup. Fiore (2020) found that fancy glass bottles pose a higher risk of pathogen survival compared to plastic bottles due to factors such as their smaller volume and greater thermal conductivity. Conversely, plastic containers tended to cool more gradually, with larger plastic bottles spending a notably longer time in the temperature range lethal to pathogens that were studied. As a result, survival rates across all analyzed bacterial genera were reduced (Fiore, 2020).

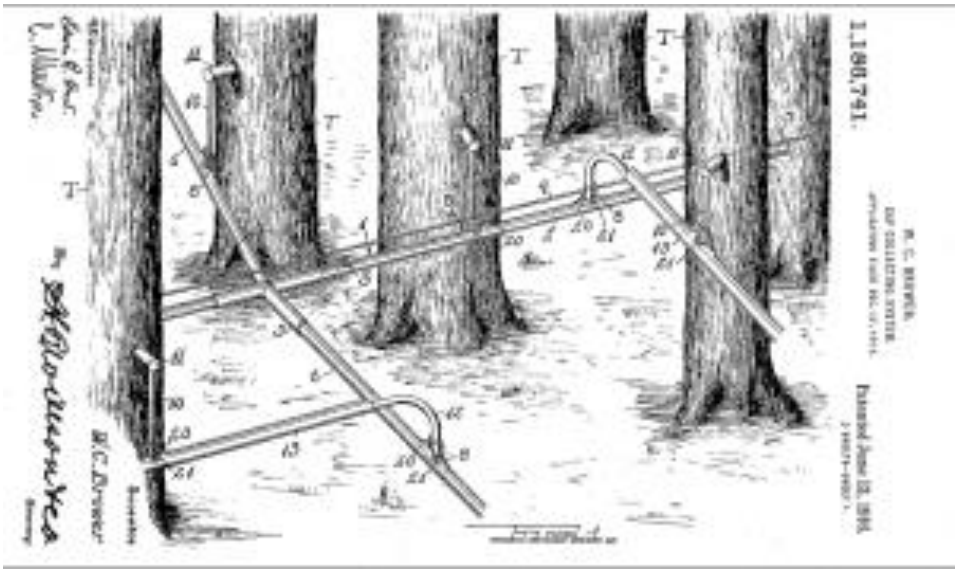

Figure 1. Gravity-fed plastic tubing and pipeline system; Thomas, M.M. (2021).

1.1.4. Grading system

It is widely known that Grade A maple syrup is available in different color and flavor classes. As the season progresses, the color of the syrup undergoes some changes; the light golden colored syrup with a light maple flavor is typically produced when the sap initially starts flowing, and later in the sugaring season, the sugar profile changes and develops a more amber to darker colored syrup as the season progresses along with a stronger, more robust maple flavor (Willits and Hills, 1976). The color and flavor changes come from the different sugar composition changes that occur during the sap season. At the beginning of the sap season, the predominant sugar is sucrose and due to the low amount of reducing sugars, produces a light-colored syrup. As the sap season progresses and daytime temperatures increase more during the day, microbes that come in contact with the sap after collection will convert sucrose into invert sugars (glucose and fructose). Due to the increased presence of these reducing sugars, the syrup will undergo Maillard browning reactions during the evaporation process, as the glucose and fructose will react with amino acids found naturally in the sap to develop a darker robust maple flavor (Perkins et al., 2009). Therefore, the final syrup color is a complicated process that is influenced by interrelated elements such as the sugar concentration, the pH level of the boiling sap, the types of sugars present in the sap, the length of boiling time, microbial activity, and even the external temperature conditions (Ubersicht and Franca, 1988).

Traditionally, the quality of maple syrup is evaluated by using a specific grading system that is set by the USDA; and the intensity of the syrup's color and flavor is an important part of this classification (Ubersicht and Franca, 1988, USDA, Saraiva et al., 2022). However, due to syrup adulteration issues in the past, a standard identity has been set for maple syrup by the FDA. Maple syrup, as defined by the U.S. Food and Drug Administration (FDA, 2018), is a natural sweetener produced from the concentrated sap of sugar maple trees (*Acer*). The FDA

mandates that maple syrup be produced solely from maple sap and adhere to precise criteria regarding density, color, and flavor.

The syrup classification system has two grades: grade A for quality syrup; while relegating the darker syrups to grade B, which is a processing grade syrup. Grade A syrup parameters must have a final Brix between 66-68.9℃, must be clear (no cloudiness or sediment), have a uniform color, a normal flavor intensity for its class, and be free from off-odors or flavors (USDA, 2015). Lighter-colored syrups are usually considered as higher quality and could command higher prices (Ubersicht and Franca, 1988). Although limited research has explored consumer preferences, studies have indicated that consumers tend to prefer darker maple syrups more than lighter ones due to their richness in maple flavor (Sendak, 1978, 1982). The dark-colored syrups are also enriched with more advantageous bioactive substances like polyphenols (Singh et al., 2014, Saraiva et al., 2022). According to Singh and colleagues (2014), autofluorescence and phenolic content exhibited notable negative correlations with light transmittance, suggesting that darker syrups typically contain higher levels of phenolics. The researchers further explained that this observation parallels findings in other plant foods, such as raspberries (Liu et al., 2002) and figs (Ercisli et al., 2012), where darker cultivars tend to possess greater antioxidant activity compared to lighter varieties (Singh et al., 2014).

Before 2015, the U.S. and Canada had their own grading systems (Nimalartne et al., 2020), and the same grade was sometimes named differently in other regions, such as the "Fancy" grade in Vermont, would be called "Light Amber" in New York and Maine, which was confusing to consumers. Maple producers and regulatory agencies in the U.S. and Canada have worked together to develop international standards for maple syrup color and flavor grades. This classification has four colors and flavor classes: Golden (delicate flavor), Amber (rich flavor), Dark (strong flavor), and Very Dark (robust flavor) (Saraiva et al., 2022; Table 3). The grades

can be checked visually against a color standard and the color intensity can be measured via an instrument to check on light transmittance. This measurement represents the amount of light that passes through the syrup in comparison to a reference substance, glycerol, that is set at one hundred percent light transmission. A greater transmittance value shows that the syrup is lighter in color; on the other hand, a lower transmittance occurs when the syrup is darker and thicker (Forest et al., 2020).

While the USDA grades are not obligatory, processors, producers, and handlers/packers who desire to label maple syrup with a specific U.S. grade must be forthright about what grade their syrup is according to the federal standards and ensure that the maple syrup aligns with prevailing standards set for that grade (AMS, 2015). The new grading system is used in almost all the U.S. states and Canada.

Table 3: Maple Syrup Grades

GRADE COLOR CLASS	**TASTE DESCRIPTOR**	**CULINARY SUGGESTIONS**	**PREVIOUSLY CALLED**
Golden	Delicate	A mild-tasting syrup to sweeten plain yogurt or top ice cream, pancakes, or waffles.	US Grade A Light Amber; Vermont Fancy; Canada No. 1 Extra Light
Amber	Rich	A full-bodied syrup for a slightly more intense maple taste.	US Grade A Medium/Dark Amber; Canada No. 1 Light/Medium
Dark	Robust	A strong-tasting syrup that works well in baking, vinaigrette, or barbecue sauce.	US Grade B; New York Extra Dark for Cooking; Canada No. 2 Amber
Very Dark	Strong	Sometimes described as “maple molasses.” For reprocessing or commercial cooking.	US Grade B; Commercial Grade; Extra Dark for Cooking; Canada No. 3 Dark

Source: USDA Standards for Grades of Maple Syrup, International Maple Syrup Institute (2015).

1.1.5. Physical properties (maple)

Foods are comprised of a complex mixture of biological and chemical constituents. All these components interact in complicated ways, and maple syrup is no exception. Plus, it is subject to seasonal and climate differences each year, and it is difficult to measure these compounds precisely. To determine the quality, chemical and nutritive value, and authenticity of pure maple syrup, many studies have investigated these characteristics.

1.1.5.1. Brix

Ensuring an accurate maple syrup density is important to ensure the product meets the standard identity for syrup and is a critical responsibility for producers. Maple syrup is more susceptible to fermentation when the density is too low (<66° Brix), and when the syrup is cooked too long and the density is too high (>69° Brix), the syrup becomes more vulnerable to sugar crystallization (Willits and Hills, 1976). Brix (or the % soluble solids) is the main unit of measurement used to estimate the density of cold maple syrup by gauging the sugar (sucrose) concentration, as one degree Brix represents approximately 1% sugar content. The appropriate density established for maple syrup to meet the FDA's standard of identity falls within the range of 66 to 68 degrees Brix, although the state or local regulations in certain areas may be stricter and stipulate a specific minimum Brix to be able to sell maple syrup (Canada Food Inspection Agency, 2018). To illustrate, New York state enforces a minimum Brix of 66% soluble solids (or 66° Brix) as their requirement, while Vermont is slightly stricter with a minimum of 66.9% sugar density. These rules help to protect consumers from receiving watered-down or adulterated syrups. According to data from Nimalaratne, Blackburn, and Lada (2020), the average Brix value from 33 maple syrup samples tested was 68.7 degrees Brix (or 68.7% sucrose) with a range from 61.6 to 70.2 degrees Brix, and significant differences were noted among the Brix values of syrups

collected from various geographical states and provinces in the northeastern region of North America (Table 4).

Table 4: The degrees Brix values of maple syrup from different provinces and states in North America

Province/State	Nova Scotia	New Brunswick	Ontario	Quebec	New York	Vermont	Massachusetts	New Hampshire
No of samples	7	3	3	3	8	3	3	3
Degrees Brix	68.5 ±1.08	67.5 ±1.02	68.7 ±2.20	67.3 ± 0.98	66.9 ±2.35	68.1 ±0.54	65.8 ±3.16	67.7 ±0.80

Note: Mean Brix value with standard deviation
Source: Nimalaratne, Blackburn, and Lada (2020)

1.1.5.2. Water Activity

Water activity is another important parameter to monitor in syrups because it measures the potential shelf life and the ability to limit microbial growth. According to Brunauer et al. (1938), water activity is defined as “the proportion of water vapor pressure in a substance compared to pure water vapor pressure under normal conditions". In other words, water activity helps to measure the quantity of free water present within the syrup that may be available for microbial growth. Bacteria usually need a high water activity level in foods to persist and grow compared to fungi (molds and yeasts). Most pathogenic bacteria don't grow at water activity levels <0. 91, but most molds and yeasts can grow at lower water activity levels < 0.85 (Jay, 1996). Some xerophilic (dry-loving) molds and osmophilic (preferring high osmotic pressure) yeasts have been found to grow at very low water activity levels of 0. 65 or sometimes lower (Silva, 2015, Table 5).

Table 5: Water Activity and Microbial Growth

Microorganisms	water Activity
Most bacteria, some yeasts, pathogenic and spoilage organisms	>0.95
Most cocci, some molds, salmonella, lactic acid bacteria	0.91-0.95
Most yeast, mycotoxin-producing molds	0.87-0.90
Staphylococcus aureus may grow	>0.86
Most molds, no pathogenic bacteria	0.80-0.87
Most halophilic bacteria	0.75-0.80
Xerophilic molds	0.65-0.75
Osmophilic yeasts	0.60-0.65
No growth	<0.65

Source: Adapted from Roos (2003)

Foods with a higher sugar concentration level typically have lower water activity levels, which usually corresponds to a higher density (Jay, 1996). Maple syrup producers commonly assume that packing syrup at a density lower than 66.0 degrees Brix is the main reason behind microbial contamination in maple syrup. However, fungi contamination can still take place in syrup if the heating temperatures are not high enough and/or held for long enough periods. Bacterial and fungal contamination in syrups are further discussed in Section 1.3.

1.1.6. Chemical and nutritive composition

The composition of maple syrup is influenced by many factors such as temperature, climate/environmental conditions and stresses, tree species, and soil conditions. Consequently, maple syrup does not have a fixed chemical composition; however, it tends to contain some core ingredients that include sucrose, water, small amounts of monosaccharides, and additional elements like organic acids, minerals, phenolics, and minerals (King and Morselli, 1983).

1.1.6.1. pH

The pH level plays a vital role in controlling and affecting the proliferation of microorganisms in food products (Vijayakumar et al., 2019). Although syrup products are

typically water activity-controlled products, researchers have still investigated the pH levels in maple syrup. Stuckel and Low (1996) obtained 80 pure maple syrup samples from Canada and the U.S. and found that the pH level of these samples ranged from 5.6 to 7.9, with an average value of 6.7, and that they did not detect any significant regional differences. Radu et al. (2022) determined the pH results were quite similar to Stuckel and Low's (1996) findings after evaluating a total of 21 maple syrup samples from the United States and Canada. The pH values recorded varied from 5.6 to 7.9. Robinson and colleagues (1989) conducted a similar study and tested maple syrup samples from Eastern Canada and their results showed an average pH level of 6.60, as well as a decline in pH levels as the maple season progressed. Singh et al. (2014) investigated the connection between pH levels and different grades of maple syrup. Their analysis of commercial maple syrup from Southern Ontario, comprising 35 samples, indicated minimal variation in pH values across the different grades of syrup, with no statistical differences observed. The pH values they noted were 7.1 for amber, 6.9 for dark, and 6.7 for very dark-grade maple syrup. Overall, research studies have shown that the pH level of maple syrup can range from 4.7 to as high as 8.7, with a typical average in the 6.3-6.8 range (Perkins et al., 2009).

1.1.6.2. Sugars

Multiple studies have shown that sugar is the primary nutritive component of maple syrup, accounting for approximately 98% of the dry weight of maple syrup. The main sugar found in maple syrup is sucrose along with traces of monosaccharides (fructose and glucose) and certain oligosaccharides. St-Pierre et al. (2014) observed maple syrup's predominant sugar was sucrose at 97%, followed by glucose and traces of fructose. Van den Berg et al. (2006) examined 55 different grades of maple syrup and found no clear link between grade and sugar concentration and that all the syrup grades had about the same fructose (0.1%-0.7%), glucose (0.4%-0.7%), sucrose (65.1%-67.1%), and total invert sugar (0.9%-1.2%) concentration. Similarly, Singh et al.

(2014) found no significant differences in glucose (0. 670-0. 810 g/L), fructose (0.088-0.255 g/L), and total reducing sugars (0.870-0.878 g/L) when comparing amber, dark and very dark maple syrup samples. However, after analyzing 32 Canadian pure maple syrup samples (six extra-light, seven light, seven medium, five amber, and seven dark) obtained from the Japanese market over a span of four years, Unno (2015) observed that glucose and fructose levels increased in the syrup as the color got darker along with an inverse relationship with sucrose levels throughout the season. The researcher elaborated that this phenomenon arose from the non-enzymatic process, commonly known as the Maillard browning reaction, during which reducing sugars react with specific components containing amino groups, resulting in the formation of brown polymeric compounds known as melanoidins (Unno, 2015). Given that the levels of amino groups showed minimal variation across the different maple syrup grades, disparities in the initial reducing sugar content in the maple sap likely had the most significant impact on the Maillard reaction (Lagacé et al., 2002; Clement et al., 2010; Unno, 2015).

1.1.6.3. Organic acids

Organic acids play an important role in contributing to the flavor of foods and can impact the taste and aroma. According to Legua et al. (2016), these acids can emulate synergistic antioxidants, consequently enhancing antioxidant effects. Maple sap does not have much acidity even though it contains low levels of organic acids like citric, fumaric, oxalic, malic, aconitic, and succinic acids (Lagace et al., 2015). One study by Stuckel and Low (1996) evaluated the types of organic acids in 80 maple syrup samples using HPLC. The major organic acid identified was malic acid, which comprised 0.5% of the syrup weight, along with trace amounts of succinic acid, citric acid, and fumaric acid. Furthermore, Stuckel and Low (1996) suggested that factors such as natural variations between maple trees and the location of the sugarbush could influence the concentration of certain organic acids, which can explain the variations in the malic acid

concentration between syrup samples. The transition from sap to syrup during the production process is a significant factor influencing pH and chemical composition. Robinson et al. (1989) suggested that the pH increase observed from sap to syrup can be attributed to the removal or conversion of organic acids during the evaporation process.

1.1.6.4. Phenolic compounds

The main phytochemicals in maple sap are phenolic compounds, and the profile of these compounds depends on several factors, including the tree's location and physiology, seasonal weather variations, microbial contaminants, and soil type (Lagace et al., 2015). When the sap is boiled down into syrup, these phenolics go through important changes such as degradation and oxidation. They help contribute to maple syrup's signature color and flavor, and in the last couple of years, researchers have paid considerable attention to their health benefits, particularly their antimutagenic, anticancer, and antidiabetic properties (Budrat and Shotipruk, 2008). Many studies have demonstrated that maple syrup has over 40 different phenolic compounds, which help contribute to its unique flavors (Potter and Fagerson, 1992; Lada and Nelson, 2013). In a study conducted by Kermasha et al. (1995), ten different phenolic compounds were found including homovanillic acid, p-coumaric acid, syringic acid, sinapic acid, coniferyl alcohol, coniferylaldehyde, vanillin, ferulic acid, syringaldehyde, and vanillic acid, in both the syrup and the sap. Their research also highlighted the impact of the sap collection time on phenolic compounds with later in the season having higher concentrations of phenolics. In addition, it was noted that different producers had varying levels of phenolics in their syrups, suggesting that many factors such as the climate, soil, and even the collecting and processing techniques of maple sap may change the flavor and phenolic acid profile of the final product (Kermasha et al., 1995).

1.1.6.5. Minerals

Pure, filtered maple syrup has metal ions and minerals that offer nutritional value, as indicated by several studies (Perkins et al., 2009; Singh et al., 2014). The most abundant minerals that are present in maple syrup include calcium, potassium, and magnesium, as well as manganese, phosphorus, and zinc. A study by Stuckel and Low (1996) assessed the concentrations of potassium, magnesium, and calcium in maple syrups from producers in Canada (Ontario, Quebec) and the United States (Vermont, Massachusetts, Wisconsin, and New Hampshire). Potassium had the widest concentration range (1,005 to 2,990 ppm) compared to calcium (266 to 1,702 ppm) and magnesium (10 to 380 ppm). Another study found that the average potassium, magnesium, and calcium levels in maple syrup were similar to the previously reported concentrations, followed by low levels of phosphorus, zinc, and manganese (Nimalaratne et al., 2020). They also noticed that syrup samples from Quebec had the lowest mineral amounts of the Canadian provinces, while samples from New Brunswick and Nova Scotia had higher levels of minerals, especially in magnesium and manganese. This suggests that the mineral content of maple syrup could possibly identify the origin of syrup samples, which is a hypothesis supported by other studies. For instance, Robinson et al. (1989) analyzed 27 sap and syrup samples from different provinces in Canada (Nova Scotia, New Brunswick, and Quebec) and found noticeable differences in syrup copper levels, as values ranged between 0.09 and 8.28 μg/mL. The researchers reported that the syrup's collection location had a significant effect on the sap composition as seen in the variation of iron, lead, copper, and zinc levels. Stuckel and Low (1996) also proposed that analyzing the levels of calcium, potassium, and magnesium, which are the predominant minerals found in maple syrup, may help in determining the geographical source of maple syrup.

It is crucial to highlight that the absorption of minerals by the human body can have both positive and negative effects. There are concerns regarding the optimal intake of manganese for maintaining optimal health (typically 2-5 mg/day for adults), which has led various countries, governmental agencies, and health organizations worldwide to establish dietary guidelines (Finley and Davis, 1999; Freeland-Graves et al., 2016). In the United States, the Institute of Medicine has set the upper tolerable level for manganese at 11 mg/day. Consuming manganese above this threshold raises the risk of manganese toxicity, which has been linked to impaired neurobehavioral function in children and Parkinsonism in adults (Freeland-Graves et al., 2016).

1.2. Birch Syrup

1.2.1. Introduction

Birch syrup is another tree-based, natural syrup obtained through the concentration of birch sap extracted from birch (*Betula sp*) trees. Birch is a member of the *Betulaceae* family and of the genus *Betula*. There are about 60 species of birch trees spread over the whole northern hemisphere (Welander, 1993). Birch trees like temperate, as well as subarctic climates (Wielgolaski, 2005; Pfadenhauer et al., 2020). Its dynamic growth makes it a "colonizing" tree capable of reproducing very quickly. The main species that can be found in North America include the paper birch (*Betula papyrifera*), yellow birch (*Betula alleghaniensis*), black birch (*Betula lenta*), and river birch (*Betula nigra*) (Miller and Cahow, 1989; Perala and Alm, 1990). Due to its stability, strength, and suitability for small-stature constructions, birch wood is popular; however, there is growing interest in using birch sap as a value-added product and new innovative sweetener (Lewington, 2018). For centuries, naturalists and doctors extolled the health benefits and medicinal properties of raw unprocessed birch water; for that reason, researchers have been investigating the physical and chemical properties of this highly perishable product.

Multiple research studies have detailed the chemical composition of birch sap, including the levels of proteins (Kallio et al., 1995; Jiang et al., 2001), organic acids (Kallio et al., 1985; Kallio and Ahtonen, 1987), carbohydrates (Kallio et al., 1985), amino acids (Patzold and Bruckner, 2005), as well as micro and macronutrients (Harju and Hulden, 1990).

In the latest scientific research, concentrating the birch tree sap using new technologies, such as reverse osmosis (RO), has led to a more efficient process to create a nutritive syrup with a unique flavor profile. Panda et al. (2016) described it as "a hint of spiciness reminiscent of molasses, balsamic seasoning, or certain soy varieties". Birch syrup has been deemed more attractive and is a non-perishable product, where the healthy composition of the sap is successfully preserved (Bilek et al., 2018). Compared to the tree sap, birch syrup has received limited attention in published literature. Although some previous studies have investigated the chemistry and composition of the syrup back in the 1980s (Kallio et al., 1989a). Despite the growing popularity of birch syrup production among maple syrup producers in the U.S., few recent studies have thoroughly examined the chemical composition and properties of birch syrup, especially syrup samples obtained from trees growing in the northeastern part of the United States. The product identified as the next closest reference for birch syrup is maple syrup; however, it is important to note that birch syrup is significantly different from maple syrup in terms of both chemical properties and sensory characteristics. Similar to maple syrup, but not on the same scale, birch syrup is produced in Canada, the United States, and also in Russia. Alaska is the highest birch syrup producer worldwide, while the other U.S. states produce much lower quantities (Helfferich, 2004). In the state of Maine, which has a variety of birch tree species (Butler, 2018)), birch syrup production is a growing industry that could provide extra revenue for maple syrup operations and satisfy the specialty food consumers who want natural, artisanal, local sweeteners that can be used in a variety of food products and beverages.

1.2.2. History

The birch sap was first named "birch water" and refers to the sap collected by tapping birch trees. There are traces of the use of birch sap well before the Middle Ages when naturalists of the time and even doctors used it and extolled its merits. In the 12th century, Hildegard of Bingen (Esser, 2022), who was a German Benedictine nun and church doctor, recommended birch sap to relieve ulcers. In the 14th century in Germany, birch sap was used as a refreshing drink, and Conrad Von Megenberg, a German teacher and writer, mentioned birch water for its medicinal properties on the bladder and kidneys (Svanberg et al., 2012). In the 16th century, the Italian physician and botanist Pietro Andrea Mattioli nicknamed birch “the nephritic tree” (Svanberg et al., 2012). He praised the properties of birch sap as capable of “breaking stones in the kidneys, as well as in the bladder, and healing mouth ulcers”. The history of who exactly first tapped birch trees to make syrup isn't completely clear. There's no documentation pointing to one specific person or group that originally consumed birch sap and syrup. What is known is that birch syrup has been produced and consumed by indigenous people in various regions (North America, Europe, and Asia) where birch trees grow (Lewington, 2018). For generations, indigenous communities have traditionally tapped birch trees and gathered the sap for syrup production and other products. Birch syrup production is an old practice that has been passed down over time as part of their culture and has roots in the ancestral knowledge and customs of these indigenous communities (Lewington, 2018). The method for tapping trees has evolved over the years, but the basic idea of sustainably collecting sap from birch trees has been going on for centuries.

1.2.3. Production process

The fundamental process of transforming birch sap into syrup is similar to maple syrup production by evaporating the water to concentrate the sugar content. Birch syrup production was not very common in North America before the 19th century, mostly because birch sap has less sugar than maple sap, only about 1% compared to 2% for maple, respectively (Van den Berg et al., 2013). Consequently, it takes more fuel to produce birch syrup, as more sap is required to make syrup at a ratio of 120 gallons of sap to 1 gallon of syrup compared to maple syrup's 40:1 ratio. However, using reverse osmosis (RO) helps to concentrate the sap to about 8% sugar or more before boiling it down and allows for a more efficient process (Van den Berg et al., 2013). Also, the birch season is much shorter at only 3-4 weeks, which makes it difficult to obtain the same syrup yields as maple. However, by using modern maple sap collection methods on birches, such as plastic tubing with vacuum pressure and good sanitation practices, it is possible to obtain better sap yields (Van den Berg et al., 2013). After sap collection, the sap can be filtered through a 5-micron water filter before passing through the reverse osmosis process to concentrate the sap from 1 to about 10 degrees Brix. The RO-concentrated sap is then boiled down in the evaporator to the desired Brix level. The syrup passes through another filter (usually through a filter press) and is hot-filled (82.2-87.7ºC) into storage or retail containers (Helfferich, 2004) (Figure 2).

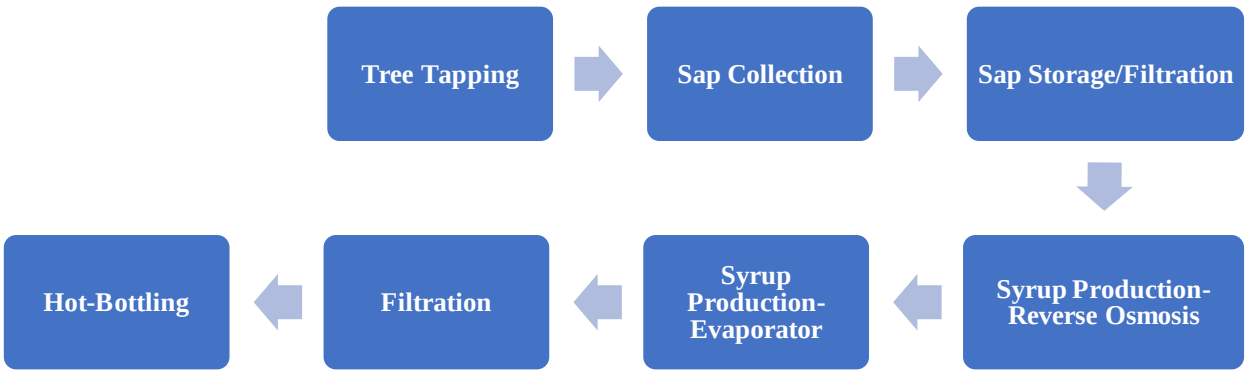

Figure 2. Major Processing Steps of Birch Syrup
Source: Adapted from Helfferich (2004)

1.2.4. Physical properties (birch)

1.2.4.1. Brix

To meet the maple syrup standards of identity set by the FDA and also Canadian regulations, a minimum of 66 % total soluble solids (or 66 degrees Brix) is required in the maple industry. As previously mentioned, the maple syrup Brix range must be 66-68.9 degrees Brix to preserve the safety and quality of the product and to protect it from possible microbial growth. The main sugar present in maple syrup is sucrose, while in contrast, the main sugars found in birch syrup are fructose and glucose. Worth noting, Beveridge et al. (1978) mentioned the sensory qualities of birch syrup are unique because it has a higher ratio of minerals and free amino groups than maple syrup. Kallio and colleagues (1989a) observed changes in Brix values throughout the process of concentrating birch sap (1 degree Brix) to syrup (63 degrees Brix) by reverse osmosis and evaporation. Reverse osmosis resulted in concentrated sap with a light yellow color and a subtle sweet aroma. Nonetheless, the presence of elevated levels of reducing sugars and free amino acids made the syrup highly prone to darkening upon heating (Kallio et al., 1989a). Currently, little research is available to support the targeted Brix value range to set regulatory standards for a minimum and maximum Brix level to be used to produce a safe and quality syrup without scorching since this syrup is easy to scorch (Cascio and Barber, 2014).

1.2.4.2. Water activity

The ratio of water to sugar in birch syrup affects many characteristics, such as the stability of the final product against fermentation, the density, and water activity level. Although there is not much data on the water activity in birch syrup, Van den Berg (2021) investigated possible best practices for producing high-quality birch syrup. The analysis of the water activity levels showed that birch syrups with lower soluble solids (about 60 degrees Brix) than the standard concentration for maple syrup had water activity levels over 0.80, which suggests that more

dilute birch syrups may not be shelf stable. Birch syrup and maple syrup can have similar water activity levels, as long as the birch syrup has about the same concentration of solids (around 67 degrees Brix) as normal maple syrup (which provides water activity levels in the 0.83-0.86 range). These results suggest that both birch and maple syrups may be at the same risk of microbial contamination (Van den Berg, 2021). However, more research must investigate and further clarify this relationship between Brix and water activity levels in birch syrup and the differences between birch and maple syrup.

1.2.5. Chemical and nutritive composition

Birch syrup has a unique composition compared to maple syrup including sugars, organic acids, phenolic compounds, and mineral content. For example, Kallio (1989b) analyzed Finnish birch syrup at 75 degrees Brix and found sugars represented 60-65% of the percentage of the total soluble solids, along with 27% water, 3% ash, and about 1% protein and free amino acids.

1.2.5.1. pH

The birch sap and syrup pH values have been investigated in a few studies. Johnson (1944) mentioned that the birch sap pH level can range between 6.8 and 7.6. In another study, the pH of birch sap was significantly lower and ranged between 5.3 and 5.7 during the entire season (Essiamah, 1980). Kallio and Ahtonen (1987a) investigated the pH level of birch sap samples collected from two species (*Betula pendula* and *Betula pubescens*) in both 1983 and 1984. They found that sap collected in 1983 had a pH level of about 7.5 at the beginning of the season, which started to decrease within 14 days to around 6.0. By the end of the season, the pH decreased even more, to the lowest values fluctuating between 5.3 and 5.5. On the other hand, the pH of the sap collected in 1984 stayed constant throughout the entire season and ranged between 5.5 and 6.0. They found no significant differences in pH levels between the birch species (Kallio and Ahtonen, 1987a). These results suggest that many factors such as time of the season, temperature,

and/or weather can affect the pH levels of birch sap. Jones and Alli (1987) examined the changes in pH levels through the process of concentrating birch sap and found that pH values decreased throughout the syrup production process (from 6.6 to 5.3). In the same study, the pH level of the birch syrup samples ranged between 5.1 and 5.6 and was significantly lower than the pH of maple syrup, with an average pH value of 6.4 for maple and 5.3 for birch (Jones and Alli, 1987).

1.2.5.2. Sugars

The sugar content of birch sap typically ranges from approximately 0.5% to 2.0%, which can vary depending on factors such as the birch species, geographical location, weather conditions, and season (Kallio et al., 1989a). The sugar composition of birch syrup consists of approximately 42% to 54% fructose, around 45% glucose, and minor quantities of sucrose. Trace amounts of galactose can also be found in birch syrup (Kallio et al., 1989a). Beveridge et al. (1978) used gas and paper chromatography for the quantification of sugars in birch syrup produced from sap collected on different days. The major carbohydrate identified was fructose ranging from 197-283 mg/g, followed by glucose ranging from 179-220 mg/g, and sucrose ranging from 0.8-2.5 mg/g. A transient decline in fructose and glucose levels was observed as the season progressed, while conversely, the sucrose levels increased near the end of the tapping season (Beveridge et al., 1978; Johnson, 1944; Kallio and Ahtonen, 1987b; Korolyak and Tomchuk, 1973; Kok et al., 1978). Furthermore, according to Kok et al. (1977), the dark color of birch syrups can be linked to the presence of high levels of reducing sugars (fructose and glucose), which consequently allows for more Maillard reactions to occur during the boiling process. Additionally, fructose caramelizes faster than sucrose, and because of the low sucrose content in birch sap, this results in a much darker syrup (Cameron, 2001).

1.2.5.3. Organic acids

According to Kallio and Ahtonen (1987a), the acidity in birch sap was due to the presence of organic acids such as citric, succinic, phosphoric, fumaric, and malic acid. In the syrup, they discovered that malic acid was the most dominant organic acid, consisting of about 3% of the dry matter of the syrup (Kallio et al., 1989).

1.2.5.4. Phenolic compounds

Currently, literature is quite limited on the composition and quantity of phenolic compounds present in birch syrup. However, research conducted by Boroduski (2017) revealed that birch sap had low concentrations of phenolic compounds including homovanillic acid, (+)-catechin 3-O-glucose, and resveratrol 3-O-glucoside. They also found betulin present in the birch sap, which comprised about 2.4% of the total content, along with some unidentified tannins. Homovanillic acid, (+)-catechin 3-O-glucose, resveratrol 3-O-glucoside, and betulin are all natural compounds derived from plant sources, each with unique pharmacological properties and potential health benefits. Homovanillic may potentially offer antioxidant properties and influence neurological health (Caruso et al., 2022). Similarly, catechin is a flavonoid abundant in foods such as tea and grapes and is known for its antioxidant and anti-inflammatory effects linked to cardiovascular health (Iqbal et al., 2023). Resveratrol, a polyphenol present in grapes and berries, exhibits antioxidant and anti-inflammatory properties, with potential benefits for cardiovascular health (Fernandez-Mar et al., 2012). Lastly, betulin is a triterpene compound from birch bark associated with diverse pharmacological effects, including anti-inflammatory, anticancer, and antiviral properties, underlining its potential therapeutic applications for various health conditions (Chaniad et al., 2019).

1.2.5.5. Minerals

Birch syrup is attracting attention because of the possible health benefits attributed to not only its phenolic compounds but also because of its mineral content (Bilek et al., 2016). According to Beveridge et al. (1978), the principal minerals found in birch syrup include magnesium, calcium, potassium, and sodium. The most abundant mineral they noted was magnesium which ranged from 9,310-20,700 ug/g, followed by potassium (3,930-11,800 ug/g), calcium (2,640 and 5,590 ug/g), and a small amount of sodium (31-66 ug/g). They also observed that the mineral levels, except for sodium, steadily increased over the tapping season (Beveridge et al., 1978). Overall, these results show that birch syrup is a good source of certain minerals.

According to an article in Healthline (2019), although birch sap provides valuable minerals, it may pose a potential risk of manganese toxicity. The current recommended upper limit for manganese intake ranges from 9-11 mg/day for adults and 2-6 mg/day for children, depending on their age (Aschner and Erickson, 2017). A 300 mL serving of birch water may contain approximately 3 mg of manganese, suggesting that even one serving could exceed the recommended upper limit for some children (Healthline, 2019). Because of the process of evaporation and possible differences in mineral content due to season and geography, birch syrup may be more concentrated in manganese than sap, which may not be suitable for small children to consume until the manganese content and potential risks are further investigated.

1.3. Bacterial and fungal contamination in syrups

As previously mentioned, the quality of maple syrup is highly impacted by water activity and Brix levels, and an adequate syrup thermal treatment prior to the hot fill process, which makes it difficult for bacteria to survive (Fiore, 2020). Although the microbial activity in the sap can affect the quality and safety of the final syrup, all the microorganisms usually die from high temperatures (100-104ºC) during the evaporation process (Fiore, 2020). Spoiled syrups are often

contaminated by fungi (Annis et al., 2016; Calder et al. 2011) which suggests that they can tolerate high sugar and low water activity levels compared to bacteria. While fungal spores typically cannot survive the evaporation step, contamination after processing (e.g. improper sanitation practices) or hot filling syrup into small glass containers with a large surface area can cause the syrup to cool down too quickly and could encourage spore outgrowth (Fiore, 2020). Some studies have found certain fungal species, such as *Aspergillus* and *Penicillium*, in syrup that was not heated adequately before bottling or were stored too long after opening (Whalen and Morselli, 1984; Heald and Pool, 1908). Furthermore, consumers complain about mold growth on syrups, especially obtained from roadside stands all over the U.S. and Canada (Drake and James, 1992). Mislivec (1973) also confirmed that the main fungi most likely to infiltrate food during storage typically belong to the *Aspergillus* and *Penicillium* species. According to Murdock (1979), an additional way food can become contaminated is from spores of certain fungi that can survive high temperatures, such as 92.7°C, for short periods of time. Whalen and Morselli (1984) also investigated mold growth in maple syrup. A batch of syrup was heated up to 82°C, sealed in different containers, and stored at 4, 24, and 30°C for one year. Visible mold was observed, and the primary molds that were identified were *Aspergillus niger*, *Penicillium chrysogenum*, *Aspergillus ochraceus*, *Eurotium repens*, and yeasts. Although scientific literature has begun to investigate the possible causes and identification of spoilage microorganisms in maple syrup, no current research has begun to evaluate if these same spoilage microorganisms can persist in birch syrup.

1.4. Study Objectives

An adequate amount of research has investigated the quality, safety, physical, and chemical composition of maple syrup. However, birch syrup composition and the parameters for safe production, are not well documented. Birch syrup is often compared to maple syrup due to the similarities of these natural sweeteners, such as how they are produced and viscosity. Therefore, these two syrups were further studied in comparison from the same producer to determine:

1. the quality differences (water activity and Brix levels),
2. the chemical and nutritive composition differences (pH, sugar content, minerals, organic acids, phenolic compounds), and
3. the microbial composition and possible mycological differences between the two syrups and at what heating temperature ranges can inhibit inoculated fungal spores.

CHAPTER 2 METHODOLOGY

2.1. Materials and Methods

Maple and birch sap and syrup samples were collected and produced by Temple Tappers, Temple, ME during the 2023 production season. Due to project time constraints, and to reduce producer and geographical variability, samples were obtained from one producer. The sap collection dates were March 16-April 4 for maple, and April 11-April 25 for birch. The sap was collected via spiles and plastic tubing (under vacuum) from yellow (*Betula alleghaniensis*), gray (*Betula populifolia*), and paper birch (*Betula papyrifera*) trees, as well as red (*Acer rubrum*) and sugar (*Acer saccharum*) maple trees. Both saps were filtered twice before boiling, by first using a water filter cartridge for both saps, and then the birch sap was filtered using a wool filter, and a fabric filter was used for maple sap. After the initial filtering steps, maple sap was boiled in the evaporator to concentrate into syrup, and then the syrup was filtered using a vacuum filter and then hot-filled at 85-87.7°C into clean and sanitized 0.25 L (5 gallon) food-grade stainless steel keg barrels. Birch sap was additionally concentrated via reverse osmosis to approximately 5 degrees Brix, then boiled in the evaporator, and finally concentrated further in the finishing pan. As previously mentioned for maple syrup, birch syrup was filtered using a vacuum filter and also hot-filled at 85-87.7°C into stainless steel keg barrels. Each syrup batch (typically an early, middle, and late season batch of syrup) was hot-filled into barrels. At the end of the syrup season, three-four batches of each type of syrup were blended and then hot filled (85-87.7°C) into glass retail containers. For this study, syrup samples were hot-filled into 200 mL glass syrup bottles (eight blended samples for maple and eight blended birch syrup samples), were obtained from Temple Tapper, transported to UMaine, and all samples were analyzed during the summer of 2023. Before analysis, the syrup samples were stored at room temperature. Once opened, all syrup samples were stored under refrigerated conditions at 3-4°C.

2.1.1. Physical properties Analysis

2.1.1.1. Brix

The Brix levels of the syrups were determined using a digital refractometer (ATAGO, model RX-5000i, ATAGO CO, Bellevue, WA, USA) and following AOAC Official Method 932.14 (AOAC, 2012). Distilled water was used to zero the refractometer. Syrup samples were placed onto the prism with a clean plastic transfer pipette, and the prism of the refractometer was cleaned between measurements with distilled water and dried with a Kimwipe®. Brix was measured three times per sample and the average for each sample was calculated.

2.2.1.2. Water Activity

The syrup water activity levels were measured using an Aqualab Series 4TE (AQUALAB, Pullman, WA, USA) and following AOAC Official Methods 978.18 (AOAC, 2012). A small amount of maple or birch syrup at room temperature (24°C) was placed in a plastic water activity sample dish (100 mm) until the bottom of the dish was completely covered by the sample and halfway up the cup. The sample was then placed into the device, and the water activity level was measured. This measurement was performed three times for each sample and the average for each sample was calculated.

2.2.2. Chemical and Nutritive Analysis

2.2.2.1. pH

The pH level of the syrup samples was measured using a pH meter model HI2002-01 (Hanna edge® Dedicated pH/ORP Meter, model HI2002-01, Hanna Instruments, Woonsocket, RI, USA) and a glass pH electrode with conical tip (model FC2100, Hanna Instruments, Woonsocket, RI, USA). The pH probe had a gel body and open junction, along with automatic temperature sensor compensation. The pH levels were determined using the potentiometer method following AOAC Official Method 981.12(AOAC, 2012). Calibration of the meter was

accomplished using both pH 4.0 and 7.0 buffer solutions. The glass electrode tip was directly immersed into the birch or maple syrup sub-sample in a 75 mL plastic beaker. The pH levels were measured three times per sample and then the average for each sample was calculated.

2.2.2.2. Sugar Analysis

The three sugars of interest (glucose, fructose, and sucrose) were measured in maple and birch syrup samples using an unpublished high-performance liquid chromatography method developed by The University of Maine Food Chemical Safety Laboratory. Six samples of birch syrup (blended batches) and six samples (blended batches) of maple syrup were used. Prior to the analysis, approximately 100 mg of each birch and maple syrup sample was weighed into duplicate glass test tubes. All diluents and solvents used were HPLC grade. The three sugar standards were prepared by dissolving 100 mg of glucose, fructose, or sucrose in 100 mL of the solvent, which was acetonitrile/water (50:50, v/v). The same solvent was used to prepare the sample extract dilutions with a 1:20 ratio of syrup to solvent. An HPLC system (Agilent Technologies 1100 Series, Waldbronn, Germany) equipped with a computer, pump system, autosampler, SHODEX NH2 column (Resonac, America, Inc., NY, NY), and a refractive index detector was used for sugar analysis. The analytical conditions were: injection volume 25 µl, flow 0.3 mL/min, mobile phase 65% acetonitrile- 35% water (v/v). Each syrup sample was analyzed in duplicate and the concentrations of the sugars were calculated and then averaged.

2.2.2.3. Organic Acids

The birch and syrup samples were analyzed for organic acids using a slightly modified method from Frioni et al. (2021). Filtered maple and birch syrup samples were diluted and assayed with an HPLC system (Agilent Technologies, Waldbronn, Germany), but this time equipped with an Allure Organic Acids 300 X 4.6 MM 5 micron column (Restek Corp, Bellefonte, PA), an autoinjector, and a photo-diode array (DAD) detector. The diluent used was a

100 mM potassium phosphate solution at a pH level of 2.5 (pH adjusted with phosphoric acid) for both samples and standards. The mobile phase (potassium phosphate solution at pH 2.5) was an injection volume of 25 µl. The samples were diluted to a ratio of 1:10 with the mobile phase. The standards were citric, fumaric, malic, oxalic, tartaric, and succinic acids, and were prepared by diluting 100 mg of each standard in 50 mL potassium phosphate solution. The detection wavelength was set at 226 nm for quantification and the organic acid levels in each sample were run in duplicate and then averaged.

2.2.2.4. Phenolics Compounds

Phenolic acid analysis was performed using a slightly modified method developed by Alhallaf et al. (2022). A mixture of analytical standards that included protocatechuic, catechin, chlorogenic, vanillic, syringic, caffeic, p-coumaric, ferulic, benzoic, hydroxybenzoic, and gallic acids were used, and the working standard solution was prepared by dissolving the standards in HPLC grade methanol (80%). The same filtered syrups were analyzed using an HPLC system equipped with an autoinjector (Thermo Hypersil Gold aQ 4.6 X 250 mm 5-micron column (Thermo Fisher, Waltham, MA) and a photo-diode array (DAD) detector from Agilent Technologies. The syrup samples were diluted in methanol (1:10 ratio). The gradient method was used with two solvents: 0.1% formic acid in water (3:3000, v/v) as mobile phase A and methanol/acetonitrile (95:5) as mobile phase B. The absorbance was measured at both 280 nm and 329 nm for quantification, and each sample was run in duplicate and then averaged.

2.2.2.5. Minerals

The mineral analysis was performed by the Analytical Lab and Maine Soil Testing Service at the University of Maine (Orono, ME). The minerals and metals analyzed were Ca, K, Mg, P, Al, B, Cu, Fe, Mn, Na, S, and Zn in six birch and six maple syrup samples after acid

digestion (EPA Method 3051) (EPA, 1994b) using inductively coupled plasma optical emission spectrometry (ICP-OES) (EPA Method 200.7) (EPA, 1994a).

2.2.3. Microbiology and Mycology Analysis

2.2.3.1. Bacteria, Yeast and Mold Quantification

Six birch and six maple syrup samples were plated in duplicate for microbiology analysis. The 3M™ Petrifilm™ Aerobic Count Plates (3M Food Safety, St. Paul, MN) were used to determine the presence of bacteria in the samples following AOAC Official Method 990.12 (AOAC, 2005). The 3M™ Petrifilm™ Yeast and Mold Count Plates were used to detect yeast and molds in the syrup samples following AOAC Official Method of Analysis^{SM} (OMA) 997.02 (AOAC, 2000). The 3M™ Petrifilm™ is a pre-prepared culture medium that contains nutrients, a gelling agent soluble in cold water, and an indicator that helps in counting colonies. The APC (aerobic plate count) plates were incubated at 35℃ for 48 hours and Y&M (yeast and mold) plates were incubated at 20-25℃ for 3-5 days and plates were examined using a Quebec counter to count any bacteria, yeast, and mold colonies growing on the plates.

2.2.3.2. Fungal Analysis

Two experiments were performed to first determine if fungi can survive in syrup samples and secondly, to conduct a thermal inactivation study of inoculated fungal spores in the syrup samples. Two unopened 200 mL glass bottles of birch and maple syrup were used for the analysis. Before the experiment, each of the selected fungi, two *Aspergillus spp*, two *Penicillium spp*, and one isolate of *Rhodotorula*, were transferred to multiple MYA (Malt Yeast Agar; 500 mL H_2O, 5g Malt extract, 1.5 Yeast extract, 7.5g Agar) plates, incubated for 12 days at 20℃, and then refrigerated to keep the spores viable long enough to perform the experiments. This process was repeated when new spores were needed. All the analyses were performed in triplicate for each fungal isolate for both birch and maple syrups.

Fungal Contamination

The objective of this experiment was to observe whether the fungal spores (1,000 spores in 1 mL) could survive in the syrups. In a sterile hood, each fungal plate was saturated with 2 mL of 0.05% sterile Tween 20 (Sigma-Aldrich, St. Louis, MO), and the wet surface was gently scraped with a glass plate spreader that was previously flamed and cooled. To facilitate spore counting, serial dilutions (1:100, 1:1,000, 1:10,000) were performed to reduce the concentration of the spore solution. The spores were counted using a hemocytometer. After rinsing and carefully drying the hemocytometer and the coverslip, the two chambers at each side of the hemocytometer were filled with the spore solution (10 µl), and using a microscope, the four outer and one middle square of the central square of each side were used for counting. The average count from each side of the hemocytometer was then multiplied by 50,000 and then by the dilution factor to get the number of spores per mL of solution. A simple formula of Concentration 1*Volume 1 = Concentration 2*Volume 2 was used to calculate the amount of spore stock solution to be added to 5 mL of birch and maple syrup samples to have a final concentration of 1,000 spores in 1 mL of syrup. The syrup samples (5 mL each) were divided into multiple 15 mL sterile centrifuge tubes and inoculated with the spore stock solution. The inoculated syrups were directly transferred onto MYA plates and incubated at 20 °C for about 7 days before observation.

Thermal Inactivation

The objective of the second experiment was to evaluate the temperatures and time needed to kill the spores of the fungi *Aspergillus spp*, *Penicillium spp*, and *Rhodotorula* when inoculated in birch and maple syrup samples. These fungi were selected based on preliminary research studies conducted at the University of Maine. Typically, standard pasteurization procedures in maple syrup production, which include heating the syrup to approximately 82.2℃ or higher (Fiore, 2020) for a few minutes, eliminate microorganisms, including spores. In previous work, it

was discovered that spores of fungi found in maple syrup were able to withstand heating at temperatures below 70°C for a duration of 3 minutes (Annis, 2016). Therefore, the selected temperatures tested were 85℃ and 87.7℃, and the incubation time at those temperatures was selected at 4 minutes. For each fungus, 500 µl of birch and maple syrup samples were distributed, in triplicate, into sterile microcentrifuge tubes and labeled "fungal isolate name + temperature + syrup name". The previous step (fungal contamination) was repeated, but this time, a final concentration of 100 spores in 500 µl of syrup was used. With a heat block, half of the syrup samples were heated at 85℃ for 4 minutes, and the other half of the syrup samples were heated at 87.7℃ for 4 minutes. To use the heat block more effectively, the samples were heated in small groups to avoid any temperature fluctuations due to overloading. For the control syrup solutions, 500 µl of both birch and maple syrup were distributed in triplicate into sterile microfuge tubes and labeled M1C, M2C, M3C (maple syrup control), B1C, B2C, B3C (birch syrup control). The control samples were not inoculated with spores and were not heated. In the sterile hood, MYA plates were labeled with the same labels as on the microcentrifuge tubes. The controls were plated first by pouring the syrup directly from the tubes onto the plates, and then each set of inoculated samples as they reached room temperature. All the plates were wrapped with Parafilm, incubated at 20℃ for 6 days, and then checked for fungal colonies.

2.2.4. Statistical Analysis

Microsoft Excel software (Microsoft® Excel® for Microsoft 365 MSO, version 2403 Build 16.0.17425.20176, Redmond, WA) was used for the data analysis of this research. The data was presented as mean values, along with the standard deviation. The correlations between the different parameters (physical, chemical, and nutritive properties) were evaluated using the Pearson correlation analysis (DATAtab statistics calculator, Graz, Austria), and the significance

was established at $p < 0.05$. In addition, the tables concerning the physical properties were analyzed by Student's independent t-test to distinguish the differences among the means.

CHAPTER 3 RESULTS and DISCUSSION

3.1. pH

The pH level of the birch syrups was significantly ($p<0.001$) lower (4.62-4.64) than the maple syrup pH levels (6.59-6.60) (Figure 3) (Appendix B, Table A-1). The pH values of the maple syrup samples analyzed were close to the average values mentioned in many other studies (Robinson et al., 1989; Stuckel and Low, 1996; Takano, 2006; Ball, 2007; Perkins et al., 2009; Frasz and Miller, 2015). Some of the researchers previously cited suggested that the variations in maple syrup pH levels can be attributed to many factors (Robinson et al., 1989; Perkins et al., 2009). Microbial contamination is one potential contributor, as different microbial species can generate acidic or basic byproducts during their metabolic processes, thereby impacting the overall pH level of the syrup. Certain fungi such as *Penicillium digitatum*, *Penicillium expansum*, and *Aspergillus niger* are recognized for their ability to lower the pH level in contaminated foods by generating organic acids, facilitating tissue acidification (Jiao et al., 2022). Furthermore, during the sap evaporation process to produce syrup, organic acids may undergo chemical changes, potentially converting into flavor compounds that could further influence the pH level in syrup (Willits and Hills, 1976; Robinson et al., 1989; Perkins et al., 2009). Maple syrup and birch syrup may also have differences in pH levels due to the differences between the two tree species, the geography of the soil, and environmental and climate conditions.

According to Kallio et al. (1989) and based on other studies (Jones and Alli, 1987; Kallio and Ahtonen, 1987), the pH level of quality birch syrup typically ranges between 5.5 and 6.5. In this study, the pH values of the birch samples were much lower (~4.6). However, Kok et al. (1977) reported, after analyzing fourteen batches of birch syrup (with a concentration of 50% sugar), that the average pH level was 4.8, which is similar to our findings. Kallio et al. (1989) mentioned that factors such as the evaporation and heating process could play a role in decreasing

the pH level of the final syrup product, which suggests the evaporation of water from the sap (pH level of 6.8-7.6) may lead to the concentration of acidic compounds, such as organic acids (Johnson, 1944). Jones and Alli (1987) agreed with this interpretation, as they found that processing sap into syrup could significantly lower the pH level of birch syrup.

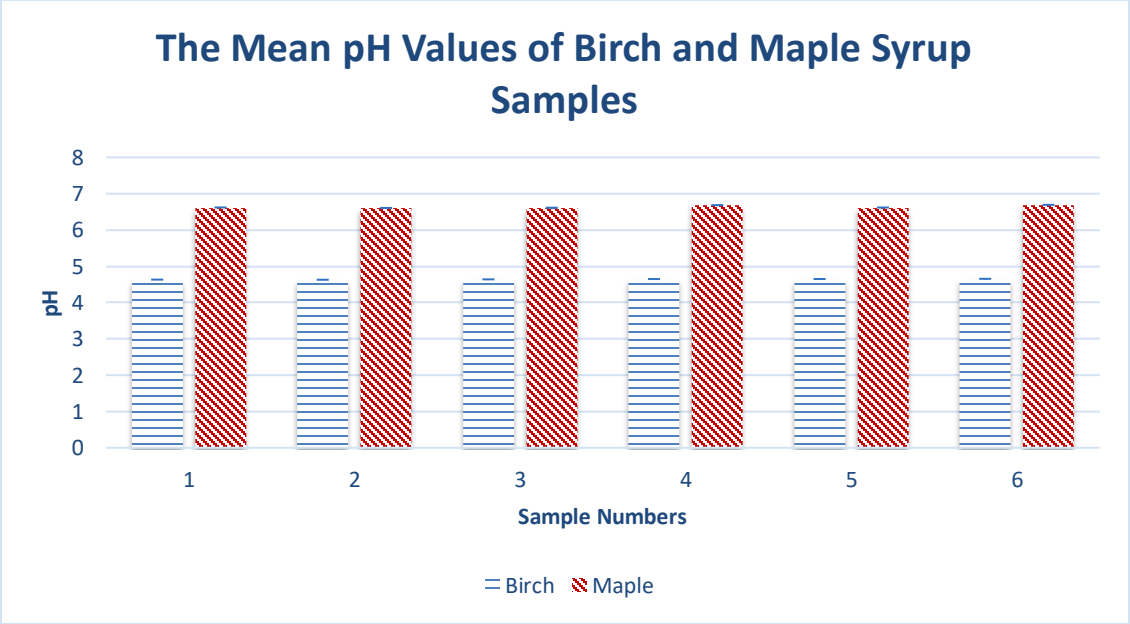

Figure 3. The Mean pH Values of Birch and Maple Syrup Samples
Each value represents the mean pH level (n=3). Error bars represent the standard deviation.

3.2. Brix

The Brix levels or percent soluble solids in the birch samples were significantly ($p<0.001$) lower (62.2-63.6 degrees Brix) than the maple syrup samples (66.4-66.8) (Figure 4) (Appendix B, Table A-2). Stuckel and Low (1996) explained that even though the Brix values do not represent the exact sugar content, it does show a definite correlation, as about 99% of the total solids found in maple syrup are mostly sugars (sucrose) (Morselli, 1975). Furthermore, according to the USDA guidelines, finished maple syrup by legal definition must have a Brix range between 66.0 and 68.9. Maple syrups with values lower than 66 degrees Brix are more susceptible to

fermentation and spoilage (Willits and Hills, 1976). In this study, all the maple syrup samples met the legal requirement for quality syrup, with Brix values between 66.4-66.8 degrees Brix. On the other hand, birch syrup samples had much lower Brix levels (62.2-63.6). Brix serves as an indicator of the total soluble solids in a solution that is mainly composed of sucrose (Kimball, 1991). According to van den Berg (2021), hydrometers and refractometers used to measure Brix levels in maple syrup production were set up just for sucrose since it is the predominant sugar in maple syrup. She further explained that because birch sap and syrup consist mostly of glucose and fructose without much sucrose, it would be impractical to use the same Brix standards for birch syrup. Therefore, the same standard density for maple syrup may not be applicable for birch syrup due to the variations in the sugar content of the corresponding syrups. In previous research, Kallio et al. (1989) concentrated birch syrup up to 75 degrees Brix, which was higher than the total sugar content of the samples (60-65% sugars). Although a Brix range of 70-75 degrees Brix was described as the "optimal consistency" by these researchers, the intensive heat treatment to obtain such Brix values may negatively affect other sensory attributes such as the taste, aroma, and possibility niter production (Kallio et al., 1989). In syrups like birch syrup, the amount of dry matter (48% glucose, 41% fructose, 0.6% sucrose, 0.5% galactose, 3.1% malic acid, 2.8% ash, and 0.4% free amino acids) can also affect the Brix measurement (Kallio et al., 1989). This phenomenon can be explained by the observation that as the concentration of sugars increases, as indicated by a higher Brix value, there is a concurrent rise in the concentration of dry matter within the solution. Consequently, this leads to a positive correlation between the two measurements (De Reijkex et al., 1984; Kallio et al., 1989).

Concentrating birch sap to the same Brix level as the standard established for maple syrup might produce a quality and safe product, although more research must investigate how to best measure Brix levels in birch syrup since most refractometers are measuring sucrose levels, while

birch sugars are predominately glucose and fructose. More birch syrup analyses over time and several seasons would help determine a Brix trend or possibly determine an offset calculation so that current refractometers can still be used to measure Brix levels in birch syrup. The real question is whether the maple Brix standards can be used for birch syrup without having a negative effect on the flavor of the syrup and/or on niter production at these Brix levels. Additionally, because birch syrup is not as widely produced as maple syrup, the USDA has yet to set specific standards or guidelines for birch syrup production and therefore, state regulatory agencies are currently struggling with how to regulate this novel syrup.

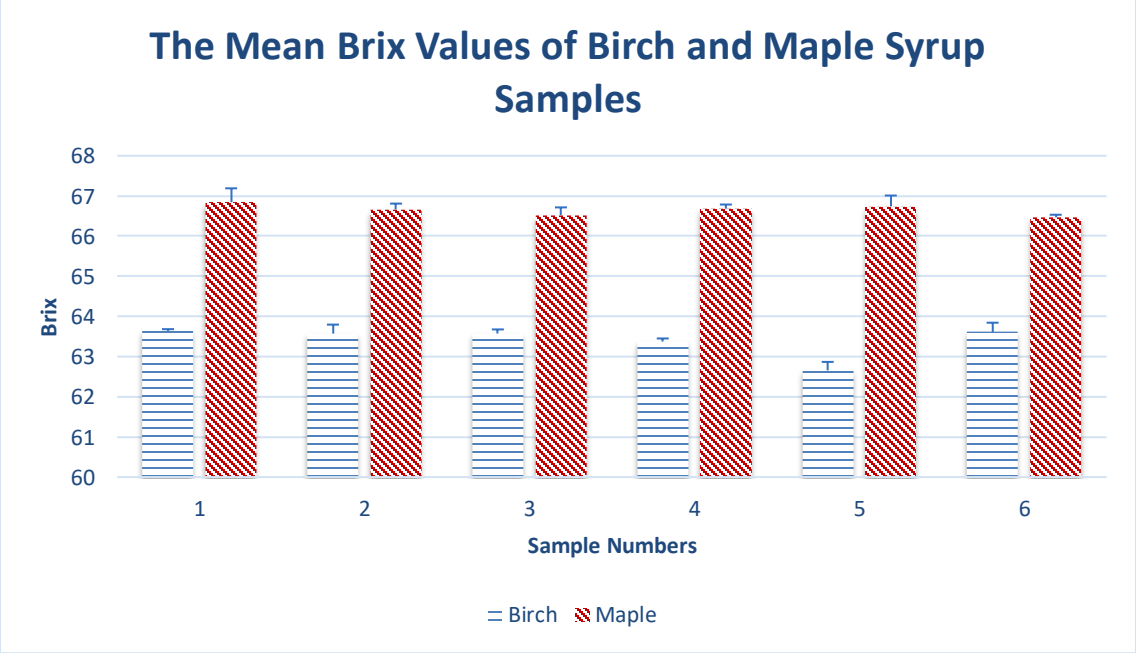

Figure 4. The Mean Brix Values of Birch and Maple Syrup Samples
Each value represents the mean Brix level (n=3). Error bars represent the standard deviation.

3.3. Water Activity

Water activity measurements are often used by the food industry as an indicator of shelf stability in carbohydrate-based foods and a way to control microbial growth. In the syrup

industry, water activity is an important parameter to consider in the production and preservation of the finished product. The water activity level of syrups is significantly affected by sugar content, and syrups with a high sugar content will often have a water activity low enough to prevent microbial growth. In this experiment, there was a significant ($p<0.001$) difference between the water activity levels of maple and birch syrup samples. All the maple syrup samples had a water activity value of 0.850 (Figure 5), and these results were similar to the maple syrup water activity levels as reported by Takano (2006). The birch syrups had much lower water activity values, which varied from 0.800 to 0.810 (Figure 5) (Appendix B, Table B-3). As previously mentioned, at these water activity levels, most bacteria will not grow, but certain fungi can persist at water activity levels nearing 0.600. Additionally, if these syrups are not properly heated at an adequate temperature and/or if the retail glass containers cool down too quickly after hot filling, then fungi spores can persist and possibly grow in the syrup (Annis et al., 2016). However, it is important to note that these values can be affected by many factors, including boiling time and processing techniques (Starzak and Mathlouthi, 2006).

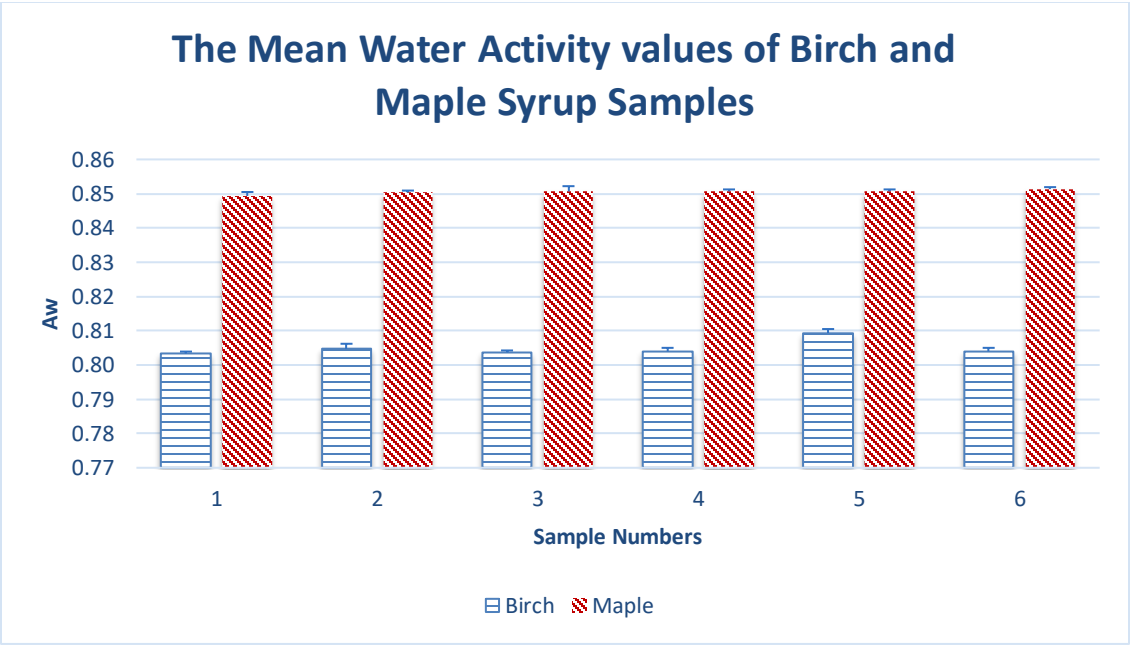

Figure 5. The Mean Water Activity Values of Birch and Maple Syrup Samples
Each value represents the mean water activity level (n=3). Error bars represent the standard deviation.

3.4. Sugars

As expected, the main sugar in the maple syrup samples was sucrose, followed by glucose, and fructose having the lowest concentration (Figure 6). Stuckel and Low (1996), Takano (2006), and van den Berg et al. (2006) reported similar findings in maple syrup. The average fructose, glucose, and sucrose levels in the tested maple syrup samples were 28.96 ± 17.39 mg/g, 31.07 ± 10.15 mg/g, and 647.31 ± 15.25 mg/g, respectively (Figure 6) (Appendix B, Table B-4). These results fell within the mean ranges indicated by Van den Berg et al. (2006) for the sugar concentrations in all grades of maple syrup. However, the sugar ratios were quite different in birch syrup samples, as the average levels of fructose, glucose, and sucrose in birch samples were 326.53 ± 7.79 mg/g, 241.53 ± 23.63 mg/g, and 19.04 ± 14.56 mg/g, respectively. In contrast to maple syrup, the birch syrup samples had more fructose (the predominant sugar), followed closely by glucose, and then sucrose had the lowest concentration. These results agree with the order of sugar concentration in other birch syrup samples, as reported by Beveridge et al.

(1978), Kallio et al. (1989), and Kuka and Gersebeka (2013). Johnson (1944) observed differences in the concentrations of sugars between birch species. In yellow birch sap, fructose and glucose had roughly the same concentration, while white birch sap had a higher concentration of fructose compared to glucose. Moreover, Johnson (1944) concluded that the variations in sugar concentrations may possibly depend on tree species and seasonal differences.

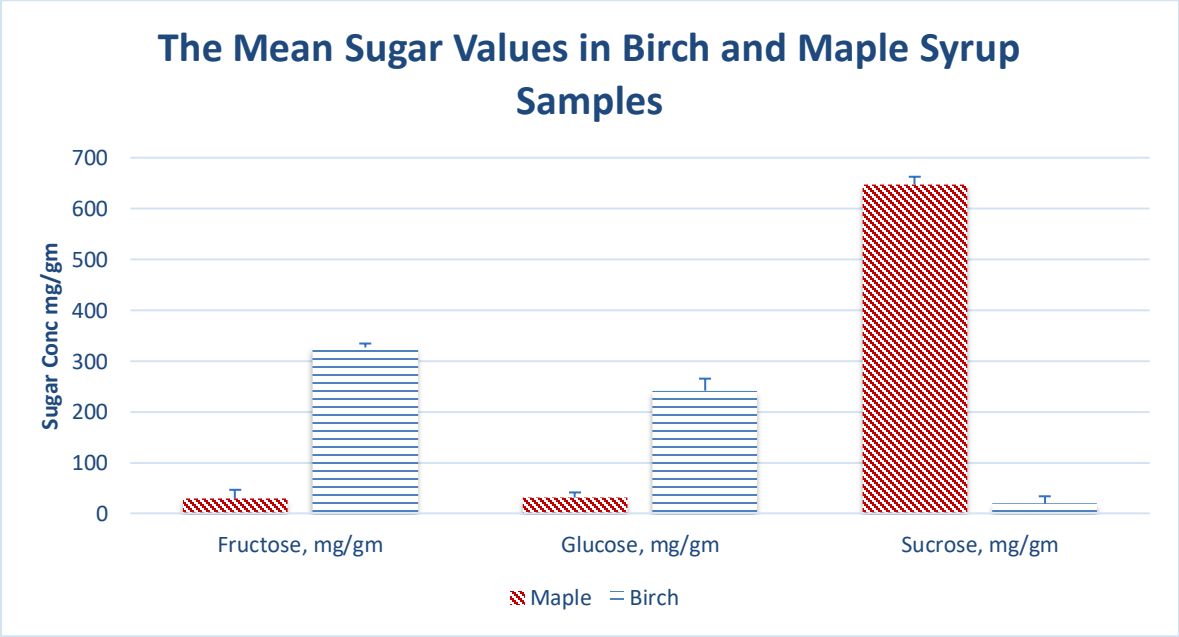

Figure 6. The Mean Sugar Values in Birch and Maple Syrup Samples
Each value represents the mean sugar values (n=12). Error bars represent the standard deviation.

3.5. Organic Acids

Tree syrups naturally contain a mixture of organic acids, which can contribute to the pH level and acidity of syrups, while also helping stabilize syrups and prevent them from degradation (Derouich et al., 2020). The concentration of six selected organic acids, based on previous research, was evaluated in this study. Citric, fumaric, malic, and tartaric acids were detected in birch syrup samples, with mean values of 0.61 ± 0.15 mg/g, 0.14 ± 0.02 mg/g, 5.59 ± 0.68 mg/g, and 0.58 ± 0.07 mg/g, respectively (Figure 7) (Appendix B, Table B-5). In the maple syrup

samples, the only organic acids detected were citric, fumaric, malic, and oxalic with mean values of 0.12 ± 0.01 mg/g, 0.04 ± 0.00 mg/g, 2.43 ± 0.03 mg/g, and 0.04 ± 0.00 mg/g, respectively. Compared to maple syrup, birch syrup samples had an overall higher total organic acid content, which may explain why this syrup had lower pH levels. Malic acid was the most abundant acid in both syrups, with birch syrup having more than twice the level than in maple syrup, and this observation is similar to what other researchers have reported in the literature (Kallio et al., 1985; Kallio and Ahtonen, 1989; Perkins et al., 2009). The second most abundant acid in both syrups was citric acid, but at a much lower concentration (<1 mg/g. Tartaric acid was about the same concentration as citric acid in the birch syrup samples but was not detected in the maple syrup samples. The higher organic acid content may explain the tart, sour, and tangy flavors that have been described for birch syrup when compared to the sweeter maple syrup (Martin and Issanchou, 2019).

The organic acid concentrations and exact composition can vary in both syrups due to many factors including tree species, geographical location, and seasonal differences (Kuka and Gersebeka, 2013; Lagace et al., 2015). For instance, tree species can influence the composition and concentrations of organic acids via a complex interplay of genetic, metabolic, environmental, and physiological factors. This phenomenon arises from the essential role organic acids play in the physiological functions of trees, including growth, nutrient uptake, defense against both biotic and abiotic stresses, and overall adaptation to their surroundings (Adeleke et al., 2017).

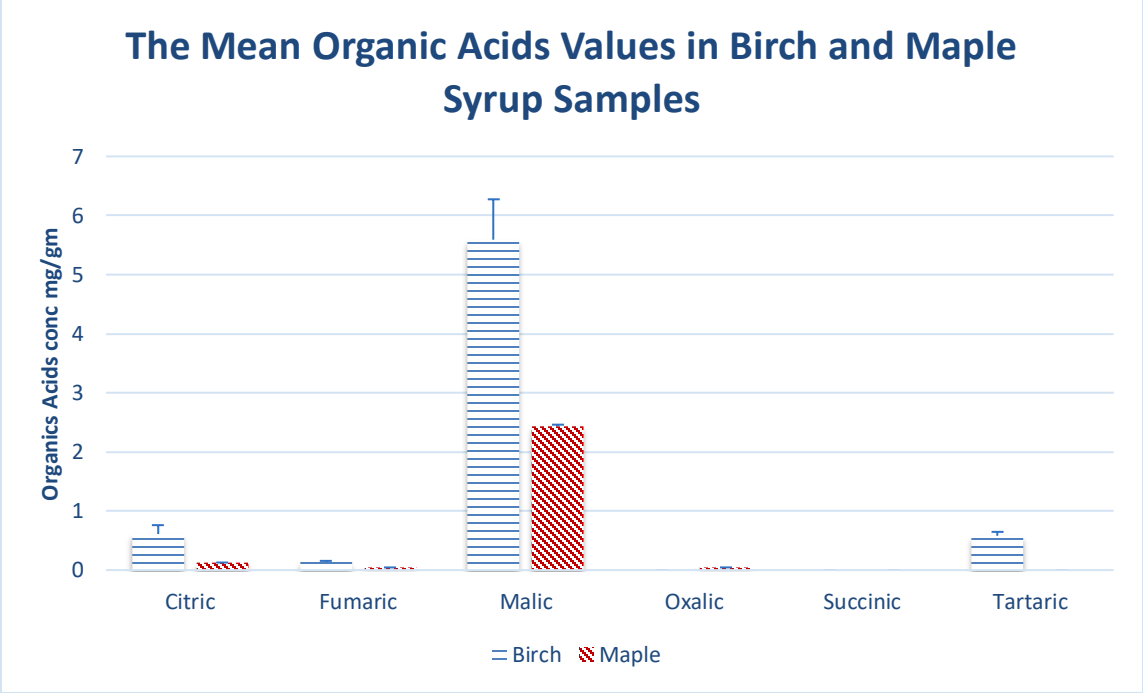

Figure .7. The Mean Organic Acid Values in Birch and Maple Syrup Samples
Each value represents the mean organic acid values (n=12). Error bars represent the standard deviation.

3.6. Phenolic Compounds

Maple and birch syrup samples were examined for phenolic compounds of interest, including protocatechuic, catechin, chlorogenic, vanillic, syringic, caffeic, p-coumaric, ferulic, benzoic, hydroxybenzoic, and gallic acids. These compounds were selected because they have been identified in the literature as the most prevalent in maple syrup, as there was limited data on the phenolic composition of birch syrup (Abou-Zaid, 2008).

While all the standards were detected, none of these phenolics were detected in either syrup. Challenges associated with identifying phenolic compounds in syrups, including maple syrup, were addressed in some previous studies (Iswaldi et al., 2012, Rodríguez-Pérez et al., 2015, Lama-Muñoz and Contreras., 2022). Some of those hurdles to analyze these compounds include the complexity of the syrup matrix (effect of the syrup components on each other), the stability of the chemical compounds, and possibly the sensitivity of the detector, method of

sample preparation, and chromatographic parameters (Iswaldi et al., 2012, Rodríguez-Pérez et al., 2015, Lama-Muñoz and Contreras., 2022).

3.7. Minerals

Of the minerals analyzed, selected based on previous research, potassium, calcium, and magnesium were the most abundant minerals present in maple syrup, with mean values of 2,658 ppm, 853.67 ppm, and 136.17 ppm, respectively (Figure 8) (Appendix B, Table B-6). These values fell within the range of potassium (1,005-2,990 ppm), calcium (266-1,707 ppm), and magnesium (10-380 ppm) content in maple syrup, as reported by Stuckel and Low (1996). The maple syrup samples also contained manganese, and small amounts of aluminum, phosphorus, boron, iron, sodium, sulfur, zinc, and with copper being the least abundant mineral.

Birch syrup had the same minerals present as maple syrup, but at much higher concentrations of potassium, calcium, and magnesium with mean values of 5,745.17 ppm, 5,541.83 ppm, and 1,407.67ppm, respectively (Figure 8). Beveridge et al., (1978) analyzed 14 birch syrup samples and reported the following ranges: potassium (3,930-11,900 ug/g), calcium (2,640-5,590 ug/g), and magnesium (9,310-20,700 ug/g). They also detected manganese, phosphorus, sulfur, and zinc, with small amounts of sodium, boron, aluminum, iron, and copper in their birch syrup samples. As previously mentioned, the high manganese content (357-365 ppm) found in birch syrup raises potential concerns regarding manganese toxicity (Healthline, 2019). Given these high concentrations, 30 mL per serving of birch syrup can approach or exceed the tolerable upper intake level for manganese, which is 11 mg/day for adults and even lower for children (Freeland-Graves et al., 2016). Consequently, it is advised to exercise caution and ensure prudent consumption practices, especially for young children. Overall, both syrups contain essential minerals, and the composition and concentration of minerals can vary depending on the tree species, season, and soil geography (Beveridge et al., 1978; Robinson et al., 1989; Stuckel

and Low, 1996; Nimalaratne et al., 2020). For instance, precipitation levels can influence the availability of minerals in the soil and their uptake by the tree roots (Burch et al., 1996). The differences in mineral concentration could also be due to the differences in evaporation time. Birch sap is boiled for longer periods of time compared to maple because it can take longer to concentrate the sap into syrup, which could cause increases in mineral concentration.

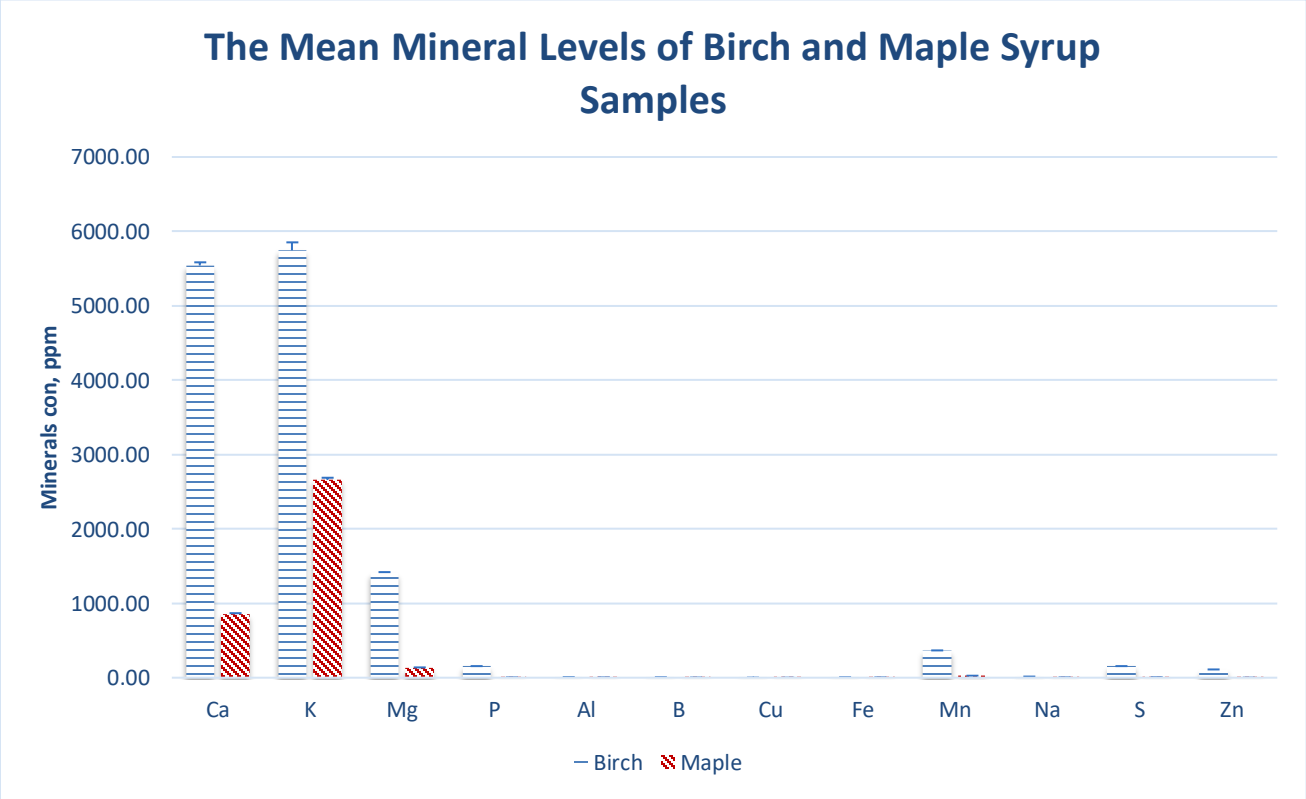

Figure 8. The Mean Mineral Levels in Birch and Maple Syrup Samples
Each value represents the mean mineral values (n=6). Error bars represent the standard deviation.

3.8. Relationships between main constituents of birch syrup

The physical, chemical, and nutritive components of birch syrup were further analyzed to determine any significant relationships and the strength of these associations. In Table 6, a strong correlation was noted between Brix and water activity in which higher Brix levels are associated

with lower water activity levels (-0.96; $p < 0.01$), which is expected. The entire Pearson correlation table is listed under Appendix B, Table B-7. During syrup production, the sugars will tend to bind to the free water molecules available as the sap is evaporated causing a reduction of free water available (Zaitoun et al., 2018). The correlation between Brix level and the dominant sugars present in the syrups (fructose and glucose) showed neither a strong nor significant relationship, and no relationship was noted with sucrose. Currently, most of the instruments available (such as hydrometers or refractometers) help measure the density/soluble solids (sucrose) in maple syrup. Birch syrup mainly contains invert sugars, and these sugars are different from sucrose when it comes to how they affect the density and viscosity of the final product. Therefore, these instruments used to assess Brix or maple sugar density might understate the overall sugar concentration in birch syrup (Van den Berg, 2021). A study conducted by Van den Berg (2021) compared density measurements of birch syrup using both a regular refractometer (Brix) that is more sucrose-based and an invert refractometer (Invert %). They found differences in birch syrup density estimates by both refractometers, with the sucrose refractometer displaying lower densities than the invert refractometer. It is worth noting that the accuracy of the invert refractometers relies on pure invert sugar solutions as impurities (non-sugar content), such as minerals and organic acids, can affect their precision (Van den Berg, 2021). Actual sugar content in birch samples was found to be significantly lower than estimated total solids, indicating high impurity levels. Van den Berg (2021) recommended the usage of invert refractometers for birch syrup density measurements, with a note to be aware that these tools estimate the percentage of total invert sugars and impurities that can affect the accuracy of these measurements.

As expected, significant negative correlations were noted between pH and some of the organic acids, including malic and tartaric acids. These results support the idea that a higher

organic acid content creates a more acidic environment by lowering the pH level. Additionally, strong and significant correlations were observed amongst some of the organic acids. Strong negative relationships were noted between citric-tartaric (-0.85; $p<0.05$) and malic-tartaric acids (0.97; $p <0.01$). According to Shi et al. (2022), the interactions between the organic acids can highly affect the overall flavor, aroma, and composition of foods. However, the reason for these organic acid associations in syrup is not known other than the way these organic acids may potentially interact or the result of reactions during the boiling and evaporation process. More research should investigate the properties of malic acid in birch syrup and its relationship with other organic acids since it is a dicarboxylic acid.

Other observed correlations were between the different minerals. Calcium appeared to have a strong positively correlated association with magnesium (0.99; $p <0.01$). Potassium was noted to have a positive correlation with most of the other minerals, while iron and aluminum had a strong negative correlation with nearly all of the other minerals ($P <0.05$). These correlations might be due to many factors such as environmental conditions, natural sources (sap, soil), tree varieties, and chemical interactions. Minerals are concentrated in syrup during the boiling process and some of these minerals, such as calcium complexes (calcium malate), are found in the niter sediment in the finishing pan (Perkins and van den Berg, 2009).

Table 6: Pearson's correlation: Strength and significance between the different Birch syrup parameters and components.

Correlation	**Value**	**Significance (P-value)**
Brix vs. Water Activity	-0.96	<0.01
Brix vs. Fructose	Not significant	Not significant

Table 6 Cont. Pearson's correlation: Strength and significance between the different Birch syrup parameters and components.

Brix vs. Glucose	Not significant	Not significant
Magnesium vs. Calcium	0.99	<0.01
Citric Acid vs. Tartaric Acid	-0.85	<0.05
Malic Acid vs. Tartaric Acid	0.97	<0.01

3.9. Microbial Population

The birch and maple syrup samples used in this study were tested to determine the microbial load in the samples. According to the standards established by the FDA, ready-to-eat foods should have less than 250 colony-forming units (CFU/g) for total aerobic plate counts and less than 10 CFU/g for yeast and mold counts (FDA, 2001). Six samples of blended birch and maple syrup samples were analyzed in duplicate, and the results showed no detectable counts at the suitable count range of 25-250 CFU/mL for aerobic bacteria, yeasts, and molds. These results suggest that temperatures of 85-87.7℃ used to hot fill these samples are adequate to control bacteria, yeasts, and molds in both syrups and why this temperature range has been widely used by maple syrup producers. However, more research needs to be conducted with more birch syrup samples to further validate these results because of the small sample size used in this study.

3.10. Mycology Analysis

Birch and maple syrup samples were initially inoculated with fungal spores and poured onto MYA plates. After incubation for one week, the plates were visually checked for evidence of fungal growth. Robust fungal colonies were observed, which indicates successful fungal propagation (Figure 9). At temperatures of 85℃ or 87.7℃, four minutes appeared to be adequate

to inactivate the inoculated fungal spores of *Eurotium* (*Aspergillus* representing the asexual stage), *Penicillium brevicompactum*, and *Rhodotorula mucilaginosa* when the contamination level was 100 spores in 500 µl for both syrups. The controls were neither inoculated with spores nor thermally treated. While no fungal growth was observed, yeast growth was noticed on some of the plates, which included the controls and some of the heat-treated samples. In the previous microbiology analysis, neither yeast nor mold was identified in the birch and maple samples sourced from the same batch utilized in the mycology analysis. When two more unopened samples of birch and maple syrup containers from a different batch were plated out to see if they were contaminated, yeast growth was observed in all the samples.

For the mycology analysis, malt yeast agar (MYA) was used for the isolation and cultivation of the spores, which is different from the media used in the microbiology analysis (3M™ Petrifilm™ Aerobic Yeast and Mold Count plates). While the 3M™ Petrifilm™ Yeast and Mold Count Plates do not specifically have maltose as a constituent, MYA is commonly used for the cultivation of yeast due to the presence of maltose, which is a good source of energy for yeasts (Gientka et al., 2016). The syrup could be contaminated with spores since the syrup was produced in several batches, then blended and heated a second time. When there are multiple processing steps, this exposes the product to possible spore contamination. The syrup samples appear to be heated at an adequate temperature but could potentially harbor partially injured fungal spores. Yeasts are commonly occurring microorganisms in diverse environments, and spores can persist in the air, on food contact surfaces, in the sap and can persist under high sugar concentrations, such as syrups (Filteau et al., 2012). While both the MYA and yeast and mold count plates were incubated at the same temperature (20°C), it is plausible that the syrups already contained yeast. When cultivated under the right conditions, such as malt yeast agar (a maltose-rich environment), after seven days of incubation, the yeasts could thrive and become discernible.

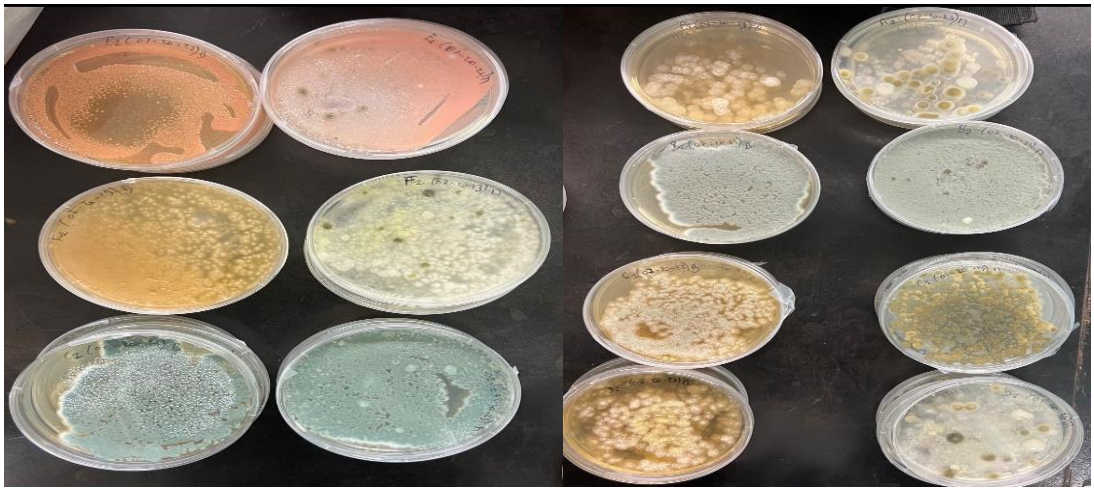

Fig.9. Growth of Fungal Spores. **Left picture**: top set of plates contains Rhodotorula mucilaginosa, middle set Eurotium sp2, bottom set Penicillium sp2. **Right picture**: top set, third set, and bottom set Eurotium sp1 and second set Penicillium brevicompactum.

CHAPTER 4 SUMMARY AND CONCLUSIONS

Research on maple syrup is extensive and covers various aspects such as quality, safety, and chemical composition. However, there is limited documentation on birch syrup and what is considered safe production parameters. Birch syrup is often compared to maple syrup since they are both tree sap-based natural sweeteners despite having very few similarities, such as color and flavor. The overall aim of this study was to take initial steps to determine the possible Brix and water activity relationships between the birch and maple syrup samples analyzed, the microbial safety of the hot fill process and temperatures commonly used by the maple industry, the temperature and time needed to control fungal spore outgrowth, and the chemical and quality differences between these syrups.

The Brix levels of maple syrup typically range between 66-68.9 degrees Brix to meet regulatory requirements. However, the birch syrup samples analyzed had much lower Brix levels ranging from 62.2-63.6 degrees Brix, below the standard Brix level for maple syrup. A significant relationship was noticed between Brix and water activity, which indicates their mutual interaction. However, correlations between Brix values and sugar content were not strong or significant. The instruments used for density analysis may understate sugar concentration in birch syrup due to its invert sugar composition. The water activity level for birch syrup was below 0.850 at this Brix level. This difference could be attributed to variations in sugar composition between the saps. Overall, due to birch syrup's limited production compared to maple syrup, no specific USDA standards have been set yet for birch syrup production. More studies need to be conducted, but results suggest that birch syrup could be produced safely at a lower Brix level (62-65 degrees Brix).

Both maple and birch syrups had differences in pH levels, with birch syrup being more acidic than maple syrup. Birch syrup exhibited higher levels of organic (citric and tartaric) acids compared to maple syrup, which may contribute to birch syrup's distinctive tangy flavor.

Maple and birch syrups were also analyzed for their mineral composition, revealing potassium, calcium, and magnesium as the most abundant minerals in both syrups. Maple syrup contained manganese and trace amounts of several other minerals. Birch syrup exhibited higher concentrations of potassium, calcium, magnesium, manganese, phosphorus, sulfur, and zinc compared to maple syrup. These mineral concentration differences could be due to the differences in tree species, time of season, or due to temperature/tree physiology differences since the birch trees are tapped in April. Birch sap is also boiled longer than maple and therefore, minerals could be in higher concentrations due to the more prolonged boiling time to concentrate the sap into birch syrup.

All the syrup samples analyzed appeared to meet the FDA microbial limits set for shelf stable food products (aerobic bacteria, yeast, and molds), with colony-forming units below specified limits. Mycology results revealed that thermal treatment probably deactivated inoculated fungal spores, but uninoculated yeast growth was observed on some plates, which indicates potential residual yeast spores in the syrup samples. This underscores the importance of thorough microbial testing and monitoring in syrup production processes and the importance of a thorough heating and hot fill process when filling retail containers.

Future work could further investigate seasonal variations, producer geography, soil differences, and climate to determine if these variables can affect the Brix, pH, water activity levels, sugar, and mineral concentrations in other birch syrup samples. With more data,

regulatory agencies can move forward to determine how to best assess an adequate Brix level range and processing parameters to begin creating regulatory definitions to best regulate the growing birch syrup industry.

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APPENDICES

APPENDIX A: CHEMICAL ANALYSIS (SUGARS AND ORGANIC ACIDS) OF FILTERED AND UNFILTERED BIRCH SYRUP SAMPLES

In total, eleven unfiltered and two filtered/blended 20 mL birch syrup samples were supplied by Temple Tappers in Temple, ME to determine potential nutrient differences throughout the 2023 syrup season. The sap was collected from April 11 through April 25. The daily temperature ranges are listed below, ranging between 5.6 to 24.4ºC. The sap was mainly obtained from yellow, gray, and paper birch trees. The birch sap passed first through reverse osmosis to be concentrated to approximately 5 degrees Brix and then was concentrated further with an evaporator and the finishing pan. A few of the syrup samples were filtered using a vacuum filter and hot-filled at 85-87.7°C. Each syrup sample was separately processed, and some of the filtered birch syrups were blended and bottled into two containers, while the unfiltered syrups were not blended. The finished birch syrup samples were named based on the collection date of each sap sample + the filtered/blended and unfiltered/unblended characteristics.

Daily Temperature Range (in ºC) for the 2023 Birch Syrup Season:

04/11 37: 17.2º

04/12 42: 5.6º

04/13 41: 23.3º

04/14 46: 24.4º

04/15 40: 21.1º

04/16 44: 13.9º

04/17 43: 11.1º

04/19 34: 7.8º

04/20 32: 10.6º

04/21 32: 13.9º

04/22 33: 15º

04/23 39: 13.9º

04/24 41: 5.6º

04/25 38: 6.7º

Legend for the Birch Syrup Samples and Date Collected:

First Run Filtered (1FR F)

Day1-Day 4 Filtered and Blended (1-4FB)

Day 2 Unfiltered (2Unf)

Day 3 Unfiltered (3Unf)

Day 4 Unfiltered (4Unf)

Day 5 Unfiltered (5Unf)

Day 3-Day 5 Filtered and Blended (3-5FB)

Day 6 Unfiltered (6Unf)

Day 7 Unfiltered (7Unf)

Day 8 Unfiltered (8Unf)

Day 9 Unfiltered (9Unf)

Day 10 Unfiltered (10Unf)

Day 11 Unfiltered (11Unf)

METHODOLOGY

For the sugar analysis, the levels of glucose, fructose, and sucrose were evaluated using high-performance liquid chromatography. Birch syrup samples (100 mg) were weighed into glass

test tubes in duplicate. All diluents and solvents used were HPLC grade. The three standards were prepared by dissolving 100 mg of glucose, fructose, and sucrose in 100 mL of the solvent, which was acetonitrile/water (50:50, v/v). The same solvent was used to prepare the sample extract dilutions with a 1:20 ratio of syrup and solvent. As for the mobile phase, it consisted of acetonitrile/water (65:35, v/v). An HPLC system (Agilent Technologies 1100 Series) equipped with a computer, pump system, autosampler, SHODEX NH2 column, and a refractive index detector was used for sugar analysis. The analytical conditions were an injection volume of 25 µl and a flow of 0.3 mL/min.

The organic acids analysis was performed with the same birch syrup samples using an HPLC system (Agilent Technologies), but this time equipped with an Allure Organic Acids 300 X 4.6 MM 5 micron column (Restek Corp, Bellefonte, PA), an autoinjector, and a photo-diode array (DAD) detector of a 100 mM potassium phosphate solution at pH 2.5 was used as the diluent for the samples and the standards, and the mobile phase injection volume was 25 µl. The samples were duplicated and diluted in a 1:10 potassium phosphate dilution, and the standards (citric, fumaric, malic, oxalic, tartaric, and succinic acids) were prepared by diluting 100 mg of each standard in 50 mL of potassium phosphate solution. The wavelength was set at 226 nm.

RESULTS AND DISCUSSION

As expected, most of the birch samples contained fructose and glucose (Figure A-1, Table A-2) since these were the predominant sugars identified in the blended birch syrups analyzed during the actual experiment. Worth noting that sucrose was occasionally detected in some of the syrup samples near the middle and later part of the season. Sugar levels and temperatures varied over the course of the season without any noticeable trends.

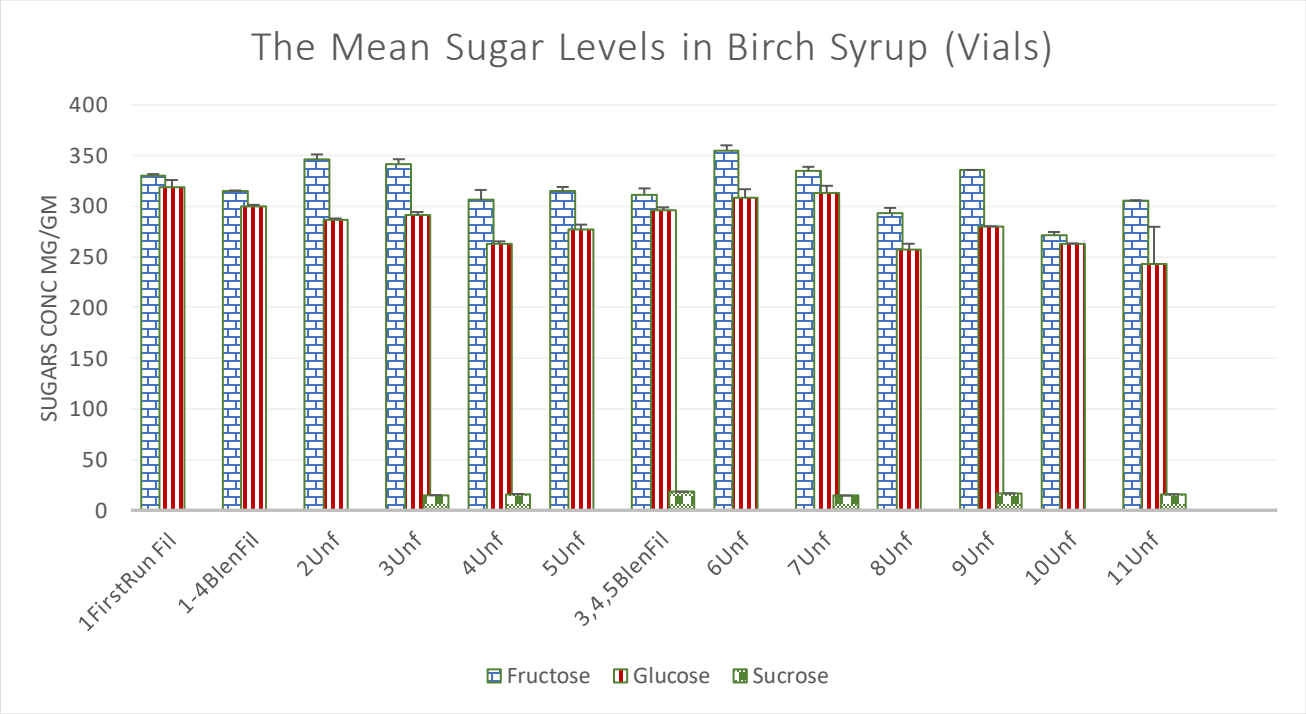
No temperature trends were noted. However, when the temperatures increased to >21ºC some sucrose was noted, but later in the season, sucrose was also seen in birch syrup samples when the temperature levels decreased to ≤15ºC.

Table A-1: The Mean Sugar Levels in Birch Syrup Samples

Samples	**Fructose**	**Glucose**	**Sucrose**
1FirstRun Fil	330.41 mg/g	319.06 mg/g	Not Detected
1-4BlenFil	315.415 mg/g	299.82 mg/g	Not Detected
2Unf	346.355 mg/g	286.525 mg/g	Not Detected
3Unf	341.74 mg/g	290.925 mg/g	14.735 mg/g
4Unf	306.06 mg/g	262.65 mg/g	15.86 mg/g
5Unf	314.68 mg/g	276.89 mg/g	Not Detected
3,4,5BlenFil	311.635 mg/g	296.19 mg/g	18.115 mg/g
6Unf	355.05 mg/g	308.7 mg/g	Not Detected
7Unf	334.895 mg/g	313.505 mg/g	14.36 mg/g
8Unf	293.23 mg/g	257.435 mg/g	Not Detected
9Unf	335.61 mg/g	279.715 mg/g	16.655 mg/g
10Unf	271.76 mg/g	262.73 mg/g	Not Detected
11Unf	305.195 mg/g	243.085 mg/g	15.73 mg/g

Values are Mean ± SD (n=2)

Figure A-1: The Mean Sugar Levels in Birch Syrup (Vials)



* Each value represents the mean sugar values (n=2). Error bars represent the standard deviation.

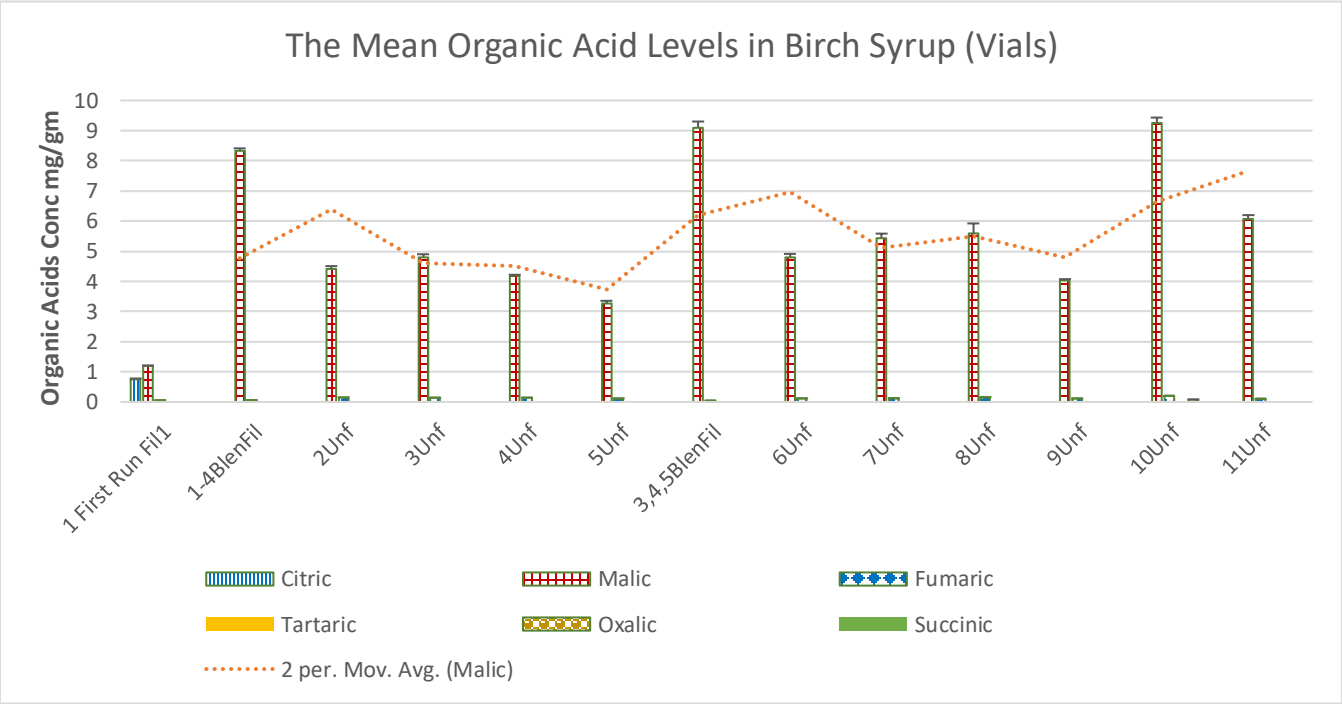
For the organic acid results (Figure A-2, Table A-2), citric acid was only detected in the First-run-filt sample. Malic acid was present in most samples, but in much higher concentrations in the blended and filtered samples and then again later on in the season, with the highest concentration in the 10Unf sample. Although tartaric, oxalic, and succinic acids were not detected in any of the samples, fumaric acid was found in trace amounts in all syrup samples. As expected, these results were similar to the ones obtained from the birch samples used in the main study. The variation in organic acid content across samples may affect the perceived taste and overall quality of the syrup (del Nozel et al., 1998; Doores, 2005), although this producer blends his syrups before hot filling into retail containers. Most likely the variability in organic acid levels is due to tree physiological and/or temperature changes over the course of the season.

Table A-2: The Mean Organic Acid Levels in Birch Syrup Samples

Samples	**Citric**	**Malic**	**Fumaric**	**Tartaric**	**Oxalic**	**Succinic**
1 First Run Fil1	0.75 mg/g	1.21 mg/g	0.06 mg/g	ND	ND	ND
1-4BlenFil	ND	8.34 mg/g	0.06 mg/g	ND	ND	ND
2Unf	ND	4.42 mg/g	0.145 mg/g	ND	ND	ND
3Unf	ND	4.8 mg/g	0.14 mg/g	ND	ND	ND
4Unf	ND	4.19 mg/g	0.14 mg/g	ND	ND	ND
5Unf	ND	3.27 mg/g	0.12 mg/g	ND	ND	ND
3,4,5BlenFil	ND	9.095 mg/g	0.04 mg/g	ND	ND	ND
6Unf	ND	4.805 mg/g	0.115 mg/g	ND	ND	ND
7Unf	ND	5.435 mg/g	0.13 mg/g	ND	ND	ND
8Unf	ND	5.575 mg/g	0.155 mg/g	ND	ND	ND
9Unf	ND	4.03 mg/g	0.12 mg/g	ND	ND	ND
10Unf	ND	9.255 mg/g	0.2 mg/g	ND	0.08 mg/g	ND
11Unf	ND	6.07 mg/g	0.1 mg/g	ND	ND	ND

Values are Mean ± SD (n=2)

Figure A-2: The Mean Organic Acid Levels in Birch Syrup (Vials)



* Each value represents the mean sugar values (n=2). Error bars represent the standard deviation.

APPENDIX B: BIRCH AND MAPLE SYRUP DATA TABLES FROM THE MAIN STUDY

Table B-1: The Mean pH Values of Birch and Maple Syrup Samples

Samples	**pH**
B1	4.63 ± 0.01
B2	4.62 ± 0.01
B3	4.63 ± 0.01
B4	4.64 ± 0.01
B5	4.64 ± 0.01
B6	4.64 ± 0.01
M1	6.61 ± 0.01
M2	6.59 ± 0.02
M3	6.59 ± 0.02
M4	6.67 ± 0.01
M5	6.61 ± 0.01
M6	6.69 ± 0.01

Values are Mean ± SD (n=3)

Table B-2: The Mean Brix Values of Birch and Maple Syrup Samples

Samples	**Brix**
B1	63.64 ± 0.05
B2	63.57 ± 0.22

Table B-2 Cont. The Mean Brix Values of Birch and Maple Syrup Samples

B3	63.57 ± 0.10
B4	63.37 ± 0.08
B5	62.66 ± 0.21
B6	63.61 ± 0.23
M1	66.84 ± 0.35
M2	66.67 ± 0.14
M3	66.52 ± 0.19
M4	66.68 ± 0.10
M5	66.74 ± 0.27
M6	66.47 ± 0.07

Values are Mean ± SD (n=3)

Table B-3: The Mean Water Activity Levels of Birch and Maple Syrup Samples

Samples	**Aw**
B1	0.80 ± 0.00
B2	0.80 ± 0.00
B3	0.80 ± 0.00
B4	0.80 ± 0.00
B5	0.81 ± 0.00
B6	0.80 ± 0.00
M1	0.85 ± 0.00
M2	0.85 ± 0.00
M3	0.85 ± 0.00

Table B-3 Cont. The Mean Water Activity Levels of Birch and Maple Syrup Samples

M4	0.85 ± 0.00
M5	0.85 ± 0.00
M6	0.85 ± 0.00

Values are Mean ± SD (n=3)

Table B-4: The Mean Sugar Levels of Birch and Maple Syrup Samples

Syrup	**Fructose, mg/g**	**Glucose, mg/g**	**Sucrose, mg/g**
Maple	28.96 ± 17.39	31.07 ± 10.15	647.31 ± 15.25
Birch	326.53 ± 7.79	241.53 ± 23.63	19.04 ± 14.56

Data are presented as mean ± SD (N=12)

Table B-5: The Mean Organic Acid Levels of Birch and Maple Syrup Samples

Syrup	**Citric, mg/g**	**Fumaric, mg/g**	**Malic, mg/g**	**Oxalic, mg/g**	**Succinic, mg/g**	**Tartaric, mg/g**
Birch	0.61 ± 0.15	0.14 ± 0.02	5.59 ± 0.68	ND	ND	0.58 ± 0.07
Maple	0.12 ± 0.01	0.04 ± 0.00	2.43 ± 0.03	0.04 ± 0.00	ND	ND

Data are presented as mean ± SD (N=12)

Table B-6: Mineral Composition of Birch and Maple Syrup Samples

Samples	**Ca, ppm**	**K, ppm**	**Mg, ppm**	**P, ppm**	**Al, ppm**	**B, ppm**	**Cu, ppm**	**Fe, ppm**	**Mn, ppm**	**Na, ppm**	**S, ppm**	**Zn, ppm**
B1	5567	5826	1412	155	5.21	5.64	1.23	3.89	365	14.60	155	111
B2	5540	5745	1406	154	6.14	5.40	1.24	4.61	363	14.00	151	108
B3	5539	5880	1407	157	5.48	6.21	1.27	4.21	369	15.50	157	111
B4	5462	5566	1386	153	6.14	5.39	1.25	4.85	357	14.00	153	108
B5	5568	5722	1416	154	6.86	5.49	1.20	4.87	357	14.00	154	109
B6	5575	5732	1419	153	7.28	5.29	1.24	5.34	362	14.00	152	109
M1	841	2642	136	3.84	4.65	2.02	0.210	2.27	26.4	3.62	5.64	0.604
M2	860	2641	135	3.76	1.59	1.91	0.249	< 1.00	26.2	3.68	5.34	0.605
M3	848	2637	135	3.90	3.10	2.43	0.260	< 1.00	26.3	3.75	5.16	0.610
M4	863	2694	138	3.92	3.14	1.80	0.282	3.87	26.8	3.47	6.02	0.629
M5	872	2699	139	3.89	2.71	1.92	0.265	< 1.00	26.7	4.47	5.45	0.636
M6	838	2635	134	3.50	2.00	1.83	0.215	< 1.00	26.2	2.27	5.20	0.657

Table B-7: Pearson's Correlation Table for the Relationship Among Birch Syrup Parameters

		pH	Brix	Aw	Fructose	Glucose	Sucrose	citric	fumaric	Malic	Tartaric	Ca	K	Mg	P	Al	B	Cu	Fe	Mn	Na	S	Zn
pH	Correlation	**1**	-0.56	0.49	0.16	0.17	0.46	0.37	-0.26	**-0.65**	**-0.65**	0.1	-0.33	0.23	-0.35	0.75	-0.21	-0.3	0.71	-0.5	-0.25	0.02	-0.16
	p		0.252	0.329	0.769	0.746	0.361	0.471	0.617	**0.16**	**0.16**	0.848	0.517	0.662	0.491	0.089	0.686	0.565	0.111	0.31	0.636	0.963	0.768
Brix	Correlation	-0.56	**1**	**-0.96**	0.31	-0.13	0.29	-0.4	-0.11	0.45	0.6	-0.07	0.3	-0.12	0.2	-0.42	0.16	0.74	-0.29	0.67	0.34	-0.04	0.26
	p	0.252		**0.002**	0.553	0.81	0.574	0.433	0.836	0.371	0.209	0.902	0.557	0.824	0.706	0.402	0.757	0.093	0.572	0.142	0.509	0.945	0.622
Aw	Correlation	0.49	-0.96	**1**	-0.31	-0.04	-0.43	0.33	0.16	-0.25	-0.43	0.26	-0.18	0.31	-0.2	0.5	-0.21	-0.8	0.33	-0.59	-0.38	-0.08	-0.28
	p	0.329	0.002		0.55	0.935	0.397	0.522	0.757	0.637	0.395	0.619	0.726	0.553	0.708	0.309	0.686	0.056	0.525	0.214	0.451	0.877	0.588
Fructose	Correlation	0.16	0.31	-0.31	**1**	0.44	0.57	0.07	0.15	-0.06	0.03	-0.53	-0.5	-0.44	-0.4	0.41	-0.29	0.55	0.64	-0.15	-0.26	-0.52	-0.64
	p	0.769	0.553	0.55		0.38	0.238	0.89	0.783	0.91	0.956	0.283	0.313	0.378	0.431	0.414	0.584	0.262	0.175	0.777	0.625	0.294	0.171
Glucose	Correlation	0.17	-0.13	-0.04	0.44	**1**	0.17	0.76	0.57	-0.75	-0.72	-0.74	-0.25	-0.69	0.27	-0.17	0.46	0.56	0.02	-0.03	0.37	0.37	-0.1
	p	0.746	0.81	0.935	0.38		0.742	0.082	0.239	0.084	0.108	0.092	0.64	0.128	0.601	0.749	0.358	0.251	0.968	0.949	0.469	0.468	0.854
Sucrose	Correlation	0.46	0.29	-0.43	0.57	0.17	**1**	-0.23	-0.61	-0.28	-0.09	-0.42	-0.58	-0.35	-0.55	0.34	-0.39	0.31	0.53	-0.3	-0.28	-0.24	-0.27
	p	0.361	0.574	0.397	0.238	0.742		0.66	0.2	0.592	0.871	0.406	0.23	0.499	0.257	0.507	0.441	0.545	0.28	0.559	0.595	0.643	0.605
citric	Correlation	0.37	-0.4	0.33	0.07	0.76	-0.23	**1**	0.73	-0.77	**-0.85**	-0.14	0.22	-0.07	0.56	-0.02	0.68	0.25	-0.01	0.15	0.55	0.6	0.22
	p	0.471	0.433	0.522	0.89	0.082	0.66		0.1	0.071	**0.034**	0.798	0.677	0.889	0.252	0.972	0.139	0.626	0.986	0.771	0.255	0.204	0.671
fumaric	Correlation	-0.26	-0.11	0.16	0.15	0.57	-0.61	0.73	**1**	-0.18	-0.3	-0.13	0.36	-0.13	0.62	-0.26	0.63	0.35	-0.24	0.38	0.5	0.3	0.06
	p	0.617	0.836	0.757	0.783	0.239	0.2	0.1		0.727	0.561	0.807	0.486	0.803	0.186	0.624	0.184	0.493	0.643	0.462	0.313	0.56	0.91
Malic	Correlation	-0.65	0.45	-0.25	-0.06	-0.75	-0.28	-0.77	-0.18	**1**	**0.97**	0.37	0.18	0.29	-0.2	-0.07	-0.4	-0.15	-0.14	0.2	-0.32	-0.58	-0.16
	p	0.16	0.371	0.637	0.91	0.084	0.592	0.071	0.727		**0.001**	0.472	0.732	0.577	0.702	0.899	0.427	0.774	0.796	0.701	0.531	0.225	0.763
Tartaric	Correlation	-0.65	0.6	-0.43	0.03	-0.72	-0.09	-0.85	-0.3	0.97	**1**	0.27	0.12	0.19	-0.24	-0.11	-0.42	-0.02	-0.13	0.23	-0.3	-0.58	-0.14
	p	0.16	0.209	0.395	0.956	0.108	0.871	0.034	0.561	0.001		0.611	0.822	0.721	0.64	0.839	0.405	0.975	0.807	0.666	0.557	0.227	0.786
Ca	Correlation	0.1	-0.07	0.26	-0.53	-0.74	-0.42	-0.14	-0.13	0.37	0.27	**1**	0.66	**0.99**	0.2	0.21	0.05	-0.45	-0.05	0.33	0.08	0.08	0.44
	p	0.848	0.902	0.619	0.283	0.092	0.406	0.798	0.807	0.472	0.611		0.151	**<.001**	0.703	0.686	0.931	0.372	0.929	0.524	0.874	0.882	0.382
K	Correlation	-0.33	0.3	-0.18	-0.5	-0.25	-0.58	0.22	0.36	0.18	0.12	0.66	**1**	0.6	0.84	-0.46	0.72	0.19	-0.62	0.88	0.76	0.6	0.83
	p	0.517	0.557	0.726	0.313	0.64	0.23	0.677	0.486	0.732	0.822	0.151		0.205	0.037	0.355	0.105	0.717	0.193	0.021	0.077	0.211	0.039
Mg	Correlation	0.23	-0.12	0.31	-0.44	-0.69	-0.35	-0.07	-0.13	0.29	0.19	0.99	0.6	**1**	0.14	0.33	0.01	-0.45	0.08	0.27	0.04	0.05	0.38
	p	0.662	0.824	0.553	0.378	0.128	0.499	0.889	0.803	0.577	0.721	<.001	0.205		0.787	0.521	0.983	0.369	0.882	0.608	0.933	0.925	0.454
P	Correlation	-0.35	0.2	-0.2	-0.4	0.27	-0.55	0.56	0.62	-0.2	-0.24	0.2	0.84	0.14	**1**	-0.7	0.97	0.42	-0.75	0.82	0.95	0.84	0.81
	p	0.491	0.706	0.708	0.431	0.601	0.257	0.252	0.186	0.702	0.64	0.703	0.037	0.787		0.123	0.001	0.406	0.084	0.048	0.003	0.036	0.051
Al	Correlation	0.75	-0.42	0.5	0.41	-0.17	0.34	-0.02	-0.26	-0.07	-0.11	0.21	-0.46	0.33	-0.7	**1**	-0.65	-0.43	0.96	-0.6	-0.68	-0.6	-0.61
	p	0.089	0.402	0.309	0.414	0.749	0.507	0.972	0.624	0.899	0.839	0.686	0.355	0.521	0.123		0.158	0.4	0.003	0.207	0.137	0.209	0.201
B	Correlation	-0.21	0.16	-0.21	-0.29	0.46	-0.39	0.68	0.63	-0.4	-0.42	0.05	0.72	0.01	0.97	-0.65	**1**	0.51	-0.66	0.75	0.98	0.9	0.77
	p	0.686	0.757	0.686	0.584	0.358	0.441	0.139	0.184	0.427	0.405	0.931	0.105	0.983	0.001	0.158		0.298	0.15	0.089	0.001	0.016	0.071
Cu	Correlation	-0.3	0.74	-0.8	0.55	0.56	0.31	0.25	0.35	-0.15	-0.02	-0.45	0.19	-0.45	0.42	-0.43	0.51	**1**	-0.21	0.61	0.6	0.27	0.21
	p	0.565	0.093	0.056	0.262	0.251	0.545	0.626	0.493	0.774	0.975	0.372	0.717	0.369	0.406	0.4	0.298		0.688	0.195	0.209	0.61	0.689
Fe	Correlation	0.71	-0.29	0.33	0.64	0.02	0.53	-0.01	-0.24	-0.14	-0.13	-0.05	-0.62	0.08	-0.75	0.96	-0.66	-0.21	**1**	-0.62	-0.68	-0.64	-0.71
	p	0.111	0.572	0.525	0.175	0.968	0.28	0.986	0.643	0.796	0.807	0.929	0.193	0.882	0.084	0.003	0.15	0.688		0.189	0.14	0.169	0.112
Mn	Correlation	-0.5	0.67	-0.59	-0.15	-0.03	-0.3	0.15	0.38	0.2	0.23	0.33	0.88	0.27	0.82	-0.6	0.75	0.61	-0.62	**1**	0.84	0.52	0.74
	p	0.31	0.142	0.214	0.777	0.949	0.559	0.771	0.462	0.701	0.666	0.524	0.021	0.608	0.048	0.207	0.089	0.195	0.189		0.038	0.288	0.091
Na	Correlation	-0.25	0.34	-0.38	-0.26	0.37	-0.28	0.55	0.5	-0.32	-0.3	0.08	0.76	0.04	0.95	-0.68	0.98	0.6	-0.68	0.84	**1**	0.88	0.84
	p	0.636	0.509	0.451	0.625	0.469	0.595	0.255	0.313	0.531	0.557	0.874	0.077	0.933	0.003	0.137	0.001	0.209	0.14	0.038		0.022	0.038
S	Correlation	0.02	-0.04	-0.08	-0.52	0.37	-0.24	0.6	0.3	-0.58	-0.58	0.08	0.6	0.05	0.84	-0.6	0.9	0.27	-0.64	0.52	0.88	**1**	0.86
	p	0.963	0.945	0.877	0.294	0.468	0.643	0.204	0.56	0.225	0.227	0.882	0.211	0.925	0.036	0.209	0.016	0.61	0.169	0.288	0.022		0.029
Zn	Correlation	-0.16	0.26	-0.28	-0.64	-0.1	-0.27	0.22	0.06	-0.16	-0.14	0.44	0.83	0.38	0.81	-0.61	0.77	0.21	-0.71	0.74	0.84	0.86	**1**
	p	0.768	0.622	0.588	0.171	0.854	0.605	0.671	0.91	0.763	0.786	0.382	0.039	0.454	0.051	0.201	0.071	0.689	0.112	0.091	0.038	0.029	

*Table depicts the correlation strength and significance between the different parameters.

BIOGRAPHY OF THE AUTHOR

Djeneba Diarra was born in Bamako, Mali on September 6, 1997. She attended Ondokuz Mayis University in Samsun, Turkey, and graduated with a Bachelor's degree in Molecular Biology and Genetics. She came to Maine and entered the Food Science graduate program at the University of Maine. During that time, she worked as a teaching assistant for both the Anatomy and Biology courses. After receiving her degree, Djeneba will be joining Bureau Veritas in Quebec, Canada to begin her career in the field of food safety. Djeneba is a candidate for the Master of Science degree in Food Science and Human Nutrition from the University of Maine in May 2024.